

Additive Manufacturing of Intraocular Lenses and their Functionalisation for Ophthalmic Drug Delivery



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Table of Contents

List of Figures	8
List of Tables	18
List of Abbreviations	19
Acknowledgements.....	24
Abstract.....	26
1. Thesis Overview	29
1.1 Introduction.....	29
1.2 Aim and Objectives.....	31
1.3 Thesis structure	32
2. Introduction.....	34
2.1 Anatomy and physiology of the human eye.....	34
2.2 The Lens.....	35
2.3 Cataract	38
2.3.1 Prevalence and Pathophysiology	38
2.3.2 Risk Factors	39
2.3.3 Socio-economic impact.....	41
2.3.4 Cataract treatment	42
2.4 The IOL: Materials and Designs.....	44
2.4.1 Materials	44
2.4.2 Designs.....	52
2.5 Posterior Capsule Opacification.....	61
2.5.1 Prevalence of PCO	61
2.5.2 Pathophysiology of PCO.....	62
2.5.3 PCO treatment.....	64
2.5.4 PCO prevention.....	65
2.6 Drug Delivery Systems	78
2.6.1 Drug release mechanisms.....	80
2.6.2 Drug release kinetics	84
2.7 Ophthalmic Drug Delivery Systems	88
2.7.1 Drug Delivery Systems for the Prevention of PCO	90
2.8 Production of IOLs	96
2.8.1 General Considerations for the Design and Production of IOLs	96
2.8.2 Techniques currently employed in the production of IOLs	100
2.9 3D-printing technologies.....	101
2.9.1 Stereolithography	104

2.10	Free Radical Photopolymerisation	111
2.10.1	Initiation	113
2.10.2	Propagation	117
2.10.3	Termination.....	120
2.11	Controlled Radical Polymerisation	121
2.12	Reactivity of Radicals	125
2.13	Influence of the Polymer Structure on its Physico-Chemical Properties	127
2.13.1	Molecular weight of the polymer chains.....	129
2.13.2	Side chains	130
2.13.3	Characteristics of the polymer backbone	132
2.13.4	Crosslinking	132
2.13.5	Intermolecular forces	133
2.13.6	Polarity of the polymer chains	134
3.	Development of 3D-Printable Hydrophilic Formulations.....	136
3.1	Introduction.....	136
3.2	Aim and Objectives.....	140
3.3	Materials and Methods.....	140
3.3.1	Manufacture of IOLs.....	140
3.3.2	ATR-FTIR.....	144
3.3.3	Differential Scanning Calorimetry.....	145
3.3.4	Thermogravimetric analysis.....	145
3.3.5	Swelling tests	146
3.3.6	Scanning Electron Microscopy	146
3.4	Results.....	146
3.4.1	3D printing IOLs.....	146
3.4.2	ATR-FTIR.....	149
3.4.3	Differential Scanning Calorimetry.....	151
3.4.4	Thermogravimetric analysis.....	151
3.4.5	Swelling tests	153
3.4.6	Scanning Electron Microscopy	156
3.5	Discussion.....	158
3.6	Conclusions.....	160
4.	Development of 3D-Printable Hydrophobic Formulations.....	163
4.1	Introduction.....	163
4.2	Aim and Objectives.....	167
4.3	Materials and Methods.....	168
4.3.1	Manufacture of IOLs.....	168

4.3.2	Optical microscopy	171
4.3.3	Analysis of the efficiency of the post-curing process	171
4.3.4	ATR-FTIR.....	172
4.3.5	Water Contact Angle.....	172
4.3.6	Differential Scanning Calorimetry.....	173
4.3.7	Thermogravimetric analysis.....	173
4.3.8	Swelling tests	173
4.3.9	Scanning Electron Microscopy	174
4.3.10	Cytotoxicity assays	174
4.3.11	Statistical analysis.....	175
4.3.12	Implantability test	175
4.4	Results.....	176
4.4.1	Manufacture of IOLs.....	176
4.4.2	Analysis of the efficiency of the post-curing process	182
4.2.3	ATR-FTIR.....	184
4.2.4	Water Contact Angle.....	186
4.2.5	Differential Scanning Calorimetry	187
4.2.6	Thermogravimetric analysis.....	191
4.2.7	Swelling tests	195
4.2.8	Scanning Electron Microscopy	197
4.2.9	Cytotoxicity assays	199
4.2.10	Implantability tests.....	204
4.5	Discussion.....	205
4.6	Conclusions.....	210
5.	Optimisation of the 3D-Printing Resolution	213
5.1	Introduction.....	213
5.2	Aim and Objectives.....	216
5.3	Materials and Methods.....	217
5.3.1	Cure depth measurements	217
5.3.2	¹ H-NMR.....	218
5.3.3	ATR-FTIR.....	219
5.3.4	Manufacture of IOLs.....	219
5.3.5	Optical microscopy	221
5.3.6	Differential Scanning Calorimetry.....	221
5.3.7	Thermogravimetric analysis.....	221
5.3.8	Scanning Electron Microscopy	222
5.3.9	Statistical analysis.....	222

5.4	Results.....	222
5.4.1	Cure depth measurements	222
5.4.2	¹ H-NMR.....	224
5.4.3	ATR-FTIR.....	225
5.4.4	Manufacture of IOLs.....	227
5.4.5	Differential Scanning Calorimetry.....	235
5.4.6	Thermogravimetric analysis.....	237
5.4.7	Scanning Electron Microscopy	239
5.5	Discussion.....	243
5.6	Conclusions.....	247
6.	Surface Functionalisation of Intraocular Lenses.....	250
6.1	Introduction.....	250
6.2	Aim and Objectives.....	255
6.3	Materials and Methods.....	256
6.3.1	Synthesis of pHEMA for spin coating	256
6.3.2	¹ H-NMR	257
6.3.3	Gel Permeation Chromatography.....	257
6.3.4	Spin coating IOLs	258
6.3.5	Optical microscopy	258
6.3.6	Production of polymer brushes	258
6.3.7	Scanning Electron Microscopy	259
6.3.8	Ellman's reaction	260
6.3.9	Water Contact Angle.....	260
6.3.10	ATR-FTIR.....	261
6.3.11	Statistical analysis	261
6.4	Results.....	261
6.4.1	Coatings produced by spin coating	261
6.4.2	Synthesis of polymer brushes	271
6.5	Discussion.....	287
6.6	Conclusions.....	292
7.	Conclusions and Future Directions.....	295
7.1	Conclusions.....	295
7.1.1	Additive manufacturing of IOLs.....	295
7.1.2	Optimisation of the 3D-printing resolution.....	298
7.1.3	Surface modification of IOLs	300
7.1.4	General conclusions	301
7.2	Future directions	302

8. Bibliography	308
9. Appendixes	342
Appendix 1.....	342
Appendix 2.....	343
Appendix 3.....	344
Appendix 4.....	345
Appendix 5.....	346
Appendix 6.....	347
Appendix 7.....	348
Appendix 8.....	349
Appendix 9.....	350
Appendix 10.....	351
Appendix 11.....	352

List of Figures

Figure 2.1: Anatomy of the human eye ³⁰	35
Figure 2.2: Structure of the human lens ⁴⁰	36
Figure 2.3: Transmission of light into the retina in a healthy (a) and in a cataractous eye (b) , where the light scattering reduces the quality of vision. Adapted from Thompson et al ³¹	38
Figure 2.4: Diagram of the different types of cataract that can develop in the lens ⁵⁹	39
Figure 2.5: Diagram showing the procedure followed during phacoemulsification. The lens fibres are extracted (a) prior to the injection of the IOL (b) , which is implanted in the capsular bag (c) . (Source: aaravmedicare.com).	43
Figure 2.6: Diagram of an intraocular lens formed of an optic portion and two haptics. The materials that are commonly used in the manufacture of these structures are detailed ¹⁸	44
Figure 2.7: Chemical structure of PMMA.	46
Figure 2.8: Chemical structure of PDMS.	47
Figure 2.9: Chemical structure of pHEMA.....	48
Figure 2.10: Chemical structure of pPhEMA.	50
Figure 2.11: Three-piece IOL design ¹¹⁴	53
Figure 2.12: Single-piece IOL design ¹¹⁴	53
Figure 2.13: Angulated IOL haptics ⁸²	54
Figure 2.14: Plate-haptic IOL ¹¹⁴	54
Figure 2.15: Staar toric plate-haptic IOL. (a) Original design with shorter haptic length and smaller fixation holes. (b) Improved design with larger fixation holes and longer haptic. The alignment marks are indicated with an arrow. Adapted from Dewey, 2015 ⁷	57
Figure 2.16: Comparison of spherical aberration between spherical (a) and aspheric IOLs (b) . Adapted from Yu et al., 2018 ¹³⁰	57
Figure 2.17: Schematics of refractive and diffractive multifocal IOLs. (a) Refractive lens showing the five zones in which it is divided, each providing optimal vision at different distances and light conditions. (b) Diffractive IOL comprised of a central area, which contains concentric levels of different heights that allow for distant and near focus, and a peripheral zone that mediates distance vision. Adapted from Yu et al., 2018 ¹³⁰	58
Figure 2.18: Crystalens® accommodative IOL (Source: bausch.com) (a) and its mechanism of action once implanted in the capsular bag (b) ¹³⁰	59
Figure 2.19: Development of IOLs over the past 60 years ¹³⁰	60
Figure 2.20: Retroillumination of an IOL immediately after cataract surgery (a) and after the development of PCO (b) . Adapted from Thompson et al., 2015 ³¹ and Colvard, 2012 ⁸³	61
Figure 2.21: Schematic diagram of the cellular responses that take place in the capsular bag during the development of PCO: proliferation, migration and transdifferentiation.....	62
Figure 2.22: Diagram of the signalling pathways involved in the development of PCO. Adapted from Nibourg et al., 2015 ¹⁴⁹	64
Figure 2.23: Anterior capsulorhexis ¹⁶⁵	66
Figure 2.24: Effect of the sharpness of the edge of an IOL on the migration of LECs. (a) Round-edge IOL implanted in the capsular bag, allowing the migration of LECs to the posterior capsule. (b) Inhibition of the migration of LECs upon implantation of a sharp-edge IOL. Adapted from Aghamollaei et al., 2019 ⁸⁶	68
Figure 2.25: Front (a) and side view (b) of the modified Zephyr® disk-shaped IOL (Anew Optics, Bristol, USA), with perforations in the haptics ¹⁸⁵ . (c) Schematic diagram showing a comparison between the "shrink-wrap" effect that occurs when an open-loop IOL with a square edge is implanted in the capsular bag and the open-bag configuration attained upon insertion of	

a disk-shaped IOL. While both designs have the capability to block the migration of LECs to the posterior capsule, the open-bag configuration has the added advantage of increasing the aqueous humour flow, hence reducing the concentration of cytokines on the posterior capsule. Adapted from Eldred et al., 2014 ¹⁸⁶	70
Figure 2.26: Reaction scheme depicting the grafting of poly(ethylene glycol) methacrylate (PEG-MA) on the surface of hydrophobic acrylic IOLs. The resulting coating can resist protein and cell adhesion. Adapted from Lin et al., 2010 ²⁰⁶	73
Figure 2.27: Endocapsular ring with a square edge and a groove on the inner surface that facilitates the IOL fixation ²⁰⁹	75
Figure 2.28: Representation of the concentration of substance vs. time, showing the fluctuations in drug concentration that occur upon administration of uncontrolled release systems and the maintenance of a constant level when a controlled release device is administered to the patient ²³⁹	80
Figure 2.29: Schematic of two diffusion-based delivery systems: reservoir (a) and matrix system (b). Adapted from Sun et al., 2014 ²⁴⁹	81
Figure 2.30: Mechanisms of drug release. Adapted from Kumar Patra et al., 2018 ²⁵⁸	83
Figure 2.31: Graphical representation of zero-order kinetics; f is the fraction of the drug dissolved during the time t and K_0 is the apparent velocity of dissolution ²²⁴	85
Figure 2.32: Graphical representation of first-order model of drug release: cumulative drug released (%) versus time ²²⁴	86
Figure 2.33: Barriers for ocular drug delivery and routes of drug administration to the eye: (1) topical, (2) intravitreal and (3) systemic. Adapted from Santos et al., 2018 ²⁷¹	89
Figure 2.34: IOL combined with two delivery systems inserted in the haptic ²⁸³	92
Figure 2.35: IOLs before (a) and after impregnation with cefuroxime sodium via supercritical impregnation (b) ²⁹⁵	93
Figure 2.36: Schematic representation of the layer-by-layer deposition of poly (sodium 4-styrenesulfonate) (PSS) and poly (ethylenimine) (PEI) ²⁹⁷	94
Figure 2.37: Schematic illustration of the working principle of the Nano-IOL, a lens functionalised with Au@SiO ₂ for the prevention of PCO via photodynamic therapy ³⁰⁷	95
Figure 2.38: Schematic of the processes involved in the foreign body reaction that occurs upon implantation of a medical device ³¹⁵	97
Figure 2.39: (a) IOL with glistenings ³²⁷ . (b) Comparison between the edge of an IOL that causes dysphotopsias (left) and another IOL which prevents this phenomenon (right) ³²⁸	99
Figure 2.40: (a) Drawing of a mould used for the production of the optic part of an IOL ³³⁸ . (b) IOL blank mounted on the mandrel prior to lathing ³³⁹ . (c) lathing of an IOL ¹³⁰	100
Figure 2.41: Diagram of a bottom-up (a) and a top-down (b) stereolithography system.	105
Figure 2.42: Parameters of stereolithography: (a) Schematic diagram of the production of two printed layers, showing the hatch spacing and hatch over-cure. Adapted from Khorasani et al., 2013 ³⁷⁶ . (b) Schematic diagram of the production of three printed layers, depicting the curing pattern obtained with stereolithography. Adapted from Salmoria et al., 2009 ³⁷⁷ . (c) Diagram depicting the importance of achieving the optimum cure depth in stereolithography, since it ensures the adequate attachment of each layer to the previous one. Insufficient cure depth would lead to failure of adhesion of the constitutive layers, while an excessive depth of cure would result in dimensional inaccuracies caused by the curing of resin in spaces that were designed to be empty. Adapted from van Bochove et al., 2016 ³⁷⁸	107
Figure 2.43: (a) Schematic diagram of a DLP-based micro-SLA system containing a digital micromirror device (DMD) which reflects the light onto the photopolymerisable resin ³⁸⁶ . (b) Schematic illustration of the experimental setup for 2PP. Adapted from Ferreras Paz et al., 2012 ³⁸⁷	109

Figure 2.44: Diagram showing the physical phenomena involved in photopolymerisation. The shrinkage of the polymer upon curing and the inhibitory effects exerted by oxygen have detrimental effects on the physico-chemical properties of the printed part. Adapted from Hafkamp et al., 2018 ³⁹² .	110
Figure 2.45: Scheme of the free radical polymerisation reaction. I represents the initiator, M is the monomer, R [•] is a primary radical, P _n [•] and P _m [•] are growing radical chains of length n and m, respectively, and P _{n+m} , P _n and P _m represent polymers without any active centre. Adapted from Goodner et al., 1999 ⁴⁰² .	111
Figure 2.46: Scheme of the thiol-ene reaction and the effects of oxygen on this process. Adapted from Cramer et al., 2001 ⁴⁰⁷ and Ligon et al., 2017 ³⁵⁰ .	113
Figure 2.47: Type I and II photoinitiators commonly used in free radical photopolymerisation. Adapted from Ligon et al., 2017 ³⁵⁰ and Allen, 1996 ⁴¹⁴ .	115
Figure 2.48: Schematic of the mechanism of generation of free radicals with Type I and Type II photoinitiators. Adapted from Dadashi-Silab et al., 2015 ⁴¹³ .	116
Figure 2.49: Schematic diagram of the mechanistic pathways for the inhibition of polymerisation by oxygen and the strategies that can be adopted to prevent this phenomenon. The green arrows represent the processes involved in the polymerisation reaction. The red arrows highlight the mechanisms of oxygen inhibition: (a) quenching of the excited state of the photoinitiator (PI), (b) formation of inactive peroxy radicals from initiating or propagating radicals. The blue arrows indicate the mitigation strategies: (c) termination via radical-radical recombination; (d) termination via hydrogen abstraction; (e) initiation stage strategies (inerting, light source, photoinitiators, singlet oxygen scavengers); (f) hydrogen donors; (g) reducing agents (RA) and (h) peroxide decomposition ³⁹¹ . “P” denotes the polymer chain, while “M” denotes the monomer.	119
Figure 2.50: Diffusion and reaction steps during termination between two macroradicals (red) ⁴⁰⁰ .	121
Figure 2.51: Equilibrium between active and dormant species in ATRP catalysed by Cu/2,2'-bipyridine complex ⁴³¹ .	122
Figure 2.52: (a) Mechanism of action of RAFT agents: the reactive S=C bond in the RAFT agent reacts with the growing polymer chain P _n [•] to form an intermediate from which the leaving group (R) can reinitiate polymerisation. The curly arrows depict the movement of the radical centre towards the leaving group R. (b) Schematic diagram of the RAFT process, where the monomer reacts with the RAFT agent to form the polymer chain. Adapted from Moad et al., 2013 ⁴³⁵ .	123
Figure 2.53: Mechanism of the RAFT polymerisation reaction. Following initiation, the radicals add to the RAFT agent and an equilibrium is established between active and dormant species. The chain transfer steps involve a reversible transfer of the chain end-group (Z – C(=S)S – R) between the dormant chains and the propagating radicals. The rate of addition/fragmentation should be higher than that of the propagation reaction in order for all chains to have a similar degree of polymerisation. Adapted from Perrier, 2017 ⁴³² .	124
Figure 2.54: (a) Molecular orbital diagram of the methyl radical. (b) Diagram showing the trend of increasing radical stability with increasing degree of substitution. Adapted from Ouellette et al., 2018 ⁴⁴⁹ .	126
Figure 2.55: Schematic diagram depicting the polymerisation reaction between alkene and thiol groups. The thiol groups can act as chain transfer agents that reduce the molecular weight of the resulting polymer chains ⁴⁶⁷ .	130
Figure 2.56: Effect of the increase in the length of the side chains on the T _g of the polymer..	131
Figure 2.57: Schematic representation of the formation of chemical and physical crosslinks between polymer chains.	133
Figure 2.58: Chemical structure of Kevlar®. Adapted from Kabir et al., 2013 ⁴⁷⁹ .	134

Figure 3.1: IOL opacification due to the formation of calcium deposits on its surface ⁸²	137
Figure 3.2: CAD model of the AcrySof [®] IOL and the support structure created in order to protect the lens from the forces created by the movement of the resin during the printer's peel process.	141
Figure 3.3: Reaction scheme detailing the photopolymerisation reaction of HEA, MA and PEG-DA.	142
Figure 3.4: Form1+ stereolithography system.	142
Figure 3.5: Diagram depicting the production of IOLs by stereolithography. The process involves the creation of the CAD model and the preparation of the formulation which is used for the manufacture of the IOLs. Once these are produced, a post-curing process is performed in order to eliminate the unreacted monomers that remain within the polymer after printing and which compromise the biocompatibility and mechanical properties of the implants.	144
Figure 3.6: Optical images of some of the first 3D printed hydrophilic IOLs that resulted from the experiments described in this chapter. (a) Replica of the AcrySof [®] IOL printed without any support structures. (b) Same IOL design printed with a custom-made, frame-like support. A distortion in the shape with respect to the original model can be seen in both cases.	147
Figure 3.7: (a) Image of the AcrySof [®] IOL. (b) 3D printed hydrophilic acrylic IOLs with the support structure still attached to them. (c) 3D printed hydrophilic acrylic IOLs after detaching the supports, with their haptic-to-haptic length measured in centimetres.	149
Figure 3.8: ATR-FTIR spectra obtained from the analysis of the resin used for the production of the IOLs (blue) and from printed IOLs before and after being post-cured (red and green, respectively). A reduction in the intensity of the signal generated by the C=C functional groups from acrylate monomers was observed after curing and post-curing.	150
Figure 3.9: Thermogram obtained from the DSC analysis of the polymer that forms the printed IOLs. This graph was generated by the second heating cycle. The value of the T_g is 4.97 °C.	151
Figure 3.10: Thermogram obtained from the thermogravimetric analysis of the polymer that forms the printed IOLs. The initial degradation temperature (IDT) of this material is 415.19 ± 18.9 °C. Two different phases can be discerned in this graph: one that corresponds to the degradation of approximately 15% of the polymer and another one that results from the degradation of the remaining 81% of material. These values match those of the theoretical composition of the polymer (82% HEA and 16% MA), hence confirming that the IOLs have the desired composition.	152
Figure 3.11: Increase in the weight of the 3D-printed hydrophilic IOLs incubated in artificial aqueous humour for one day.	154
Figure 3.12: Increase in the degree of swelling of the hydrophilic IOLs during the incubation in artificial aqueous humour for one day. The equation used for the calculation of the swelling percentages is shown on the graph, where W is the weight of the polymer after swelling and W_0 is the weight of the material before the swelling test.	154
Figure 3.13: Optical images of a hydrophilic IOL subjected to a swelling test, at each of the stages of this experiment: dry (a) and after 1 h (b) , 2 h (c) or 24 h of incubation in artificial aqueous humour (d)	156
Figure 3.14: SEM micrographs of the AcrySof [®] SA30AL IOL. (a) shows the optic-haptic junction and one of the haptics of the implant (original magnification X15). (b) shows the sharpness of the optic edge (original magnification X200). Adapted from Escobar-Gomez et al ⁴⁰⁷	157
Figure 3.15: SEM images of the optic part (a, b) and optic-haptic junction (c, d) before (a, c) and after post-processing and post-curing (b, d) . An increase in the smoothness is noticeable after these procedures.	158

Figure 4.1: Histopathological examination of the interactions between a round-edge AcrySof® IOL (a) and a sharp-edge analogue (b). The latter induces a tight wrapping of the capsular bag around the IOL, which prevents the migration of LECs to the posterior capsule ⁴⁹¹	164
Figure 4.2: (a) Diagram of a capsular bag preparation from a human donor lens ¹⁴¹ . (b) Modified dark-field image of a capsular bag implanted with a three-piece AcrySof® MA60BM IOL ⁴⁹⁴	167
Figure 4.3: CAD model of the AcrySof® IOL.	168
Figure 4.4: Reaction scheme detailing the photopolymerisation reaction of 2-phenoxyethyl acrylate (POEA) and poly(ethylene glycol) dimethacrylate (PEG-DMA) to form the 3D-printed crosslinked polymer structure that the IOLs are made of.	170
Figure 4.5: Reaction scheme detailing the photopolymerisation reaction of 2-phenoxyethyl acrylate and the aromatic urethane diacrylate crosslinker to form the 3D-printed polymer structure that the IOLs are made of.....	170
Figure 4.6: Form 2 stereolithography system.	171
Figure 4.7: Optical microscopy images of IOLs printed with formulation C1 (a, b) and C2 (c, d) with their dimensions measured using the microscope. (a) and (c) show the top view of the implants while (b) and (d) show a side view of the same.....	179
Figure 4.8: Variability in the optic diameter (a) and the thickness (b) of the IOLs printed with formulations C1 and C2. The dimensions that are denoted on the Y-axis with an asterisk correspond to the diameter and thickness of the CAD model.....	179
Figure 4.9: Optical images of IOLs printed with formulation C1 (a) and C2 (b). An increase in the optical quality is perceptible with formulation C2 in comparison with C1.	180
Figure 4.10: Digital images of IOLs printed with formulations C1 (a, b) and C2 (c, d). Images (a) and (c) show the IOLs incubated in PBS at 35 °C, while images (b) and (d) show the same implants after incubation and cooled down to room temperature. A decrease in transparency is noticeable in the latter.	181
Figure 4.11: Optical images depicting the foldability of an IOL printed with formulation C1 (a) and an analogous IOL produced with formulation C2 (b).....	182
Figure 4.12: ¹ H-NMR spectra obtained from the analysis of the efficiency of the post-curing process using IOLs printed with formulation C1. A depletion in the amount of residual monomers was observed with longer post-curing times, hence confirming the effectiveness of this protocol to eliminate the leachable molecules from the polymers (a). An enlarged view of the signal produced by the acrylate groups from the unreacted monomers is shown (b). The same results were obtained when repeating this procedure using IOLs that had been printed with formulation C2 (See Appendix 4).....	183
Figure 4.13: ATR-FTIR spectra obtained from the analysis of the resin used for the production of C1 IOLs (blue) and from lenses printed with this formulation, both before (red) and after being post-cured (green). A reduction in the intensity of the signal generated by the C=C functional groups from acrylate monomers was observed after both curing and post-curing. The same results were obtained when repeating this analysis with IOLs that had been printed with formulation C2 (Appendix 5).....	185
Figure 4.14: Comparison between the ATR-FTIR spectra obtained from the analysis of IOLs printed with either formulation C1 (blue) or C2 (red) and subjected to post-curing.	186
Figure 4.15: Water contact angle of (a) C1 and (b) C2 IOLs.	187
Figure 4.16: Thermogram obtained from the third cycle of the DSC analysis of the polymer that forms the IOLs printed with formulation C1. The value of T _g is 4.73 ± 0.32 °C.	188
Figure 4.17: Thermogram obtained from the third cycle of the DSC analysis of the polymer that forms the IOLs printed with formulation C2. The value of T _g is 10.27 ± 0.59 °C.....	189
Figure 4.18: Thermogram obtained from the third cycle of the DSC analysis of the polymer that forms the IOLs printed with formulation C1 and incubated in an aqueous environment at 35 °C for one month. The value of T _g is 4.85 ± 1.09 °C.	190

Figure 4.19: Thermogram obtained from the third cycle of the DSC analysis of the polymer that forms the IOLs printed with formulation C2 and incubated in an aqueous environment at 35 °C for one month. The value of T_g is 11.29 ± 0.31 °C.	191
Figure 4.20: Thermogram obtained from the thermogravimetric analysis of C1 IOLs. The IDT is 382.4 ± 2.17 °C.	192
Figure 4.21: Thermogram obtained from the thermogravimetric analysis of C2 IOLs. The IDT is 389.7 ± 2.63 °C.	193
Figure 4.22: Thermogram obtained from the thermogravimetric analysis of C1 IOLs after incubation in an aqueous environment at 35 °C for one month. The IDT is 383.69 ± 0.57 °C.	194
Figure 4.23: Thermogram obtained from the thermogravimetric analysis of C2 IOLs after incubation in an aqueous environment at 35 °C for one month. The IDT is 388.41 ± 3.85 °C.	195
Figure 4.24: Line graph showing the variation in weight of C1 and C2 IOLs incubated in an aqueous environment for one month.	196
Figure 4.25: Line graph showing the swelling percentage of IOLs made with formulations C1 and C2.	197
Figure 4.26: SEM micrographs of an IOL printed with formulation C1 showing its different parts: (a) left haptic; (b) left optic-haptic junction; (c) optic part of the IOL; (d) angle formed by the optic-haptic junction; (e) side view of the optic-haptic junction and (f) side view of one of the haptics. The scale bars represent 500 μm	198
Figure 4.27: SEM micrographs showing the optic-haptic junction and optic edge of an AcrySof® IOL (a, b, respectively) and same for a printed IOL (c, d).	198
Figure 4.28: Bar graphs showing the LDH release after a 48-hour incubation of FHL-124 cells in the presence of IOLs made with formulation C1 (a) or C2 (b) and which had been subjected to different stages of the post-curing process: not post-cured, UV-treated for 1 hour, or UV-irradiated and subjected to different heating times in vacuum (3, 4, 8 hours and overnight). One-way analysis of variance (ANOVA) was performed in order to evaluate the statistical significance. Tukey's multiple comparison post-hoc test was used to determine the differences within the experimental groups. The asterisk indicates that the levels of LDH release detected in the not post-cured group were significantly different to those detected in the rest of the experimental groups.	201
Figure 4.29: Optical microscopy images of lens cells from the control group and cells which had been cultured in the presence of C1 IOLs that had not been post-cured or which had been subjected to different stages of this process. As can be seen, after two days of incubation with IOLs that had not been post-cured or which had been treated only with UV light, the number of healthy cells was lower than on the control group, which did not contain any IOL. However, the thermal treatment performed after the irradiation with UV light led to a considerable increase in the number of cells that survived the incubation with the printed IOLs. The author would like to thank Dr Julie Eldred for obtaining these images.	202
Figure 4.30: Optical microscopy images of lens cells from the control group and cells which had been cultured in the presence of C2 IOLs that had not been post-cured or which had been subjected to different stages of this process. As can be seen, after two days of incubation with IOLs that had not been post-cured or which had been treated only with UV light, the number of healthy cells was lower than on the control group, which did not contain any IOL. However, the thermal treatment performed after the irradiation with UV light led to a considerable increase in the number of cells that survived the incubation with the printed IOLs. The author would like to thank Dr Julie Eldred for obtaining these images.	203
Figure 4.31: Surgical injector DualTec, used in the implantation tests described herein.	204
Figure 4.32: Digital images showing the delivery of IOLs from a surgical injector. (a-c) IOL, indicated with an arrow, inside the injector and being delivered to the aqueous medium. (d, e)	

IOL unfolding upon release from the injector. (f) image showing the IOL completely unfolded in the aqueous medium.	205
Figure 4.33: Digital images showing the implantation of a printed IOL in a human capsular bag model in vitro. (a) Delivery of the IOL into the capsular bag with a surgical injector; (b) IOL implanted in the human capsular bag.....	205
Figure 5.1: Digital image showing the method followed for the production of the cylinders used for the evaluation of the effect of Eusorb UV-1995 on the cure depth of the resin.	218
Figure 5.2: (a) CAD model of the AcrySof® IOL; (b) AcrySof® IOLs with the supports generated by PreForm; (c, d) CAD model of the prototype with cylindrical haptics; (e, f) CAD model of the disc-design prototype with topography on its surface; (g) IOL with cylindrical haptics mounted on the supports; (h) disk-design IOL with the supports generated by PreForm. These designs were chosen due to their potential ability to reduce the incidence of PCO after cataract surgery.	220
Figure 5.3: Line graph showing the reduction in the cure depth of formulation C1 with increasing concentrations of Eusorb UV-1995. The results from the non-parametric Kruskal-Wallis test indicated that the differences in cure depth between the experimental groups evaluated in this study were statistically significant ($p < 0.0001$).	224
Figure 5.4: ¹ H-NMR spectrum of Eusorb UV-1995 and possible chemical structure of this compound. The signals detected indicate the presence of a monosubstituted benzyl ring ($\delta = 8.06, 7.94 - 7.91$ and $6.69 - 6.67$), an ethyl group located near an electron-withdrawing group ($\delta = 4.33$ and 1.37) and two equivalent methyl groups ($\delta = 3.09$).	225
Figure 5.5: ATR-FTIR spectrum of Eusorb UV-1995.....	226
Figure 5.6: ATR-FTIR spectra of formulation C1 (blue) and C1+0.006% Eusorb UV-1995 added to it (red). The same spectrum was obtained when analysing the polymer that contained 0.012% Eusorb (Appendix 8).....	227
Figure 5.7: Digital images showing a comparison between an AcrySof® IOL printed with formulation C1 (a) and the same model printed with C1 + 0.012% Eusorb UV-1995 added to the formulation (b) . Image (c) shows the side view of an IOL printed with formulation C1, while image (d) shows the same for another IOL printed with C1 + 0.012% Eusorb. Image (e) shows the IOLs composed of formulation C1 + 0.012% Eusorb printed on the build platform.	229
Figure 5.8: Optical microscopy IOLs of the replicas of AcrySof® printed with formulation C1 + 0.012% Eusorb and with the supports attached to it. (a) General view of the implant; (b) detail of one of the haptics; (c) side view of the IOL and supports; (d) detail of the optic part of the implant.	230
Figure 5.9: Comparison between the original AcrySof® IOL and the printed analogues. (a) one-piece AcrySof® IOL; (b) IOL printed with formulation C1; (c) IOL printed with formulation C1 + 0.012% Eusorb. A higher fidelity respect to the original model can be observed in the latter, as the haptic-optic junction is as defined as in the AcrySof® implant and the shape of the optic is not distorted.	230
Figure 5.10: Optical microscopy images of the IOL prototype with cylindrical haptics, printed with formulation C1 + 0.012% Eusorb. Image (a) shows the IOL with the support structures attached to it, while (b) shows the implant after removing the supports.	231
Figure 5.11: Optical images showing the IOL prototype with cylindrical haptics, printed either with formulation C1 (a) or C1 + 0.012% Eusorb (b) . A decrease in the thickness of the optic portion can be observed upon addition of Eusorb to the formulation. Furthermore, the use of the absorber enabled the formation of the perforations present in the haptics, which was not possible when using the resin that did not contain this compound.	231
Figure 5.12: Optical microscopy images of a cylindrical haptic of an IOL printed with formulation C1 + 0.006% Eusorb (a) and the haptic of another implant which had been printed	

with formulation C1 + 0.012% Eusorb (b) . The perforation could only be formed when adding the highest concentration of absorber to the photopolymerisable resin.	231
Figure 5.13: Optical microscopy images of the IOL prototype with a disk-design and a linear topography on the anterior surface. (a) Side view of the IOL showing its convexity and the topography; (b) top view of the IOL attached to the supports, with the dimensions annotated; (c) detail of the optic part and haptics with topography; (d) detail of the topography created on one of the linkers between the optic and haptic.	232
Figure 5.14: Variability in the optic diameter (a) and the thickness (b) of the IOLs printed with formulations C1 + 0.006% Eusorb and C1 + 0.012% Eusorb. The dimensions that are denoted on the Y-axis with an asterisk correspond to the diameter and thickness of the CAD model. .	234
Figure 5.15: Thermogram obtained from the third cycle of the DSC analysis of the polymer that forms the IOLs printed with formulation C1+0.006% Eusorb. The value of T_g is 8.4 ± 0.31 °C.	236
Figure 5.16: Thermogram obtained from the third cycle of the DSC analysis of the polymer that forms the IOLs printed with formulation C1+0.012% Eusorb. The value of T_g is 6.46 ± 0.34 °C.	236
Figure 5.17: Thermogram obtained from the thermogravimetric analysis of IOLs printed with formulation C1+0.006% Eusorb. The IDT is 386.24 ± 1.41 °C.	238
Figure 5.18: Thermogram obtained from the thermogravimetric analysis of IOLs printed with formulation C1+0.012% Eusorb. The IDT is 384.24 ± 2.2 °C.	239
Figure 5.19: SEM micrographs showing the optic-haptic junctions (a-c, h, i) , optic part (d, j) , and haptics (e-g, k, l) of IOLs that had been printed with C1 + 0.006% Eusorb (a-f) or C1 + 0.012% Eusorb (g-l)	241
Figure 5.20: SEM micrographs of the original AcrySof® IOL (a, b) and IOLs that had been printed with formulation C1 (c, d) , C1 + 0.006% Eusorb (e, f) and C + 0.012% Eusorb (g, h) . An increase in the definition of the optic-haptic junction can be observed when adding Eusorb to the formulation (c, e, g) , making it comparable to that of AcrySof®. The sharpness of the edge increased upon addition of 0.006% Eusorb, while it decreased slightly when increasing the concentration to 0.012%.	242
Figure 6.1: Diagram depicting the deposition of a polymeric film on an IOL via spin coating.	254
Figure 6.2: Reaction scheme of the synthesis of pHEMA via RAFT polymerisation. The RAFT agent used in this reaction was 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPTPA).	262
Figure 6.3: Digital image of the pHEMA obtained via RAFT polymerisation, after drying...	262
Figure 6.4: ¹ H-NMR spectrum of pHEMA synthesised via RAFT polymerisation. The integral values for the signals produced by the benzyl ring are 1, 2.07, 1.33 and 2.31, while the integral of the signal produced by the methylene named “c” was 134.56. These values were used in the calculation of the approximate molecular weight of the polymer chains.	263
Figure 6.5: Digital image of two IOLs coated with two layers of pHEMA via spin coating. The slightly pink coloration of the coatings can be observed.	265
Figure 6.6: Optical microscopy image of a non-coated IOL (a) and an IOL that had been coated with one layer of pHEMA via spin coating (b)	265
Figure 6.7: SEM images of an IOL spin-coated with two layers of pHEMA before incubating in water (a) and after incubation (b) . It can be seen how most of the coating had detached from the surface. All scale bars represent 500 µm and the magnification was 27X.	267
Figure 6.8: SEM images of an IOL spin-coated with eight layers of pHEMA before incubating in water (a) and after incubation (b) . It can be seen how most of the coating had detached from the surface. All scale bars represent 500 µm and the magnification was 27X.	268

Figure 6.9: SEM micrographs showing the optic part (a) and the haptic (b) of an IOL coated with three layers of pHEMA at a spinning speed of 4,000 rpm by following the dynamic spin coating method. The scale bars represent 1 mm and the magnification was 25X.	270
Figure 6.10: Diagram depicting the synthesis of the polymer brushes composed of Ac-PEG-SH.	272
Figure 6.11: FTIR microscopy images of two different locations of a C1 IOL coated with Ac-PEG-SH after being photoactivated with a solution that contained 0.32% TPO. The darker areas correspond to the coating deposited on the surface of the lens.	273
Figure 6.12: SEM images of non-coated C1 IOL (a) and IOLs that had been coated with Ac-PEG-SH after being photoactivated either by pipetting 10 μ L of 10% TPO solution on their surface (b-d) or by incubating the IOL in 2 mL of this solution (e-g) . The arrows indicate the degree of coverage of the lenses, which was higher when following the second approach. Some of the surface irregularities are highlighted. The scale bar on the image of the non-coated IOL represents 500 μ m, while on the rest of the micrographs they represent 1 mm. The magnifications were 25X.	276
Figure 6.13: SEM micrographs taken on two different locations (a and b) of an IOL coated with Ac-PEG-SH after being photoactivated via incubation in a 10% TPO solution. The scale bars represent 600 μ m and the purple rectangles indicate the areas of the coating that were subjected to EDS analysis.	277
Figure 6.14: WCA of a non-coated C1 IOL (a) , an IOL coated with Ac-PEG-SH after being photoactivated by pipetting a 10% TPO solution on its surface (b) and an IOL coated with Ac-PEG-SH after incubation in a 10% TPO solution (c) . The highest hydrophilicity, and therefore the highest coating efficiency, was achieved when the IOL had been incubated in the photoactivating solution.	278
Figure 6.15: ATR-FTIR spectra obtained from the analysis of Ac-PEG-SH (blue), a non-coated IOL (red) and C1 IOLs which had been coated with Ac-PEG-SH after depositing the photoinitiator either by pipetting the 10% TPO solution on their surface or by incubating the implants in this solution. Four spectra were obtained for each of the coated IOLs.	279
Figure 6.16: Optical images showing a dry IOL coated with Ac-PEG-SH (a) and the same implant immersed in water (b)	280
Figure 6.17: SEM images of a non-coated IOL (a) and IOLs that had been coated with Ac-PEG-SH after incubating them in either 13% (b-e) or 26% TPO solutions (f-i) . The arrows indicate the degree of coverage of the surface, which was similar in both cases. However, the coating density was higher with 26% TPO. The scale bars represent 500 μ m and the magnification is 27X.	282
Figure 6.18: WCA of a non-coated IOL (a) and IOLs coated with Ac-PEG-SH after being incubated in a solution containing either 13% (b) or 26% TPO (c) . The coating efficiency, inferred from the increase in the hydrophilicity of the surface, was achieved when photoactivating the IOLs with a 26% TPO solution.	284
Figure 6.19: Reaction scheme of the Ellman's reaction. The reaction between DTNB and a thiol group results in the production of TNB ²⁻ , which absorbs light with a wavelength of 412 nm and, consequently, has an intense yellow colour.	285
Figure 6.20: ATR-FTIR spectra obtained from the analysis of Ac-PEG-SH (blue), a C1 IOL that had not been coated (red) and IOLs which had been coated with Ac-PEG-SH after incubation in either 13% TPO (green) or 26% TPO solutions (purple). The higher intensity in the peak produced by the C-H ₂ bond in the coated samples with respect to the non-coated IOL showed that the polymer brushes were present on the surface of the lenses.	287
Figure 9.1: Thermogram obtained from the thermogravimetric analysis of pMA. Adapted from Brostow et al., 2008 ⁴⁸⁵	343
Figure 9.2: Thermogram obtained from the thermogravimetric analysis of pHEA ⁵⁴⁵	343

Figure 9.3: ^1H -NMR spectra obtained after the analysis of IOLs printed with formulation C2 and which had been subjected to different stages of the post-curing process (UV irradiation only or complemented with different heating times) or had not been post-cured. A reduction in the intensity of the signal produced by the acrylate groups (6.47 – 5.84 ppm) was observed with longer times of post-curing (a) . An enlarged view of the acrylate signals is shown (b)	345
Figure 9.4: Spectra obtained from the analysis of the resin used for the production of C2 IOLs (blue), the printed IOLs before post-curing (red) and the post-cured IOLs (green). A reduction in the intensity of the signal produced by the C=C bonds was observed after each step of the production process.	346
Figure 9.5: (a) IOLs printed with formulation C1 on the build platform of the SLA system and (b) same IOLs once they had been detached from the platform.	347
Figure 9.6: UV absorption spectrum of Eusorb [®] UV-1995.	348
Figure 9.7: ATR-FTIR spectrum obtained from the analysis of an IOL that was composed of formulation C1 + 0.012% Eusorb UV-1995.	349
Figure 9.8: ATR-FTIR spectra obtained from the analysis of non-coated C1 IOLs (blue) or IOLs which had been coated with Ac-PEG-SH polymer brushes which had been produced after the photoactivation of the lenses with either 0.16% (red), 0.32% (green) or 0.64% (purple) TPO solutions. No additional signals were detected on the spectra that could be attributed to the coating.	351

List of Tables

Table 2.1: Risk factors for cataract formation. Adapted from Hanson et al ⁶¹	41
Table 2.2: Intraocular lens materials and their properties.	51
Table 2.3: Properties of different IOL haptic designs.	55
Table 2.4: List of therapeutic agents and techniques tested in studies for PCO prevention. Adapted from Nibourg et al., 2015 ¹⁴⁹	77
Table 2.5: Comparison of various 3D printing technologies ^{8,342,350,351}	103
Table 2.6: Effect of the steric effects exerted by bulky side groups on the T_g of a polymer. ..	131
Table 2.7: Effect of the structure of the polymer backbone on the T_g of the material.	132
Table 3.1: Formulations tested during the optimisation of the composition of the 3D printed hydrophilic IOLs. The formulation composed of 82% HEA, 16% MA, 1.5% PEG-DA and 0.5% TPO yielded the best results when printing IOLs with it.	148
Table 4.1: Formulations tested during the development of the printable formulations C1 and C2. The crosslinker used for formulation C1 was PEG-DMA (M_n 750 Da), while for C2 an aromatic urethane diacrylate crosslinker (M_n 1,500 Da) was used instead.	177
Table 6.1: Molecular weight and polydispersity of pHEMA synthesised via RAFT polymerisation.	264
Table 6.2: Summary of the results obtained during the optimisation of the spin coating process.	270
Table 6.3: Elemental composition of the coatings, as obtained by EDS analysis.	277
Table 9.1: Thickness of the 3D-printed IOLs and dimensional accuracy with respect to the CAD model.	342
Table 9.2: Dimensions of the IOLs printed with formulation C1.	344
Table 9.3: Dimensions of the IOLs printed with formulation C2.	344
Table 9.5: Dimensions of the IOLs printed with formulation C1 + 0.006% Eusorb UV – 1995.	350
Table 9.6: Dimensions of the IOLs printed with formulation C1 + 0.012% Eusorb UV – 1995.	350
Table 9.7: IR group frequencies. Adapted from Coates, 2000 ⁴⁹⁶	352

List of Abbreviations

2PP	Two-Photon Polymerisation
3D	Three dimensional
3DP	3D-Printing
Đ	Polydispersity
δ	Chemical shift
Ac	Acetylation or Acrylate
Ac-PEG-SH	Acrylate-PEG-Thiol
AFM	Atomic Force Microscopy
AGEs	Advanced Glycation End-products
AIBN	2,2'-azobis (2-methylpropionitrile)
AM	Additive Manufacturing
AMD	Age-related Macular Degeneration
ANOVA	Analysis of variance
ATR	Attenuated Total Reflectance
ATRP	Atom Transfer Radical Polymerisation
Au@SiO₂	Silica-coated gold nanorods
B&L	Bausch & Lomb
BAPO	Phenylbis(2,4,6-trimethylbenzoyl) phosphine oxide
BDDA	1,4-Butanediol diacrylate
c-KIT	Receptor tyrosine-protein kinase
CAD	Computer-Aided Design
CPTPA	4-cyano-4-(phenylcarbonothioylthio) pentanoic acid
d	Doublet
dd	Doublet of doublets
Da	Dalton
DLP	Digital Light Processing
DMD	Digital Micromirror Device
DMF	<i>N,N</i> -Dimethylformamide

DMSO	Dimethyl sulfoxide
DSC	Differential Scanning Calorimetry
DTNB	5,5-dithio-bis-(2-nitrobenzoic acid)
ECM	Extracellular matrix
EDS	Energy Dispersive X-ray Spectroscopy
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal Growth Factor
EMEM	Eagle's Minimum Essential Medium
EMT	Epithelial-to-Mesenchymal Transition
ERK	Extracellular-signal-regulated kinase
FCS	Foetal Calf Serum
FDA	Food and Drug Administration
FDM	Fused Deposition Modelling
FGF	Fibroblast Growth Factor
FHL 124	Human lens epithelial cell line
FTIR	Fourier Transform Infrared Spectroscopy
GPC	Gel Permeation Chromatography
GSH	Glutathione
h	hour
HEA	2-hydroxyethyl acrylate
HEMA	2-hydroxyethyl methacrylate
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
HGF	Hepatocyte Growth Factor
HPLC	High-Performance Liquid Chromatography
IDT	Initial Decomposition Temperature
IGF	Insulin-like Growth Factor
IOL	Intraocular lens
IPA	Isopropanol
LbL	Layer-by-layer
LCD	Liquid Crystal Display

LDH	Lactate dehydrogenase
LECs	Lens Epithelial Cells
LOM	Laminated Object Manufacturing
M	Molar
MA	Methyl acrylate
MAPK	Mitogen-Activated Protein Kinase
Micro-SLA	Microstereolithography
min	Minute
MMA	Methyl methacrylate
MMP	Matrix metalloproteinase
M_n	Number average molecular weight
MPC	2-methacryloyloxyethyl phosphoryl-choline
M_w	Weight average molecular weight
MHz	Megahertz
N	Newton or Normal
Nd: YAG	Neodymium-doped Yttrium Aluminium Garnet
NIH	National Institutes of Health
NMR	Nuclear Magnetic Resonance
NSAIDs	Non-steroidal anti-inflammatory drugs
O/N	Overnight
PBMA	Poly(butyl methacrylate)
PBS	Phosphate-Buffered Saline
PCO	Posterior Capsule Opacification
PDGF	Platelet-Derived Growth Factor
PDMS	Polydimethylsiloxane
PEG	Poly(ethylene glycol)
PEG-DA	Poly(ethylene glycol) diacrylate
PEG-DMA	Poly(ethylene glycol) dimethacrylate
PEI	Polyethylenimine
PEMA	Poly(ethyl methacrylate)

pHEA	Poly(2-hydroxyethyl acrylate)
PhEA	2-Phenylethyl acrylate
pHEMA	Poly(2-hydroxyethyl methacrylate)
PhEMA	2-Phenylethyl methacrylate
PI3K	Phosphoinositide-3-kinase
PLGA	Poly lactic-co-glycolic acid
POEA	2-Phenoxyethyl acrylate
ppm	Parts per million
PPMA	Poly(propyl methacrylate)
PSS	Poly(sodium 4-styrenesulfonate)
PTFE	Poly(tetrafluoroethylene)
PVDF	Poly (vinylidene fluoride)
RAFT	Reversible addition-fragmentation chain transfer polymerisation
rpm	Revolutions per minute
s	Singlet or Second
scCO₂	Supercritical CO ₂
SEM	Scanning Electron Microscopy
SF	Serum-free
SLA	Stereolithography
SLM	Selective Laser Melting
SLS	Selective Laser Sintering
STL	Stereolithography (file format)
t	Triplet
T_g	Glass transition temperature
TGF-β	Transforming Growth Factor beta
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
TIMP	Tissue inhibitors of metalloproteinases
TNB	5-thio-2-nitrobenzoic acid

TPO	Diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide
UV/vis	Ultraviolet/visible (light)
VEGF	Vascular Endothelial Growth Factor
VEGFR	Receptor of VEGF
vs.	Versus
XPS	X-ray Photoelectron Spectroscopy
WCA	Water Contact Angle

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Abstract

Cataract is the leading cause of blindness worldwide. The only treatment currently available for this disease is surgery. This procedure consists of the removal of the cataractous lens fibres by phacoemulsification and the subsequent implantation of an artificial intraocular lens (IOL) in the resulting capsular bag. While this method results in a good restoration of vision, a high rate of recurrence of cataract is caused by the development of posterior capsule opacification (PCO). This syndrome occurs as a consequence of the wound-healing response elicited in the eye after cataract surgery, which results in the transdifferentiation and migration of lens cells to the posterior capsule. These cells deposit aberrant extracellular matrix proteins which cause the contraction and wrinkling of the capsular bag, hence causing a significant loss of vision. Whilst PCO can be treated by performing a laser capsulotomy, this technique is associated with numerous complications such as the damage to the IOL and retinal detachment. Hence, it would be highly desirable to prevent PCO in order to ensure the eradication of cataract while avoiding these issues. This is of particular importance in the context of an ageing population that is leading to an increase in the incidence of this disease.

Numerous approaches can be taken in order to reduce the incidence of PCO. These include the creation of IOLs with materials and designs that modulate the cellular responses which lead to this disease and the administration of therapeutic agents which target the signalling pathways involved in this pathology. However, the high costs and limited accessibility of the techniques currently employed in the manufacture of IOLs do not facilitate the development of novel implants with improved bioactivity. Thus, the development of alternative methods of production would be highly beneficial for the achievement of this objective.

Within this context, the present thesis aimed at demonstrating the suitability of additive manufacturing technologies for the production of functional IOLs. The technique of choice for these experiments was stereolithography. Thus, novel

photopolymerisable formulations were developed which allowed the production of IOLs by employing this technique. The process was optimised in order to obtain the highest resolution possible, hence allowing the production of implants with a high fidelity with respect to the commercial IOL used as a model. Furthermore, novel prototypes that presented structural elements which aided in the prevention of PCO were fabricated with this technique. The evaluation of the resulting lenses indicated that these implants possessed the optical and mechanical properties required for an IOL to be biofunctional. Moreover, these implants were biocompatible and implantable in a human capsular bag, as demonstrated by performing implantability tests employing a human model *ex vivo*.

Once the IOLs were printed and characterised, their surface was functionalised in order to allow the attachment of a drug delivery system which would release Axitinib[®] into the capsular bag in order to prevent the development of PCO. This was achieved by generating polymer brushes on the surface of the lenses in such a way that covalent interactions could be created between these molecules and a delivery system composed of 4-arm PEG-maleimide. The rationale behind the choice of this polymer is the possibility of conjugating the maleimide groups to MMP2-cleavable peptide crosslinkers in order to create an enzyme-responsive system which would release the therapeutic agent during the onset of PCO.

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CHAPTER 1

Thesis Overview



1. Thesis Overview

1.1 Introduction

Cataract is the leading cause of blindness worldwide¹. The treatment of this disease involves a surgical procedure in which the cataractous lens is removed and an artificial intraocular lens (IOL) is implanted in the capsular bag that remains in the eye after the extraction of the lens fibres. This implant restores the optical function of the natural crystalline lens². While patients regain sight after this procedure, there are a number of long-term complications which lead to a relapse of visual impairment. PCO, also known as secondary cataract, is the most common complication of cataract surgery and results in the recurrence of avoidable blindness³. The development of this disorder results from the wound-healing responses that are elicited by the surgical procedure¹. While PCO can be treated by performing a laser capsulotomy, this technique causes numerous complications such as damage in the IOL, retinal detachment or an increase in the intraocular pressure⁴. Therefore, the prevention of this syndrome is highly desirable in the clinic⁵.

Some of the approaches that have been taken to prevent PCO include the implantation of IOLs made of materials that modulate cell adhesion and which possess design features that hinder the migration of lens cells to the posterior capsule. The surgical technique performed to treat cataract and the administration of drugs during and after surgery have also been shown to play an important role in the prevention of PCO. However, these techniques result in the delay rather than the prevention of secondary cataract⁵. Thus, it is of paramount importance to make improvements to these methods in order to decrease the recurrence of blindness after surgery. The most promising avenue for the achievement of this objective is the creation of bioactive IOLs with improved designs and materials that increase the rates of PCO prevention by altering the behaviour of the cells that interact with these implants^{6,7}. Nevertheless, the research required to develop the next generation of IOLs is currently hindered by the limited accessibility of the manufacturing techniques used for the production of these lenses. Therefore, the utilisation of novel technologies associated with lower

expenditures and reduced production times would be highly beneficial in this endeavour.

Additive manufacturing, or 3D printing, is a method of manufacture that is based on the utilisation of computer-aided design to create prototypes that are virtually sliced into numerous layers which are subsequently generated and stacked by processing the material of choice until the desired object is formed⁸. This technology allows the production of items at a low cost and in relatively short periods of time⁹. The versatility and simplicity of additive manufacturing has boosted its popularity in numerous industrial sectors and academic environments. Medicine is one of the fields that is already benefiting from this technology. The introduction of 3D printing in research laboratories and clinical settings is enabling the development of personalised therapies, such as custom-built implants and drug delivery devices, which are improving the results of numerous surgical procedures^{10–12}.

Within this context, this thesis aimed at utilising additive manufacturing technologies to increase the success rate of cataract surgery by facilitating the fabrication of bioactive IOLs with novel materials and designs that decrease the incidence of PCO. Furthermore, these lenses would be used as drug delivery vehicles that release a therapeutic agent to block the signalling pathways which lead to the development of secondary cataract. While previous studies have reported the use of 3D printing technologies to produce IOLs, the functionality of these lenses was compromised by their poor optical and mechanical performance and the presence of dimensional inaccuracies as a consequence of the low resolution achieved with the systems employed^{13,14}. Moreover, the authors did not perform the pertinent characterisation techniques required for the evaluation of these lenses, which include physico-chemical analysis, cytotoxicity assays and mechanical testing.

The novelty of the research described in this thesis lies in the development of novel printable formulations that allow the production of foldable IOLs which can be implanted in the capsular bag by means of micro-incision surgery. In addition, the work performed in this project explored the production of novel IOL designs with

features that control the biological responses which lead to the onset of PCO. Finally, a protocol was developed for the creation of polymer brushes on the surface of the printed IOLs in order to allow the attachment of a drug delivery system which would administer Axitinib[®] into the capsular bag. This would provide a second line of defence against the recurrence of cataract, thus reducing the incidence of this disease.

1.2 Aim and Objectives

The aim of this thesis was to produce bioactive IOLs capable of preventing the development of PCO by employing additive manufacturing technologies. With the objective of increasing the therapeutic potential of these lenses, this project also focused on the functionalisation of the implants in order to allow the attachment of a drug delivery system which would release Axitinib[®] into the capsular bag. These aims were achieved through the completion of the following objectives:

1. Development of printable formulations for the production of functional IOLs. The utilisation of acrylic monomers results in the creation of transparent polymers that comply with the requirements imposed by the regulatory bodies for the fabrication of IOLs.
2. Production of IOLs with the resulting resins by using a stereolithography system.
3. Characterisation of the 3D-printed IOLs. The physico-chemical properties of the implants can be evaluated by performing thermal analysis, FTIR, NMR, SEM and swelling tests, while the biological performance of these lenses can be assessed by carrying out a cytotoxicity test *in vitro*.
4. Functionalisation of the surface of the IOLs in order to facilitate the attachment of a drug delivery system.

1.3 Thesis structure

Chapter one describes the research background and details the objectives established for the development of the project. Chapter two provides a literature review which starts by describing the clinical problem addressed in this project, the strategies currently adopted to treat this disorder and the potential solutions to the recurrence of this disease. The second part of this review focuses on additive manufacturing technologies and provides a detailed explanation of the working principle of stereolithography, the system employed in this thesis.

Chapters three and four describe the additive manufacturing of hydrophilic and hydrophobic IOLs, respectively. This includes the development of the printable resins and the characterisation of the resulting lenses. The experiments described in chapter five aim at increasing the resolution of the system by adding a light absorber to one of the formulations developed in chapter four. The physico-chemical properties of the resulting polymers were evaluated by thermal analysis and FTIR for comparison with the materials that did not contain the absorber.

Chapter six describes the experiments performed aiming at the functionalisation of the printed IOLs in order to allow the attachment of a drug delivery system to their surface. The techniques employed in this work included spin coating and the synthesis of polymer brushes.

Finally, the conclusions that can be drawn from the work presented in this thesis and the future directions that the project could take are discussed in chapter seven.



CHAPTER 2

Introduction



2. Introduction

2.1 Anatomy and physiology of the human eye

The eye is a complex organ that allows humans and other organisms to obtain visual information about their environment. This is accomplished by absorbing light and converting this radiation into electric signals that are transmitted to the visual cortex in the brain^{15–17}. The human eye is formed of a variety of specialised tissues and structures that work in fine synchronisation in order to form a perceptible image of the outside world. The eyeball is a hollow structure which is formed of two cavities: the anterior and posterior segments (Figure 2.1)¹⁸. The anterior segment is filled with aqueous humour and is divided into the anterior and posterior chambers¹⁹. The anterior chamber is located between the cornea and the anterior iris surface²⁰, while the posterior chamber is the region that lies between the iris and the lens²¹. The posterior segment of the eye extends from the lens to the retina and is filled with vitreous humour, a gelatinous fluid that maintains the shape of the eye¹⁸.

The main constituents of the human eye are the cornea, iris, pupil, lens and retina^{17,22}. The iris is a pigmented tissue that functions like the diaphragm of a camera, as it regulates the light that can enter through the pupil by adjusting its diameter²². The cornea, a transparent tissue located at the front of the eye, works in conjunction with the crystalline lens to refract the light that enters the eye. Most of the refractive power is provided by the cornea, while the function of the lens is to cause small changes in refraction in order to focus objects that are located at different distances²³. As a result of these processes, the incident light is focused onto the focal point situated on the retina¹⁵. This tissue is formed of two types of photoreceptors, cones for vision in bright light and rods for dim, night-time vision²⁴. Human retina contains three types of cones, each of them sensitive to either short, medium or long wavelengths. Therefore, human vision is trichromatic and the perception of different colours is based on the composition of the stimuli perceived by each of these types of photosensitive cells^{24–26}. The sensitivity of each cell to light from a specific spectral region is caused by the presence of a different pigment in each of the three types of cones. This pigment is a transmembrane protein that belongs to the opsin family, and which is associated to a

chromophore known as retinal. This molecule is an aldehyde of vitamin A whose 11-*cis* double bond undergoes an isomerisation to its *trans* isomer in the presence of light²⁷. The conformational change leads to the activation of a series of enzymatic reactions within the cell that results in a hyperpolarisation. The electrical signals generated in this process are then transmitted via neuronal synapses into the optic nerve, which carries this information to the visual cortex in the brain^{22,28,29}.

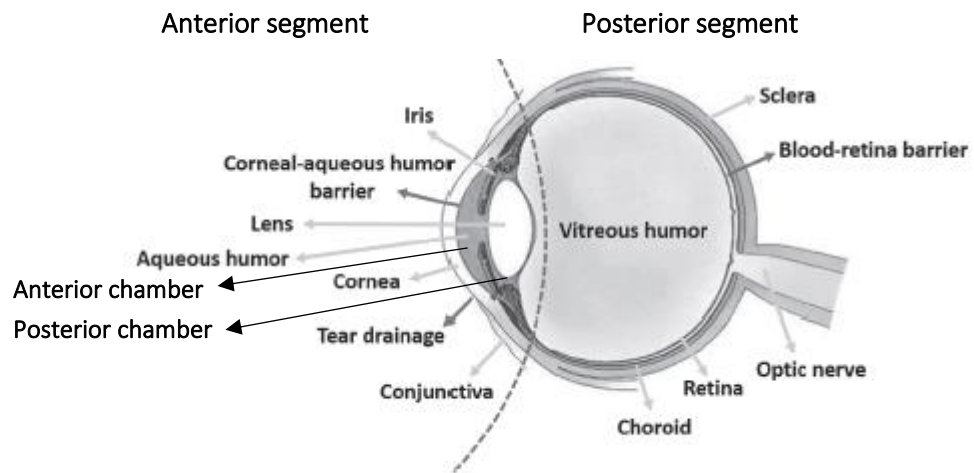


Figure 2.1: Anatomy of the human eye³⁰.

2.2 The Lens

The crystalline lens is a transparent, biconvex structure with a thickness of 4 mm and an average diameter of 9.5 mm^{31,32}. It is an asymmetric body, since the anterior half is flatter than the posterior side³³. The lens is located in the posterior chamber of the eye and it is formed of a capsule, an epithelial layer, a cortex and a nucleus^{34,35}. The capsule is a collagenous membrane that protects the lens from infections, regulates the transport of amino acids and metabolites and participates in the accommodation reflex by adjusting the shape of the lens surface^{34,36,37}. The epithelial layer is attached to the inner surface of the anterior side of the capsule. This tissue is composed of cuboidal epithelial cells that are connected via gap, tight and adherence junctions in a configuration that allows the exchange of metabolites and ions^{34,36,38}. The epithelium

is the portion of the lens that possesses the highest metabolic activity³⁴. The cells that comprise this tissue have been shown to participate in the synthesis of glutathione (GSH), an antioxidant that protects the lens from the oxidative damage caused by UV radiation^{34,39}.

The central portion of the anterior epithelium contains quiescent cells that do not undergo mitosis. In contrast, the cells that are located on either side of this region, in the germinative zones, are continuously proliferating and migrating towards the equatorial region^{36,38}. The cells that reach the equator begin to elongate and differentiate into lens fibres, which are concentrically organised within the cortical region of the lens (Figure 2.2)^{34,36}. The organelles are degraded during this process in order to increase the transparency of the cells, which start to produce large amounts of structural proteins known as crystallins^{36,40,41}. The formation of lens fibres starts during embryogenesis and continues during the entire life of an individual, although at a slower pace, causing the uninterrupted growth of this structure⁴⁰.

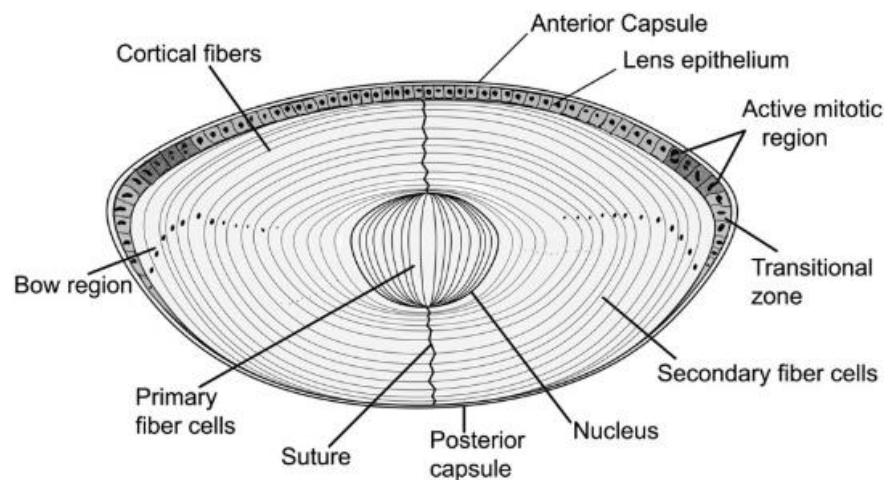


Figure 2.2: Structure of the human lens⁴⁰.

Crystallins are the most abundant proteins in the lens, accounting for 90% of the protein content in this structure^{40,42}. Three families of crystallins are present in the lens: alpha, beta and gamma crystallins. These proteins contribute to the transparency of the lens cells by maintaining a structural organisation that increases their refractive

index^{32,41–44}. Moreover, the chaperone activity of α -crystallins, the most prevalent of these proteins, prevents the aggregation of denatured proteins, which would otherwise cause light scattering⁴⁰. The dense packing of the lens fibres in an arrangement that minimises the intercellular spaces and the avascularity of this tissue are other factors that contribute to the transparency of the lens^{42,45}.

As mentioned above, the function of the lens is to adjust the focal distance in order to focus objects that are located at different distances from the eye³². This process, known as accommodation, involves a change in the lens shape in response to neural impulses that are transmitted to the ciliary muscle of the eye⁴⁶. The lens is attached to the ciliary body through a group of elastic fibres known as zonules³². The ciliary muscle, together with the zonules, exerts the forces that change the shape of the lens to focus the light on the retina^{47,48}. When the lens is not accommodating, the ciliary muscles are relaxed and they move away from the lens. A radial tension is consequently exerted on the zonules, which are in contact with the lens capsule. This decreases the curvature of the anterior side of the lens, resulting in the characteristic flatness of this face of the organ. When the lens needs to focus an object that is in close proximity to the individual, the opposite effect is observed. The ciliary muscles contract and the radial tension is released from the zonules, resulting in a reorganisation of the lens fibres that leads to an increase in the curvature of the anterior portion of the lens and a subsequent change in the refractive power^{37,49}.

Another characteristic property of the lens is its ability to filter UV light in order to protect the intraocular tissues from the damaging effects of this radiation. The young crystalline lens transmits UV and visible light, but the repeated exposure to these wavelengths leads to the development of yellow pigments that prevent the transmission of UV radiation and the shorter wavelengths of the visible spectrum^{32,50}.

2.3 Cataract

2.3.1 Prevalence and Pathophysiology

Cataract is the leading cause of blindness worldwide, accounting for 50% of the reported cases of this disability, according to data provided by the World Health Organisation (WHO)^{51,52}. This syndrome can be defined as an opacification or cloudiness of the crystalline lens that leads to visual impairment or even blindness due to changes in the refractive index of this structure⁵³. Cataract is caused by a combination of metabolic and physiological processes that result in post-translational modifications of the lens crystallins, such as oxidation, glycation, Schiff base formation or proteolysis^{36,54}. These alterations lead to the formation of protein aggregates that cause variations in the refractive index of the lens fibres and a subsequent loss of transparency in the lens^{54,55}.

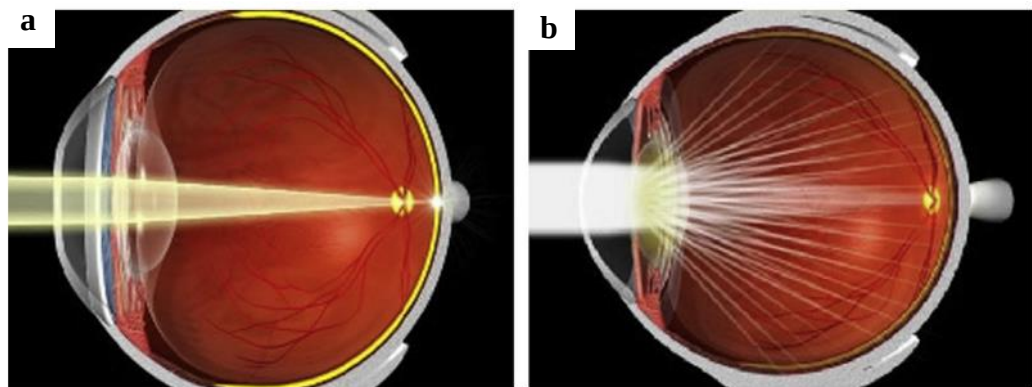


Figure 2.3: Transmission of light into the retina in a healthy **(a)** and in a cataractous eye **(b)**, where the light scattering reduces the quality of vision. Adapted from Thompson et al³¹.

The protein aggregation that leads to the development of cataract can occur in different parts of the lens. Consequently, three types of cataract have been described depending on the location of this opacity within the lens: nuclear, cortical and subcapsular (Figure 2.4)^{56,57}. Nuclear cataract shows the highest incidence and is usually related to age, while cortical and subcapsular cataracts are commonly

associated with diabetes or the oxidative stress that results from exposure to UV radiation^{31,56,58}.

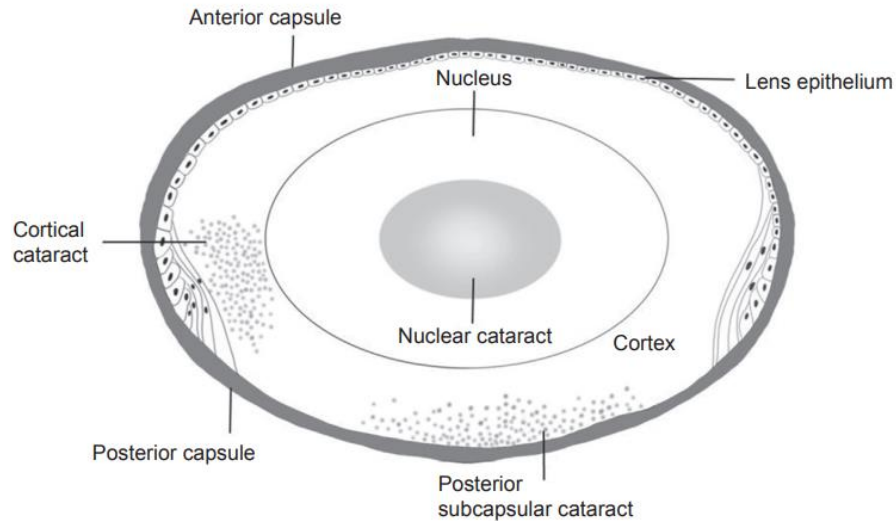


Figure 2.4: Diagram of the different types of cataract that can develop in the lens⁵⁹.

2.3.2 Risk Factors

The probability of developing a cataract increases with age, as the cumulative damage exerted by environmental factors leads to alterations in the morphology of lens proteins and cells, causing visual impairment^{53,60,61}. As the lens ages, vacuoles and multilamellar bodies accumulate between the lens fibres, causing disruptions in the plasma membrane of these cells³⁶. Moreover, the activity of the enzymes that protect the lens from oxidative insults, such as glutathione reductase, decreases with age. The subsequent increase in oxidative stress accelerates the processes that result in the chemical modification of proteins, hence contributing to the development of cataract^{33,38,41}.

Age is not the only factor that increases the risk of suffering from cataract. This disease can be genetically inherited or acquired as a consequence of physical trauma, incidence of other diseases or exposure to environmental conditions that cause oxidative damage in the eye⁶³⁻⁶⁵. Repeated exposure to UV radiation and the habit of

smoking can result in the formation of free radicals that initiate cataractogenesis^{66,67}. The incidence of diabetes is another factor that can increase the risk of cataract development⁵⁸. In hyperglycaemic conditions, the enzymes involved in metabolic processes become saturated with glucose and the levels of this monosaccharide increase dramatically. The carbonyl groups from glucose react with the free amino groups in proteins, leading to the formation of advanced glycation end-products, or AGEs. The accumulation of these molecules in the lens of diabetic individuals has been shown to promote photooxidative stress and cause modifications in the structure, activity and coloration of α -crystallins. These processes result in the light scattering and opacification that are characteristic of cataract. Another phenomenon involved in the development of diabetic cataract is the conversion of excess glucose into polyols by aldose reductase. The accumulation of these alcohols in the lens leads to the establishment of an osmotic gradient that increases the diffusion of aqueous humour into the lens. The subsequent swelling and electrolyte imbalance result in the formation of a cataract^{54,68}.

Table 2.1: Risk factors for cataract formation. Adapted from Hanson et al⁶¹.

Individual	Increasing age Female sex Racial or ethnic group
Genetic	Two loci for nuclear cataract: chromosome 3 in <i>KCNAB1</i> and chromosome 21 in <i>CRYAA</i> Gene polymorphisms
Lifestyle	UV-B exposure Smoking
Diet	Consumption of carbohydrates with high-glycaemic index Malnutrition
Systemic pathologies	Type II diabetes mellitus (cortical and posterior subcapsular cataracts) High systemic blood pressure (posterior subcapsular cataract) Metabolic syndrome Renal impairment Hypocalcaemia
Ocular disorders	Myopic refractive error (nuclear cataract) Retinal drusen

2.3.3 Socio-economic impact

While the prevalence of cataract is higher in developing countries, the socioeconomic impact of this disease in the wealthiest nations is still a significant problem⁵¹. More than 330,000 operations are performed every year in the United Kingdom⁶⁹, with an associated cost in the millions of pounds⁵¹. These numbers are only expected to rise with the increase in life expectancy in developed countries^{70,71}. The ten-fold increase in the number of cataract operations performed in England between 1968 and 2003 corroborates this hypothesis⁶⁹. Therefore, cataract represents a significant burden on public health services and difficulties will be encountered to meet the future demand for cataract surgery if preventative actions are not taken soon^{51,70}. It is for these

reasons that the eradication of cataract is considered as a global health priority by the WHO^{72,73}.

2.3.4 Cataract treatment

The only effective treatment for cataract is surgery⁷¹. Cataract surgery is one of the safest and most common clinical procedures in developed countries^{69,74,75}. The first historical reports of this practice date back to 2000 B.C. in ancient India, when cataract was treated by luxation of the affected lens. It was not until the 18th century that the first cataract extractions were performed⁷⁶. Two different surgical approaches can be differentiated depending on the methodology used for the removal of the cataractous tissue^{57,74}. Intracapsular extraction (ICCE) consists of the creation of a long incision in the limbus and the subsequent removal of the whole lens. This technique is obsolete in developed countries, but is still common practice in the developing world^{57,74,77}. On the other hand, extracapsular extraction (ECCE) involves the creation of a smaller, 12-mm incision on the anterior side of the capsule in order to facilitate the removal of the cataractous lens fibres, while the capsular bag is left in the eye^{57,74,78}. This technique benefited from the invention of the intraocular lens (IOL) in the 1940s by Sir Harold Ridley, whose inspiration to create this implant came from the absence of any immune rejection towards the pieces of poly(methyl methacrylate) that were found in the eyes of fighter jet pilots who had served in the armed forces during World War II^{74,79,80}. Before this discovery, the capsular bag was not filled with any tissue substitute⁸⁰, so the IOL was a revolutionary invention which allowed the correction of the refractive errors that the aphakic eye suffers from⁷⁹.

Further advances in the surgical technique led to the introduction of phacoemulsification, the most common procedure in developed countries since the 1990s⁷⁵. This method is a variation of ECCE in which the lens fibres are removed through a 2-mm incision made on the edge of the cornea and a continuous curvilinear capsulotomy, or capsulorhexis, created on the anterior lens capsule⁵⁷. The creation of a smaller incision led to an increase in the safety of the surgical procedure and a faster patient recovery. This was facilitated by the introduction of an ultrasonic probe and

an irrigation system in the lens in order to fragment and dissolve the cataractous fibres with vibrational energy prior to extraction (Figure 2.5)^{74,81}. Further improvements made in both the surgical equipment and the IOLs have resulted in an increase in the efficiency of the process and a reduction in the incidence of post-surgical complications⁵⁷.

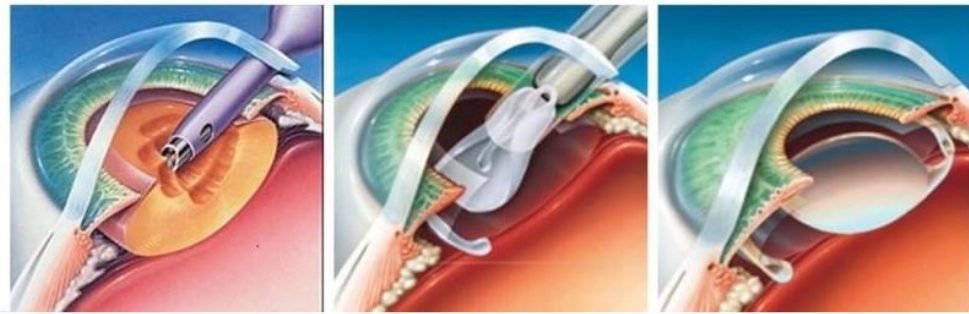


Figure 2.5: Diagram showing the procedure followed during phacoemulsification. The lens fibres are extracted **(a)** prior to the injection of the IOL **(b)**, which is implanted in the capsular bag **(c)**. (Source: aaravmedicare.com).

2.4 The IOL: Materials and Designs

As previously mentioned, an IOL is implanted in the capsular bag during cataract surgery in order to restore the refractive power of the lens^{2,5}. An IOL is an implant that is formed of an optic component and support structures, known as haptics, which hold the lens in place within the capsular bag by exerting a centripetal pressure on this structure (Figure 2.6)^{82,83}.

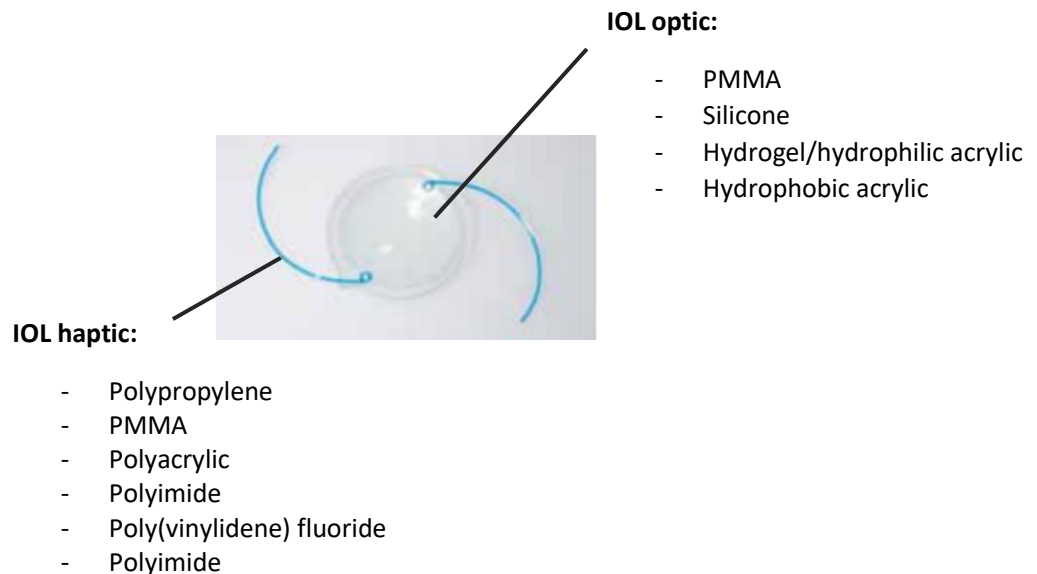


Figure 2.6: Diagram of an intraocular lens formed of an optic portion and two haptics. The materials that are commonly used in the manufacture of these structures are detailed¹⁸.

2.4.1 Materials

The materials used in the manufacture of IOLs must have outstanding optical properties, excellent biocompatibility and enough mechanical strength to withstand the stress that they are subjected to during implantation⁸⁴. These properties are determined by the side groups attached to the polymer backbone¹⁸. Therefore, the development of novel materials for the manufacture of IOLs requires a thorough understanding of the relationship between the chemical structure of these side groups and the physico-chemical properties of the polymer.

The first IOL was made of PMMA, a biocompatible material with a very high rigidity due to the steric hindrance imposed by the methyl groups present in the side chains,

which restrict the mobility of the polymer molecules⁸⁵. The evolution of the surgical technique towards a reduction in the invasiveness of this procedure was accompanied by modifications in the materials employed in the manufacture of IOLs with the objective of producing lenses that were implantable through small incisions. This resulted in the development of foldable polymers, which is the main type of material that is currently in use for the production of IOLs^{18,83}. As well as their ductility, another distinctive characteristic of these materials is their capability to recover their original shape after deformation. The fabrication of foldable polymers requires a careful monomer selection, since the glass transition temperature of the material should not be higher than 37 °C in order for it to maintain its flexibility under physiological conditions⁸⁶.

The removal of the natural crystalline lens during cataract surgery leaves the eye unprotected from UV light, as this structure contains a yellow pigment that absorbs this high-energy radiation³². Therefore, all IOLs currently available in the market contain a UV-blocking chromophore that protects the retina from the oxidative stress induced by this radiation^{7,83}. Vinyl anthracene, benzophenones and benzotriazole derivatives are some examples of these blocking agents³². Moreover, some IOLs also contain blue-filtering pigments in order to reduce the transmission of short-wavelength visible light. It has been hypothesised that radiation from this region of the spectrum can also cause retinal phototoxicity and contribute to the development of age-related macular degeneration^{7,87,88}.

As there is no universal material for the fabrication of IOLs, the selection of the lens that is to be implanted in each patient is based on the physico-chemical properties of the polymer and the biological responses that it would elicit in the host⁸⁹. A description of the materials that are used in the manufacture of IOLs is provided in the following sections.

2.4.1.1 Rigid materials

2.4.1.1.1 Poly(methyl methacrylate)

Poly(methyl methacrylate) (PMMA) is a homopolymer of methyl methacrylate (Figure 2.7) that was first synthesised by Rowland Hill and John Crawford in the 1930s^{7,90}. It is a rigid, non-foldable hydrophobic material with a refractive index of 1.49 and a very low oxygen permeability^{43,79}. As mentioned above, PMMA was the first material employed in the manufacture of IOLs and the polymer of choice for the following few decades^{79,91}. The high success rates observed after the implantation of IOLs made with this material can be attributed to their biocompatibility, mechanical strength and the low adherence of LECs to their surface^{90,92}. However, the rigidity of this polymer results in IOLs that cannot be folded and therefore are unsuitable for implantation through small incisions. Thus, this material became obsolete in developed countries with the advent of microincision surgery⁹³, although it is still utilised in the developing world due to its low cost⁸² and in paediatric surgery because of its durability^{67,68}. The high rigidity of PMMA makes it suitable for the fabrication of the haptics of three-piece IOLs^{79,94,95}, although fractures in these structures have been reported due to the brittleness of this polymer^{94,96}.

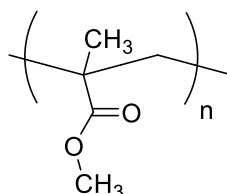


Figure 2.7: Chemical structure of PMMA.

2.4.1.2 Foldable materials

2.4.1.2.1 Silicone

During the 1980s, the surgical technique performed for the treatment of cataract started to evolve towards a reduction in the size of the incision, as this decreases the incidence of post-surgical complications⁷. The rigidity of PMMA did not allow the manufacture of flexible IOLs that could be folded to facilitate the implantation

through such small incisions³². Therefore, the IOL manufacturing industry began its journey towards the development of flexible ophthalmic materials for the production of lenses that could be implanted via microincision surgery⁷⁹. The first foldable IOL was implanted in 1989 and was made of silicone³². Silicones are synthetic, high-molecular-weight polymers that contain Si-O bonds in their backbone (Figure 2.8)^{94,97}. The rotational freedom of these bonds is responsible for the high flexibility of this polymer³². Silicone materials are hydrophobic, biocompatible and possess a low refractive index of between 1.41 and 1.46, which does not allow the fabrication of thin lenses. It is for this reason that this material lost popularity when further reductions were made in the size of surgical incisions. Moreover, the implantation of silicone IOLs has been associated with higher rates of bacterial infection⁷⁹. Despite these issues, the implantation of silicone IOLs can still be advantageous due to their low rates of PCO development, which are comparable to those of hydrophobic acrylic lenses⁹⁸. Furthermore, some of the problems herein reported can be counteracted by introducing chemical groups that provide the material with the desired physico-chemical properties. For example, the introduction of aromatic groups in the chemical structure of poly(dimethylsiloxane) leads to an increase in the refractive index of the material³².

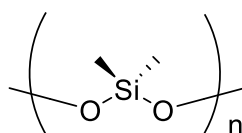


Figure 2.8: Chemical structure of PDMS.

2.4.1.2.2 Hydrophilic acrylic

Hydrophilic acrylic materials are mainly composed of hydrophilic monomers such as 2-hydroxyethyl methacrylate (HEMA) (Figure 2.9)^{32,79}. These biomaterials form a three-dimensional network of hydrophilic polymer chains that can retain relatively high amounts of water, which increases the flexibility of the structure⁹⁹⁻¹⁰². Their high porosity, soft consistency and hydrophilicity make them ideal for the fabrication of tissue replacements since these characteristics are also exhibited by the natural

extracellular matrix⁹⁹. Moreover, the addition of chemical crosslinks prevents the polymer from dissolving in water and modulates the mechanical properties of the material^{101–103}.

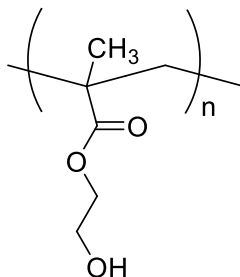


Figure 2.9: Chemical structure of pHEMA.

Soft hydrophilic polymers were first employed in the manufacture of IOLs in the 1980s, the IOgel lens (Alcon, USA) being the first implant made with this material¹⁰⁴. Since then, several modifications have been introduced in their composition that have made way for the development of copolymers with different physico-chemical properties and diverse water contents, which usually range between 18% and 26%⁷⁹. Currently, most hydrophilic acrylic IOLs are composed of copolymers of HEMA and PMMA. The introduction of a hydrophobic monomer reduces the equilibrium water content of the polymer, hence preventing moderating the reduction in the mechanical strength of the material upon implantation in an aqueous environment⁷. Generally, the hydrophilic copolymers used in the manufacture of IOLs are soft, foldable and their oxygen permeability can be modulated by changing the thickness and water content of the polymer network^{43,105}. They possess a number of properties that make them suitable for the manufacture of these lenses, such as a refractive index that is similar to that of the natural crystalline lens^{79,94,103}, an excellent uveal biocompatibility¹⁰⁶ and a high resistance to damage caused by the instruments used during surgery or the Nd: YAG laser used to treat PCO⁷⁹. However, the implantation of hydrophilic acrylic IOLs often results in a higher incidence of PCO and other opacities caused by the deposition of calcium. This will be further explained in following sections of this thesis^{7,82,107}.

2.4.1.2.3 Hydrophobic acrylic

This group comprises different copolymers of acrylate and methacrylate monomers that have been derived from methyl methacrylate with the objective of increasing the flexibility of this material^{79,104}. The first foldable IOL made with a hydrophobic acrylic material was released by Alcon in 1993 under the name of AcrySof^{®79}. This IOL is composed of a copolymer of phenylethyl acrylate and phenylethyl methacrylate (Figure 2.10). Subsequently, hydrophobic acrylic polymers went on to become the most successful materials in the IOL industry, as their utilisation results in the creation of lenses that are easy to implant and which unfold in a controlled manner once they are introduced in the capsular bag^{18,79}.

The flexibility of a material can be tuned by adjusting its glass transition temperature¹⁰⁴. This can be achieved by selecting monomers with chemical structures that result in the formation of polymers with the desired mechanical properties³². Due to the low equilibrium water content of these materials, their mechanical strength does not vary significantly upon incubation in an aqueous medium¹⁰⁸. Other distinctive properties of these polymers are their high memory and a high refractive index, up to 1.55, which enables the production of thinner IOLs^{79,108}. Moreover, they show a very good capsular biocompatibility, which leads to low rates of PCO development^{79,107–109}.

The main problems encountered during the implantation of hydrophobic acrylic IOLs are the stickiness of their surface^{18,94} and their slow unfolding in the capsular bag, which is a consequence of their mechanical strength. This can cause damage in the optic part of the implant, which would result in a decrease in the quality of vision¹¹⁰. Once implanted in the capsular bag, these IOLs induce higher rates of inflammation in comparison with lenses made of other materials¹⁰⁵. Moreover, the optical performance of hydrophobic acrylic IOLs is often compromised by the occurrence of glistenings and photopsias^{104,111,112}.

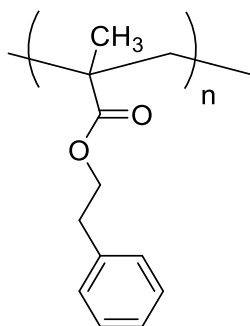


Figure 2.10: Chemical structure of pPhEMA.

Table 2.2: Intraocular lens materials and their properties.

Material	Advantages	Limitations	IOL examples	References
PMMA	Very good biocompatibility Low adhesion of LECs Mechanical resistance and durability Very good optical properties Low cost	Not flexible Haptic breakage Possible damage of the corneal endothelium	Kelman Multiflex and Monoflex (Alcon) EZE-50 (B&L)	82,90,92,113,114
Silicone	Good biocompatibility Flexibility	Low refractive index Slippery when wet Rapid release from injector	TECNIS 3-Piece CL (Amo) CeeOn 911A (Pharmacia) AA4203VF (Staar)	79,94,98
Hydrophilic acrylic	Excellent biocompatibility High oxygen permeability Good optical properties	Promotes adhesion of LECs Photopsias	Ioflex (Mediphacos) MemoryLens (Ciba vision) Hydroview (B&L)	79,84,94
Hydrophobic acrylic	Good biocompatibility Good optical properties	Glistenings and photopsias Failure to unfold Sticking to surgical instruments	TECNIS Symphony® (Amo) AcrySof® (Alcon) Clareon® (Alcon) AF-1™ (Hoya)	79,104,107,109,111,112,115

2.4.2 Designs

Since their creation, IOLs have been built with a wide variety of haptic designs and optic geometries⁸³. These have been developed in order to increase the quality of vision of patients and to improve the surgical outcomes by increasing the stability of the installation of the implant within the surrounding tissues. IOLs can be implanted in different parts of the anterior segment of the eye, which include the anterior and posterior chamber. Iris-fixated and angle-supported IOLs are inserted into the anterior chamber and held in place via attachment of the haptics to the iris or between the iris and the cornea. On the other hand, posterior chamber IOLs are usually inserted into the capsular bag. This method results in the highest positional stability. However, in cases where this is not possible, such as in the occurrence of uveitis, endophthalmitis or capsular rupture, the lens is implanted in the ciliary sulcus, which is a small space located between the posterior surface of the iris and the anterior portion of the ciliary body^{19,114}. A review of the IOL designs currently available in the clinic is herein provided.

2.4.2.1 Haptic designs

2.4.2.1.1 Open-loop IOLs

Open-loop IOLs are formed of an optical lens and two haptics for support of the implant within the capsular bag. If this tissue is damaged or ruptured, these IOLs can also be implanted in the ciliary sulcus^{83,116}. Open-loop IOLs can be subclassified into three-piece or single-piece IOLs. In three-piece IOLs, the optic part and the support structures are fabricated separately from different materials and they are subsequently assembled to form the IOL (Figure 2.11)^{79,117}. Poly(methyl methacrylate) (PMMA), poly (vinylidene fluoride) (PVDF) and polyimide are the most commonly used materials for the fabrication of the haptics^{79,83}. These structures can also be built with different shapes that facilitate the implantation in specific parts of the anterior segment of the eye. For example, C-loop IOLs are implanted inside the capsular bag, while J-loop lenses are preferred for sulcus implantation⁸³. The popularity of multipiece IOLs

has declined in the last decades due to the advent of micro-incision cataract surgery, since three-piece IOLs require a larger incision than single-piece implants. However, three-piece IOLs are still preferred in certain cases, such as sulcus implantation after posterior capsule rupture⁷⁹.

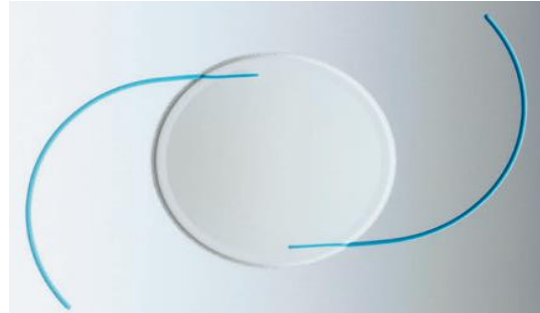


Figure 2.11: Three-piece IOL design¹¹⁴.

As opposed to three-piece IOLs, the optic and haptics of single-piece lenses are made with the same material (Figure 2.12). Furthermore, these IOLs are produced in a single step, hence reducing the costs of production. One-piece IOLs have a higher mechanical strength than their three-piece analogues, which makes them suitable for implantation through very small incisions^{79,83,118}. Moreover, the haptics of IOLs that are made with foldable materials have a bulky structure in order to increase the stability of the lens after implantation^{7,119}.



Figure 2.12: Single-piece IOL design¹¹⁴.

Some open-loop IOLs have angulated haptics in order to reduce the abrasion exerted on the iris when the lens is implanted in the sulcus. The angulation of these structures usually ranges from 5 to 10 degrees^{7,83} and it can affect the adhesion of the lens to the surrounding tissues, hence influencing the responses of the host to these implants¹²⁰.



Figure 2.13: Angulated IOL haptics⁸².

2.4.2.1.2 Plate-haptic IOLs

The plate-haptic model is a variant of the single-piece design in which the optic is built within a rectangular, plate-like support structure (Figure 2.14)⁷⁹. Instead of adhering to the lens capsule, these IOLs are held in a pocked formed between the anterior and posterior capsules, along the edge of the implant¹²¹. The robustness of this design makes it very appropriate for the production of IOLs made with silicone or hydrogels, which are materials that possess a lower mechanical strength than PMMA or hydrophobic acrylic polymers⁹⁴. Moreover, the presence of small loop-like haptics on each corner of the implant provides extra support within the capsular bag and a better adaptability to this tissue⁸³. Another design feature that contributes to its high positional stability upon implantation is the presence of perforations in the support structure, which allows fibrous adhesions to be established between the anterior and posterior capsule, thus locking the IOL in place and improving the long-term stability of these implants¹²¹.



Figure 2.14: Plate-haptic IOL¹¹⁴.

Table 2.3: Properties of different IOL haptic designs.

Haptic design	Advantages	Disadvantages	IOL examples	References
Three-piece	Barrier effect for the prevention of secondary cataract	Their implantation requires a larger incision than single-piece IOLs	TECNIS ZA9003 (Amo) AcrySof® IQ MN60AC (Alcon) AF-1 iMICS (Hoya)	79,118,122,123
Single-piece	Lower production costs than three-piece IOLs Very resistant to mechanical stress; implantable through small incisions	Lack of barrier effect for the prevention of secondary cataract	AcrySof® IQ SN60WF (Alcon) AF-1 NY-60 (Hoya) C-flex (Rayner)	79,83,118,123,124
Plate-haptic	High positional stability	Lower rates of prevention of secondary cataract	Acri.Smart 46S (Carl Zeiss Meditec) Lentis Tplus LU-313 T1-T6 (Oculentis) Staar Toric (Staar Surgical)	79,83,125

2.4.2.2 Optic designs

A functional IOL must improve the quality of vision of the patient. Initially, IOLs were designed with only one purpose: to restore the refractive capability of the lens¹²⁶. However, the advances made in the manufacturing technologies used in the production of these lenses have allowed the incorporation of features that improve the visual acuity of the patient, hence reducing their dependence on spectacles⁸³.

The refractive power of an IOL is determined by the curvature and refractive index of the optic part⁷. Most IOLs have a biconvex optic with a diameter of 6 mm, although they are also available in other shapes that provide different refractive powers^{7,82}. Moreover, a number of alterations have been made in these lenses in order to provide the patient with the highest visual acuity possible. Consequently, various types of IOLs can be differentiated when considering their optical functionality. These are explained in detail below.

2.4.2.2.1 Toric

A considerable number of patients undergoing cataract surgery suffer from corneal astigmatism, which can be corrected via surgical manipulation of the cornea or by implanting a toric IOL^{7,127}. Toric lenses have a toroidal optic design that is more efficient in reducing astigmatism than the aforementioned surgical procedure¹²⁸. The first FDA-approved toric lens was the Staar Toric IOL (Staar Surgical, Monrovia, CA), a injector-haptic lens made of silicone that was later modified with larger fixation holes and an increased length that resulted in an improved rotational stability and, consequently, an enhanced astigmatic correction (Figure 2.15)⁷.

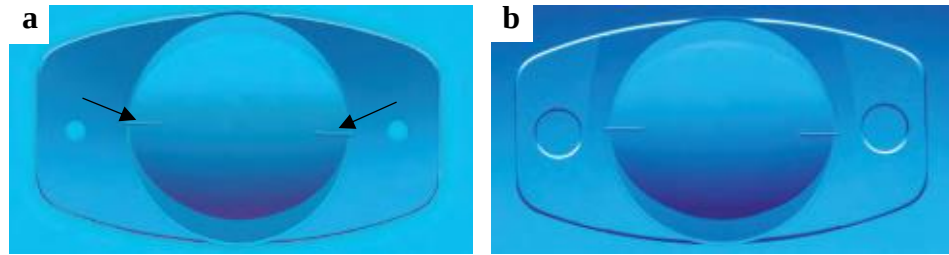


Figure 2.15: Staar toric plate-haptic IOL. **(a)** Original design with shorter haptic length and smaller fixation holes. **(b)** Improved design with larger fixation holes and longer haptic. The alignment marks are indicated with an arrow. Adapted from Dewey, 2015⁷.

2.4.2.2.2 Aspherical

Spherical aberration occurs in spherical surfaces when peripheral and axial light rays converge in different foci. This phenomenon results in the formation of blurry images and a decrease in contrast sensitivity⁷. In pseudophakic eyes, i.e., those in which the natural lens has been replaced by an artificial lens, the IOL and the cornea are the two sources of spherical aberration⁸². Aspherical lenses with an optimised surface curvature have thus been developed in order to correct these spherical aberrations and improve the quality of vision (Figure 2.16)¹²⁹.

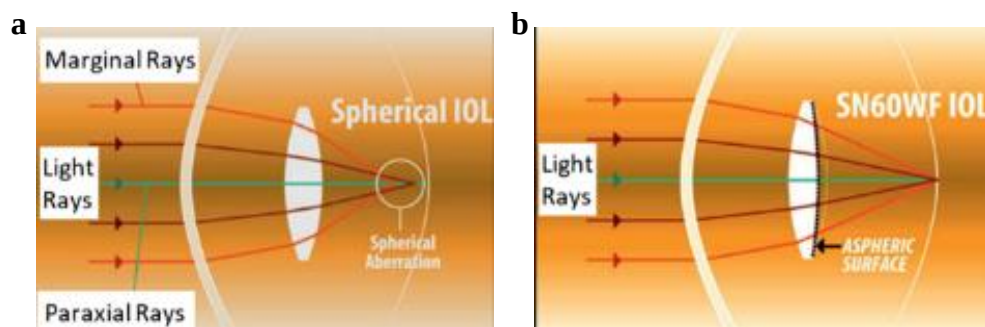


Figure 2.16: Comparison of spherical aberration between spherical **(a)** and aspheric IOLs **(b)**. Adapted from Yu et al., 2018¹³⁰.

2.4.2.2.3 Multifocal

Multifocal IOLs are designed to compensate for the loss of the accommodative ability of the lens after cataract surgery. The working principle of these lenses is based on the division of the light into two or more focal points that are used for distant, intermediate or near vision^{83,129}. This effect can be achieved with either a refractive or a diffractive mechanism. Refractive lenses are formed of a series of concentric refractive zones with different curvatures, while diffractive IOLs create two different focal points by using constructive and destructive light interference (Figure 2.17)^{7,83,128}.

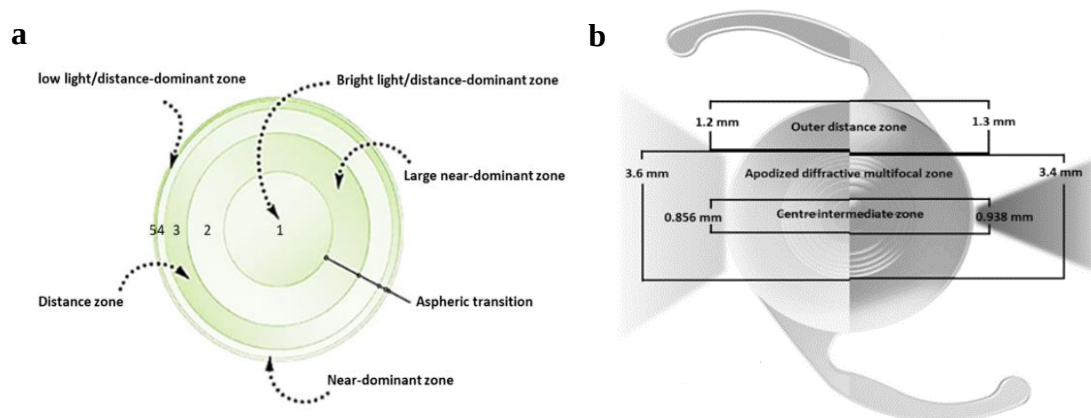


Figure 2.17: Schematics of refractive and diffractive multifocal IOLs. **(a)** Refractive lens showing the five zones in which it is divided, each providing optimal vision at different distances and light conditions. **(b)** Diffractive IOL comprised of a central area, which contains concentric levels of different heights that allow for distant and near focus, and a peripheral zone that mediates distance vision. Adapted from Yu et al., 2018¹³⁰.

2.4.2.2.4 Accommodative

Multifocal IOLs can improve vision at certain distances but they do not have the capability to restore accommodation. Accommodative IOLs have thus been created in an attempt to reproduce the accommodative reflex of the crystalline lens. These IOLs mimic some of the processes that occur during accommodation by changing their

position or curvature in response to the contraction of the ciliary muscle (Figure 2.18). One example of this type of IOL is Crystalens[®] (Bausch & Lomb, USA) However, no IOL has been developed yet that can be considered as truly accommodative^{7,128,131}.

a



b

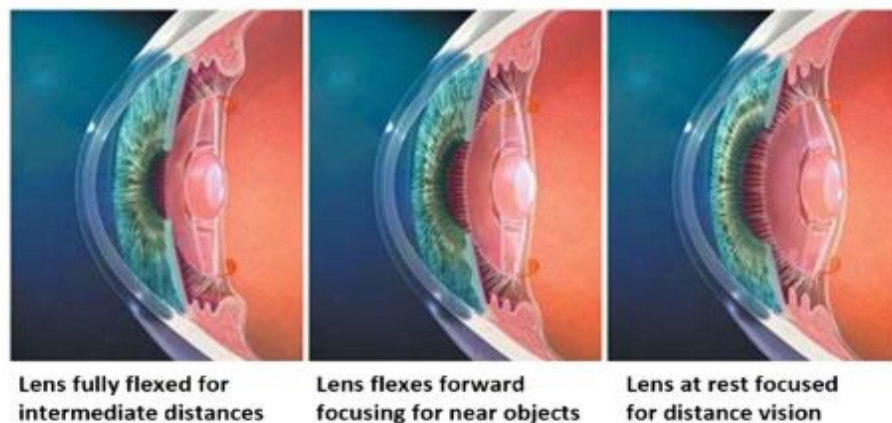


Figure 2.18: Crystalens[®] accommodative IOL (Source: bausch.com) **(a)** and its mechanism of action once implanted in the capsular bag **(b)**¹³⁰.

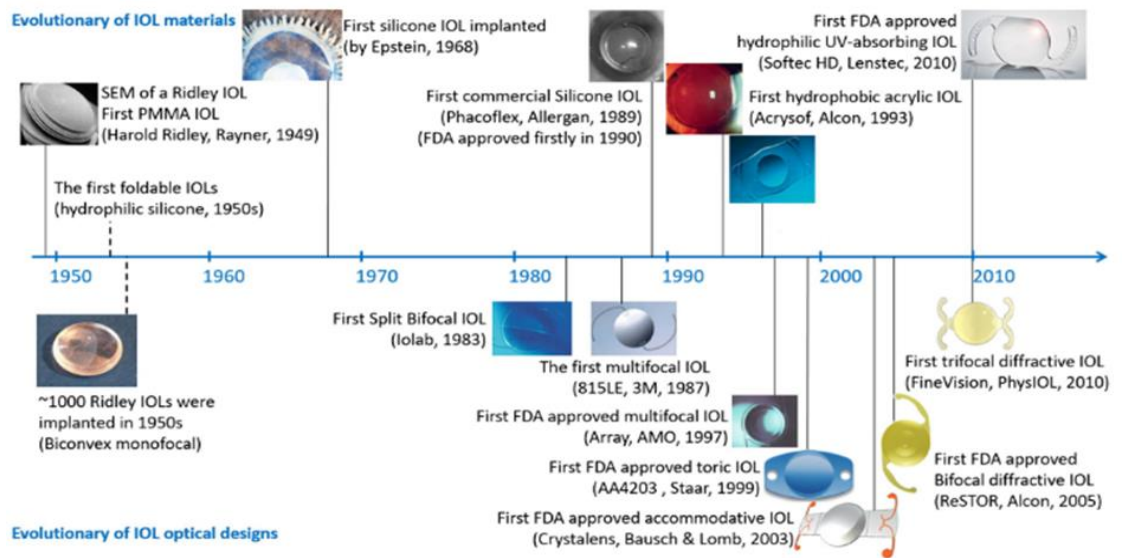


Figure 2.19: Development of IOLs over the past 60 years¹³⁰.

2.5 Posterior Capsule Opacification

2.5.1 Prevalence of PCO

Despite the high success rate of cataract surgery and the improvements made in the design and manufacture of IOLs, various post-surgical complications still occur which result in a relapse of vision loss⁵⁷. Posterior capsule opacification, or PCO, is the most common long-term post-operative complication of cataract surgery^{3,132,133}. This syndrome results in the formation of a secondary cataract on the posterior side of the capsular bag, hence causing visual impairment (Figure 2.20)¹³⁴.

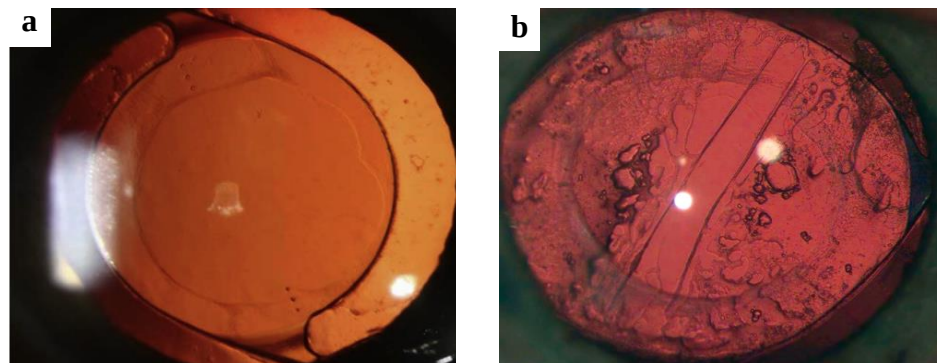


Figure 2.20: Retroillumination of an IOL immediately after cataract surgery **(a)** and after the development of PCO **(b)**. Adapted from Thompson et al., 2015³¹ and Colvard, 2012⁸³.

According to a number of reports, the incidence of this syndrome ranges from 5% to 50% within the first five years after surgery^{74,135,136}, while other studies report a three-year rate of 2%-63%^{129,137,138}. The high variability in the prevalence of PCO can be attributed to the multifactorial nature of this disease, which is influenced by the type of IOL implanted in the eye, the surgical technique performed and the age of the patient, among other factors^{129,136,139,140}. While a decrease in the short-term rates of PCO development has been achieved with the implantation of square-edge IOL designs and the improvement of the surgical technique, recent studies have reported an increase in the incidence of this disease in the long term¹⁴¹. Therefore, it is of

paramount importance to increase the success rate of PCO prevention in order to improve the outcome of cataract surgery.

2.5.2 Pathophysiology of PCO

Posterior capsule opacification develops as a consequence of the wound-healing response that occurs in the lens epithelial cells (LECs) which remain in the capsular bag after cataract surgery¹⁴¹. As the blood-aqueous barrier is broken during this surgical procedure, cytokines are released from the blood stream into the lens environment³. These proteins, together with other growth factors produced by the lens cells, induce the proliferation and transdifferentiation of LECs and their migration to the posterior side of the IOL². PCO can be subdivided into two types of opacities, regenerative and fibrotic¹⁴². Regenerative PCO develops when the LECs from the equatorial region of the capsular bag migrate to the posterior side of this tissue and differentiate into unorganised fibres that form Elschnig pearls and a Soemmering's ring, which cause visual impairment^{5,142–144}. On the other hand, fibrotic PCO occurs when LECs undergo transdifferentiation into myofibroblasts in a process known as epithelial-to-mesenchymal transformation, or EMT^{5,145,146}. As fibroblastic cells are less adherent to their substrate, they start migrating to the posterior capsule. Once they reach this region, they deposit aberrant extracellular matrix proteins that cause capsular wrinkling and contraction, resulting in a decrease in the transparency of the lens (Figure 2.21)².

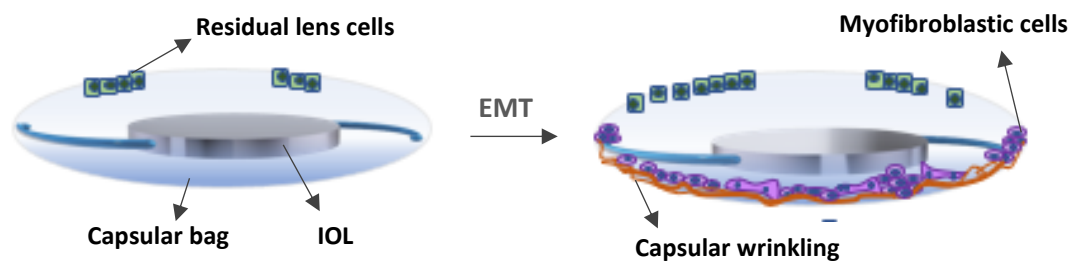


Figure 2.21: Schematic diagram of the cellular responses that take place in the capsular bag during the development of PCO: proliferation, migration and transdifferentiation.

The conversion of LECs into fibroblasts is mainly regulated by TGF- β signalling^{145,147}. TGF- β 2, the most abundant isoform in the eye, is constitutively present in the aqueous humour in an inactive form that is activated after injury or disease¹⁴⁸. When the active form of this protein binds to the serine/threonine kinase receptors that are present on the surface of the cells, the Smad-dependent pathway is activated via phosphorylation of Smad proteins. This results in the formation of a Smad complex which is transported to the nucleus of the lens cells, where it activates the transcription of TGF- β target genes. The expression of these genes results in the production of MMPs, which are involved in matrix contraction and remodeling through the MAPK pathway. The transcriptional repressor Snail, which acts as a suppressor of the epithelial phenotype, is also produced as a result of this process¹⁴⁹. Besides the Smad-dependent pathway, other signalling routes such as MAPK/ERK1/2 are also involved in the TGF- β -activated signalling routes that lead to the development of PCO^{145,149}.

While TGF- β plays a fundamental role in the onset of secondary cataract, other growth factors have also been found to regulate the processes that cause this disorder. These include FGF, EGF, IGF and PDGF. It has been reported that the combined action of these cytokines induces the proliferation of LECs, FGF being a fundamental regulator in this process. Furthermore, PDGF stimulates the migration of lens cells to the posterior capsule, while FGF is also involved in the transdifferentiation of these cells into a fibroblastic phenotype¹⁴⁹. Finally, a recent study conducted by Eldred *et al.* has shown that VEGF is another growth factor that plays an important role in EMT. According to another investigation that evaluated the effect of an anti-VEGF monoclonal antibody on LECs, VEGF could also be involved in the regulation of the expression of TGF- β ¹⁵⁰. Nevertheless, further work will be needed in order to learn more about the effect of VEGF on TGF β -induced myofibroblast formation¹⁴¹.

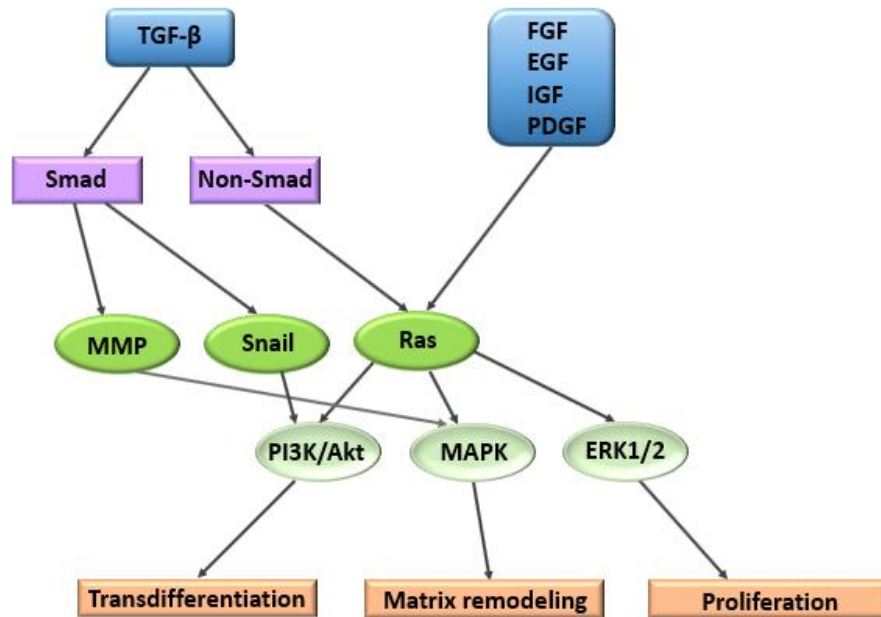


Figure 2.22: Diagram of the signalling pathways involved in the development of PCO. Adapted from Nibourg et al., 2015¹⁴⁹.

2.5.3 PCO treatment

The only treatment currently available for PCO is the creation of a perforation in the posterior capsule using a Nd: YAG laser^{5,124}. While this procedure is relatively simple, numerous complications are associated with the utilisation of this technique, such as damage in the optic part of the IOL, cystoid macular edema and retinal detachment^{4,142,151,152}. These limitations make it desirable to develop preventative approaches for the eradication of PCO¹⁵². The prevention of this disease can be achieved either by improving the surgical technique and the bioactivity of the IOLs or by administering drugs that interfere with the signalling pathways which lead to the development of a secondary cataract⁸⁴. However, these techniques have so far delayed rather than prevented the onset of PCO. Therefore, further advancements must be made in this field in order to eradicate this syndrome⁵.

2.5.4 PCO prevention

The pathophysiology of PCO is multifactorial, since the occurrence of this disease is influenced by factors such as the age and pathological history of the patient, the surgical technique and the IOL material and design^{5,84,153}. As previously mentioned, this disease can be treated by performing a laser surgery, known as Nd: YAG capsulotomy, in order to create a perforation in the posterior capsule that results in the elimination of this opacity⁴. However, several complications are associated with this technique. These include the rise in the intraocular pressure, retinal detachment, cystoid macular edema, uveitis and damage of the IOL¹⁵⁴. Therefore, it is highly desirable to prevent the development of PCO^{4,155,156}. While the improvements made recently in both the surgical technique and the implanted IOLs have led to a reduction in the incidence of PCO, this syndrome is still one of the main problems encountered in the treatment of cataract¹⁵³.

2.5.4.1 Effect of the surgical procedure

The surgical procedure carried out for the removal of the cataractous lens plays an important role in the prevention of PCO. This technique should ensure the complete removal of cortical debris from the capsular bag, since the presence of this material would impair the formation of the capsular bend that prevents the migration of LECs to the posterior capsule¹⁵⁷. Hydrodissection, which is the injection of fluid into the lens to separate the nucleus from the cortex¹⁵⁸, is an effective method for extraction of the cortical material from the capsular bag, hence contributing to the prevention of PCO^{5,159}. However, this procedure does not eliminate the LECs that remain in the capsular bag after the extraction of the lens fibres, as these cells adhere to the capsule via tight junctions¹⁶⁰. The elimination of these cells has been attempted by polishing the anterior or posterior capsule during surgery. While this technique might delay the onset of PCO, it has not proven effective in preventing this disease as it does not remove the germinative LECs located in the equatorial region, which are the cells that are involved in the formation of PCO^{5,84,161}.

Another factor that has a significant effect on PCO prevention is the creation of a capsulorhexis that overlaps completely with the anterior surface of the IOL. This enhances the preventative effect of some design features, such as the sharp posterior optic edge^{153,162}. Moreover, the capsulorhexis should be curvilinear and it must show a 360-degree overlap with the optic edge for it to be effective in promoting the formation of the capsular bend that prevents the migration of LECs to the posterior capsule. The creation of smaller incisions results in a larger overlapping area between the anterior capsule and the optic part of the IOL, which leads to a higher degree of contraction of the anterior capsule and, consequently, a tighter wrapping of the posterior capsule around the implanted lens. This limits the migration of lens cells to the posterior side of the capsular bag and contributes to the prevention of PCO. On the other hand, incisions that have irregular shapes or that exceed the diameter of the optic edge are not successful in creating this shrink-wrap effect around the IOL, resulting in higher rates of PCO due to the migration of LECs to the posterior side of the lens^{160,163,164}.



Figure 2.23: Anterior capsulorhexis¹⁶⁵.

2.5.4.2 Effect of the IOL design

Some of the design features of an IOL have the capability to elicit certain cellular responses upon implantation of this artificial implant in the capsular bag. A sharp posterior optic edge has been widely reported to have a positive effect on PCO

prevention^{139,166–168}. However, other factors such as the haptic angulation or the optic size are also known to play a fundamental role in the development of this syndrome¹⁶⁶. The explanation of the exact mechanism by which the implantation of square-edge designs leads to a reduction in the rates of PCO development has been a source of controversy for some time^{153,169}. Nishi and Nishi hypothesised that the inhibitory effect of this design feature is caused by the formation of a barrier through tight contact between the IOL and the capsular bag, which leads to the creation of a capsular bend that prevents the migration of LECs to the posterior capsule¹⁷⁰. An alternative theory was postulated by Boyce *et al.*, who suggested that the compression exerted by the square edge on the posterior capsule has a higher effect on the prevention of cell migration than the barrier effect described above¹⁷¹. Another possible explanation to the inhibitory effect of a square edge may lay in its structural similarity with the periphery of a wound, which could be recognised by the lens cells and trigger a down-regulation of TGF- β , with the subsequent reduction in the proliferation and migration of LECs¹⁷². However, the latest research supports the theory that ascribes the efficacy of the sharp edge to prevent PCO to the creation of a mechanical barrier for cell migration as the most valid hypothesis for this phenomenon^{32,161,173}.

As the haptic design of the IOL has a direct effect on the sharpness of the edge of the implant, this is another important factor that influences the capability of the lens to prevent PCO. The implantation of three-piece IOLs allows the formation of a capsular bend around the entire circumference of the optic edge, hence stretching the capsular bag in such a way that the “shrink wrap” effect is successfully achieved¹⁶⁰. Both the sharpness of this bend and the speed of its formation are important factors for the prevention of PCO¹⁷⁴. Single-piece IOLs lead to a faster wrapping of the capsular bag around the lens, which may explain the low short-term rates of PCO development observed after the implantation of these lenses^{175,176}. However, their long-term inhibitory effects can be compromised by the bulky haptics that these IOLs possess, which can disrupt the 360-degree barrier effect exerted by the sharp edge and impede the fusion between the anterior and posterior capsules at the optic-haptic junction¹⁷⁷. This may result in higher levels of migration of LECs to the posterior side of the IOL, with the subsequent increase in the rate of PCO development^{139,160,178}. However, the

studies that have been conducted in order to examine the effect of different haptic designs on the incidence of PCO have come to contradictory conclusions. While some authors reported a higher incidence of PCO with single-piece IOLs^{176,179,180}, other studies concluded that there is not a significant difference between both designs^{139,167,181–183}. However, the differences in the material used in the manufacture of these IOLs, the haptic angulation and the higher tension exerted by the PMMA haptics of the three-piece lenses could have added a bias on these results. Despite the inconclusive evidence provided by these studies, some companies have incorporated a 360-degree sharp edge at the base of the haptics of single-piece IOLs in an attempt to reduce the incidence of PCO. Studies conducted in animals with the aim of evaluating the efficacy of these IOLs have confirmed a reduction in the migration of LECs to the posterior capsule after the implantation of IOLs modified with this structural element^{153,184}. Therefore, this provides further confirmation of the importance of this design feature for the inhibition of secondary cataract formation.

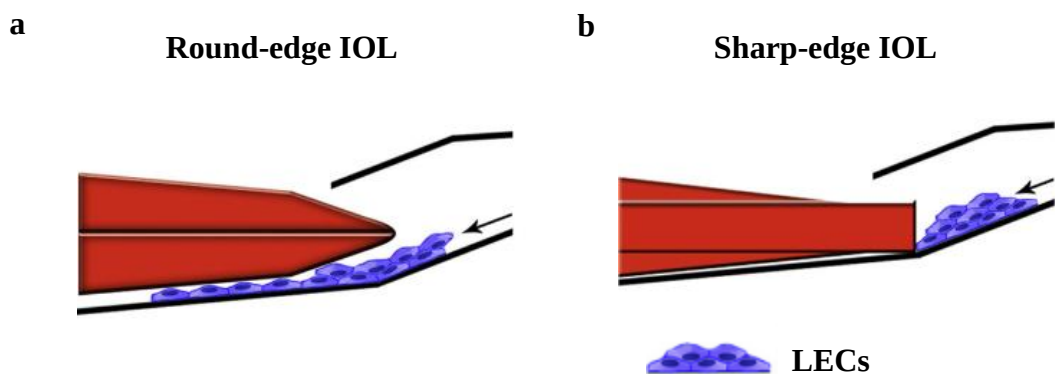


Figure 2.24: Effect of the sharpness of the edge of an IOL on the migration of LECs. **(a)** Round-edge IOL implanted in the capsular bag, allowing the migration of LECs to the posterior capsule. **(b)** Inhibition of the migration of LECs upon implantation of a sharp-edge IOL. Adapted from Aghamollaei et al., 2019⁸⁶.

The haptic angulation is another characteristic that can have an effect on the incidence of PCO. The angulation of the haptics might enhance the barrier effect caused by the sharp edge of the optic by pushing the IOL against the posterior capsule. However,

the studies conducted to test this theory showed that the implantation of IOLs with angulated haptics did not lead to a significant reduction in PCO^{83,120}.

While it has been shown that the implantation of IOLs with designs that promote the fusion between the anterior and posterior capsule has a positive effect on the prevention of secondary cataract, it has recently been postulated that the separation of these portions of the capsular bag may have a similar effect. This can be achieved by implanting disk-shaped IOLs which have a circular haptic that surrounds the optic (Figure 2.25). Recent studies have reported a substantial reduction in the incidence of PCO after implanting these IOLs in rabbit eyes¹⁸⁵. This may be due to the bulky morphology of these implants, which pushes the LECs against the anterior capsule and prevents the migration of these cells onto the posterior side of the capsular bag. Moreover, the maintenance of an open-bag configuration by separating the anterior and posterior capsule results in a higher flow of aqueous humour within the capsular bag, hence reducing the concentration of EMT-inducing cytokines in the microenvironment that surrounds the LECs. A modified version of the disk-shaped IOL includes perforations in the haptic ring that improve the circulation of fluid between both sides of the IOL. Consequently, lower PCO rates have been observed in rabbit eyes in which this type of IOL had been implanted^{185,186}.

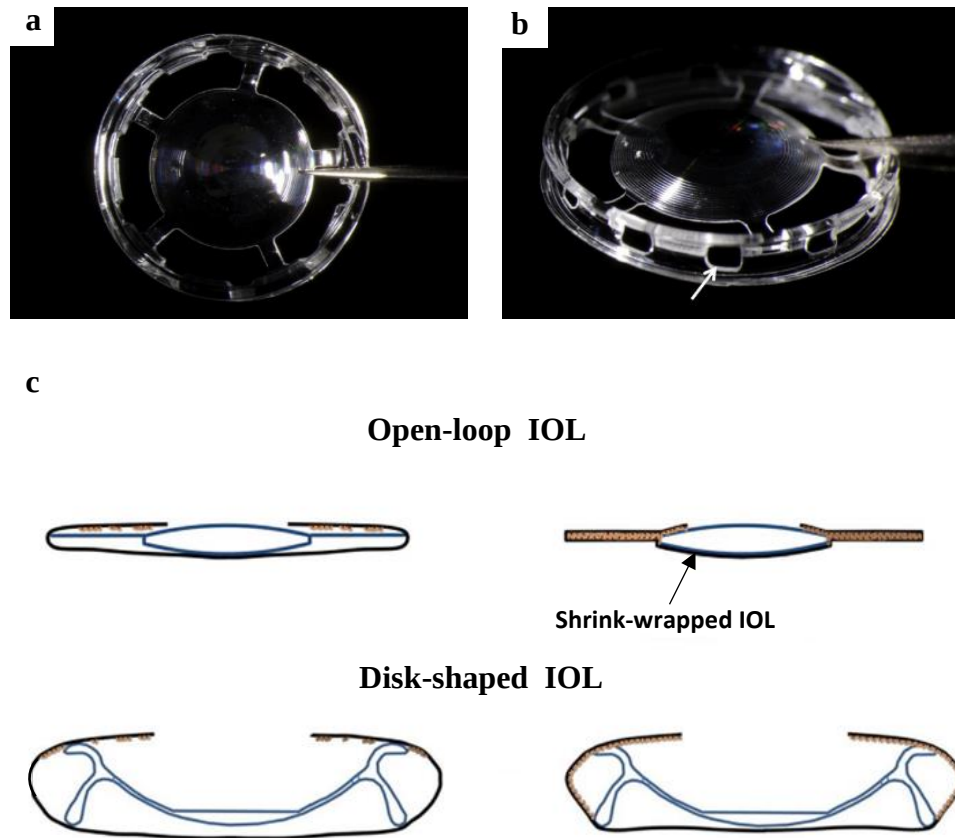


Figure 2.25: Front (a) and side view (b) of the modified Zephyr® disk-shaped IOL (Anew Optics, Bristol, USA), with perforations in the haptics¹⁸⁵. (c) Schematic diagram showing a comparison between the "shrink-wrap" effect that occurs when an open-loop IOL with a square edge is implanted in the capsular bag and the open-bag configuration attained upon insertion of a disk-shaped IOL. While both designs have the capability to block the migration of LECs to the posterior capsule, the open-bag configuration has the added advantage of increasing the aqueous humour flow, hence reducing the concentration of cytokines on the posterior capsule. Adapted from Eldred et al., 2014¹⁸⁶.

2.5.4.3 Effect of the IOL material

The materials used for the fabrication of IOLs include PMMA, soft acrylics and silicone. These materials are classified as hydrophobic or hydrophilic polymers depending on their ability to absorb water, which affects their biocompatibility. The biocompatibility of the IOLs can be classified as uveal or capsular. Uveal compatibility is related with the immune response elicited by the presence of a foreign body in the eye, while the capsular biocompatibility is determined by the interactions

of the IOL with the capsular bag. Hydrophilic IOLs show a very good uveal biocompatibility, since they do not have a high tendency to provoke a foreign body reaction. On the other hand, hydrophobic acrylic materials have a better capsular biocompatibility, which leads to a reduction in the incidence of PCO when IOLs made of these materials are implanted in the capsular bag^{139,153,155,187}. Consequently, the most successful IOL with regard to PCO prevention is Alcon AcrySof[®], a hydrophobic acrylic lens composed of poly(phenylethyl acrylate) and poly(phenylethyl methacrylate)¹⁸⁸.

As opposed to hydrophobic materials, hydrophilic polymers have a high capacity to absorb water and a low stiffness. These properties make them similar to the natural extracellular matrix. Therefore, hydrogels support the adhesion, proliferation and migration of LECs, resulting in a higher probability of developing PCO^{189,190}. Another factor that might contribute to the formation of a secondary cataract is the reduction in the sharpness of the edge of IOLs made with hydrogels upon implantation in an aqueous environment¹⁶⁰. The reduction in the incidence of PCO after the creation of an enhanced sharp edge on a hydrophilic IOL corroborates this theory¹⁹⁰. Due to the differences in the design features that can be produced with certain materials and manufacturing techniques, it is sometimes a challenge to differentiate the effects that arise as a consequence of the material from those that are caused by the design. However, the results of the studies conducted in this field seem to indicate that the prevention of PCO is dependent on both the material and the design of the IOL^{153,191}.

A number of hypotheses have been suggested in order to explain the reduction in the incidence of PCO after the implantation of hydrophobic acrylic IOLs. Linnola *et al.* published their “Sandwich theory” in 1997, which hypothesises that the LECs which remain in the capsular bag after cataract surgery can bind to both the IOL and the posterior capsule. When this occurs, a “sandwich-like” structure is generated and the migration of LECs to the posterior capsule is consequently thwarted¹⁹². Further studies have also shown that fibronectin, vitronectin, laminin and type-IV collagen adhere to a greater extent to soft hydrophobic acrylic materials than to the rest of the polymers used in the manufacture of IOLs¹⁹³. This may explain the increased adherence of

hydrophobic acrylic polymers to the posterior capsule, which results in a reduction in the rates of PCO development as a consequence of the inhibition of cell migration^{166,194–196}. However, some of the long-term studies published to date have reported comparable results in PCO prevention between silicone and hydrophobic acrylic IOLs, which may indicate a loss of efficacy of the latter after longer periods of time since the surgical procedure¹⁹⁷. Hence, further studies are needed in order to learn more about the efficacy of the implantation of hydrophobic acrylic IOLs for the prevention of PCO.

2.5.4.4 Surface modification of IOLs

Any biomedical device that is implanted in the body comes into contact with fluids that carry numerous proteins which can adhere in a non-specific manner to the surface of the implant^{198,199}. This facilitates the recognition of the material by cells such as macrophages, which trigger an immune reaction against the foreign body¹⁹⁹. Therefore, the physico-chemical properties of the material, such as surface chemistry, wettability and roughness, have a direct effect on its biocompatibility^{91,200}. Surfaces that are highly hydrophilic reduce the deposition of proteins and the adhesion of cells. An inhibition of cell adhesion has also been observed in highly hydrophobic materials, although in this case it might be due to the adsorption of adhesive proteins with conformations that the cells cannot identify^{32,200–202}. Based on this principle, various studies have been conducted in which the surface of IOLs was functionalised with highly hydrophilic or hydrophobic polymers in order to try and prevent the adhesion of proteins and LECs and thus reduce PCO¹⁰⁵. Hydrophobic fluorinated polymers or highly hydrophilic materials such as poly(ethylene glycol) and methacryloyloxyethyl phosphorylcholine (MPC) have been attached on the surface of hydrophobic IOLs, leading to a decrease in the incidence of PCO^{152,154,202–204}. Another approach that has been tested is the chemical functionalisation of hydrophilic polymers with motifs that promote cell adhesion, such as the RGD peptide. The rationale for this is based on a theory that postulates that the adhesion of a monolayer of LECs on the surface of the IOL facilitates the contact between the lens and the capsular bag and therefore

prevents the migration of LECs into the space between the IOL and the posterior capsule²⁰². Satisfactory results with regard to PCO prevention have been observed after testing IOLs whose surface had been modified by following these methodologies^{84,105,205}.

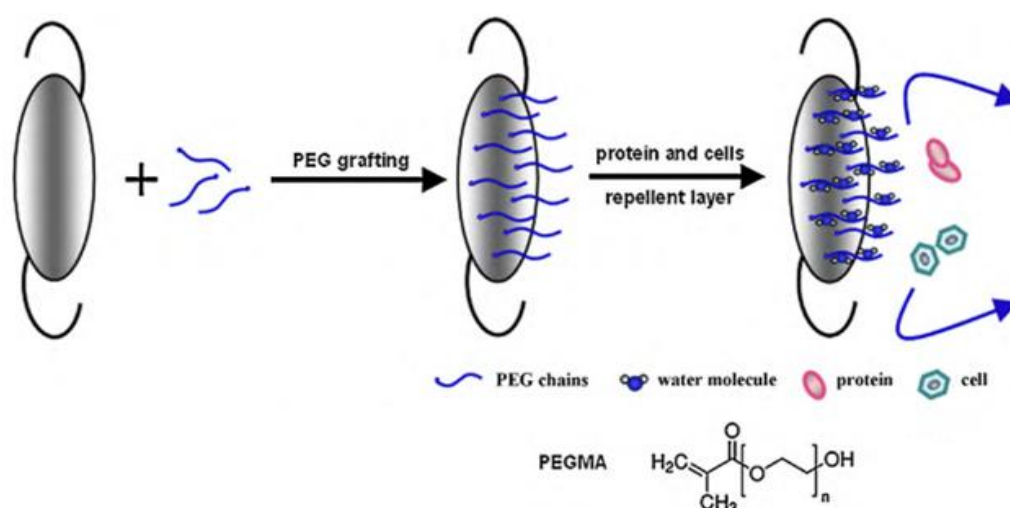


Figure 2.26: Reaction scheme depicting the grafting of poly(ethylene glycol) methacrylate (PEGMA) on the surface of hydrophobic acrylic IOLs. The resulting coating can resist protein and cell adhesion. Adapted from Lin et al., 2010²⁰⁶.

Careful consideration must be taken when modifying the surface of an IOL, as each side is exposed to a different environment and is therefore involved in different processes. The anterior surface of the lens is in contact with uveal tissue and the cells and proteins that orchestrate the immune response against the artificial material. Therefore, an increase in the hydrophilicity of this part of the IOL would prevent the attachment of macrophages, leading to an improvement in the uveal biocompatibility of the implant. On the other hand, the posterior side of the IOL is in contact with the posterior capsule and it has a higher effect on the capsular biocompatibility of the implant. The adherence of the posterior surface of hydrophobic IOLs to the capsular bag prevents the migration of LECs to this area, hence contributing to a reduction in PCO²⁰³. Therefore, the strategies envisaged for the functionalisation of the surface of the lenses should take these factors into consideration in order to achieve optimum results.

Numerous methods are available for the modification of the surface of IOLs. These include plasma treatment, layer-by-layer deposition and chemical grafting¹⁵⁴. While plasma treatment is a widespread technique in the functionalisation of biomedical implants, its complexity and the low stability of the resulting chemical groups make it desirable to seek alternative methods in certain cases¹⁹⁸. Chemical grafting is a good alternative to plasma and layer-by-layer deposition, as it results in the attachment of chemical functionalities that stay on the surface of the implants for longer times than those that are immobilised via physical methods²⁰³. Two different approaches exist for the conjugation of molecules on the surface of IOLs. The “grafting-to” technique consists in the attachment of molecules via conjugation between functional groups from the surface of the implant and the molecules that are to be grafted on it. On the other hand, the “grafting-from” method is based on the immobilisation of an initiator on the surface of the IOL and the subsequent growth of polymer brushes via polymerisation reactions such as RAFT or ATRP^{154,199}.

2.5.4.5 Other devices used for PCO prevention

Capsular tension rings with sharp edges can also prevent PCO after implantation in the capsular bag by creating a capsular bend²⁰⁷. Hara *et al.* were the first to develop an intracapsular ring made of silicone, which caused a significant reduction in the incidence of PCO after implantation in rabbits, monkeys and humans^{208,209}. The circular shape of these devices contributes to the stretching of the capsular bag at the equatorial region, which prevents the collapse of this structure and leads to the maintenance of an open-bag configuration. An increase in the flow of aqueous humour occurs in this situation, which maintains the relative levels of cytokines in the microenvironment of the LECs under the threshold for stimulation of cell proliferation. Moreover, the square edge of these rings contributes to the prevention of cell migration to the posterior capsule, while the compressive forces exerted on the capsular bag result in a reduction in cell proliferation. These effects have also been observed after the implantation of an IOL in conjunction with a protective membrane whose surface had been patterned in order to prevent cell adhesion²¹⁰.

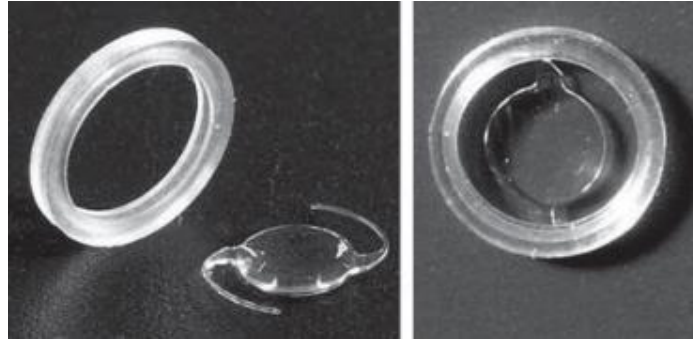


Figure 2.27: Endocapsular ring with a square edge and a groove on the inner surface that facilitates the IOL fixation²⁰⁹.

2.5.4.6 Pharmacological approaches

The administration of pharmacological agents during the surgical procedure or the post-operative period is another method that can contribute to the prevention of PCO²¹¹. These drugs can either be dissolved in the irrigation fluids used during surgery, injected in the posterior chamber or incorporated into the IOLs^{5,84,161}. The studies that have been published so far to report the effects of pharmacological agents on the prevention of PCO have been conducted either *in vitro* or using animals²¹¹. Some of the drugs that have been shown to reduce the incidence of secondary cataract, such as hypoosmolar and cytotoxic agents, exert their effects by destroying the LECs that remain in the capsular bag after surgery, while other therapeutic agents inhibit the proliferation, migration and transdifferentiation of these cells^{5,18}. The proteins that are involved in these cellular responses include TGF- β , integrins and MMPs^{3,212–214}. Therefore, numerous therapeutic agents, such as receptor inhibitors, immunotoxins or inhibitors of epigenetic mechanisms, have been developed in order to interfere with the expression or activity of these proteins^{149,215,216}. Furthermore, other types of drugs such as anti-inflammatory agents have also been tested *in vitro*, resulting in positive outcomes^{18,161}. However, their use in the clinic is restricted due to the toxic effects that they exert in the surrounding tissues at the concentrations that are required for them to be effective *in vivo*^{5,161}. The use of drug delivery systems could be a solution to this problem, and it will be discussed in depth in the following sections.

The administration of novel non-pharmacological therapies is another approach that is being currently explored for the prevention of secondary cataract. Gene therapy can inhibit the formation of PCO by blocking the expression of proteins that are involved in the signalling cascades that trigger this disease^{231,233}. However, the translation of these techniques to the clinic is hampered by ethical regulations and the side effects that this type of therapy can elicit²³⁴. The utilisation of nanomaterials is another innovative technique that can prevent secondary cataract. Zinc oxide nanoparticles administered in conjunction with UV radiation have been shown to inhibit LEC proliferation²¹⁹, and a wide variety of nanomaterials have been utilised as drug delivery systems for various therapeutic agents²²⁰. Moreover, the implantation of nanofibers in the inner surface of the capsular bag could affect the activity and morphology of the neighbouring LECs^{149,221}.

Table 2.4: List of therapeutic agents and techniques tested in studies for PCO prevention. Adapted from Nibourg et al., 2015¹⁴⁹.

Mechanism of action					
Anti-proliferation	Experimental model	Anti-inflammatory	Experimental model	Apoptosis inducers	Experimental model
Cytostatic drugs		NSAIDs		Cytotoxins	
<i>Anti-metabolites</i>		Celecoxib	Animal cells, <i>ex vivo</i> animal	<i>Immunotoxins</i>	
5-fluorouracil	Human cell, line, <i>ex vivo</i> human, <i>in vivo</i> animal	Diclofenac sodium	<i>In vivo</i> animal	FGF2-saporin	<i>In vivo</i> animal
Methotrexate	<i>In vivo</i> animal, <i>ex vivo</i> human	Indometacin	<i>In vivo</i> animal	MDX-RA (Ricin A)	<i>In vivo</i> human
<i>Other cytostatics</i>		Rofecoxib	Animal cells, <i>ex vivo</i> animal	Polylysin-saporin	<i>In vivo</i> animal
Colchicine	<i>In vivo</i> animal	Steroids		Gene therapy	
Paclitaxel	<i>In vivo</i> animal	Dexamethasone	<i>In vivo</i> animal	Transfer β -galactosidase gene	Animal cells, <i>in vivo</i> animal
TGF-β inhibitors		Immunosuppressants		Transfer BMP-7	<i>In vivo</i> animal
Anti-TGF- β antibody CAT-152	Human cell line	Cyclosporin A	Human and animal cells, <i>in vivo</i> animal	Transfer HSVtk gene	Animal cells, <i>in vivo</i> animal
Pirfenidone	Human cell line	Rapamycin	Animal cells, <i>in vivo</i> animal	Target Snail	Human cell line
Zebularine	Human cell line	Anticoagulants		Target TGF- β receptor	Human cell line
Rho inhibitors		Heparin	<i>In vivo</i> human and animal	Photodynamic therapy	
H-7	<i>Ex vivo</i> human			Indocyanin green	Human cell line
Lovastatin	Animal cells			Trypan blue	Human cell line
EGF inhibitors					
Erlotinib	Human cell line				
Tyrosine kinase inhibitors					
Genistein	Human cell line				
Local anesthetics					
Lidocaine	<i>Ex vivo</i> animal				

2.6 Drug Delivery Systems

The administration of drugs with traditional immediate-release dosage forms results in a rapid absorption and elimination of the active agent, which causes large fluctuations in its concentration. A multiple-dosing regime is thus necessary when employing these systems if the levels of drug are to be maintained within the therapeutic range for extended periods of time^{222,223}. However, this practice is associated with some negative consequences, such as a low patient compliance and a poor control over the administered dose, which can affect the efficiency of the therapy and cause an increase in the occurrence of systemic side effects^{224,225}. To solve these issues, drug delivery technology has been developed with the aim of achieving a higher degree of control over the dispensed dose. This would increase the efficacy of the drug and decrease the incidence of toxic side effects in the body by directing the therapeutic agent to the desired target^{226–229}. The mechanisms of action of these systems include the increase in the solubility of the drug, the reduction in the degradation rate, the targeted delivery to the affected cells or tissues, the modulation of the release rate and the facilitation of the penetration through biological barriers such as the cell membrane^{226,230}. Depending on their release mechanism, drug delivery devices can be classified as immediate or modified-release systems. The latter can be further divided into delayed-, targeted- or extended-release forms, which can provide either a sustained or a controlled release profile²³¹.

Sustained-release formulations are available in the form of drug complexes, suspensions, emulsions and coated tablets, among others^{225,232,233}. These systems are designed to release the drug continuously and at slower rates in order to maintain the therapeutic concentrations for a prolonged period of time^{229,231,234}. Thus, the frequency of administration and the required dose can be reduced, hence contributing to a decrease in the incidence of side effects²³⁵. An additional advantage of employing these systems is the minimisation of the fluctuations in plasma drug concentrations²³¹. However, while these systems are effective in prolonging the therapeutic effect a drug, they are not capable of maintaining a constant plasma concentration²²⁵. Moreover, the release kinetics and the termination of the delivery cannot be finely controlled with these devices²³⁶. Consequently, sustained-release systems are highly susceptible to

environmental conditions, hence increasing the patient-to-patient variations upon administration of these formulations^{233,237}. Thus, controlled-release systems were developed in order to address these problems.

Controlled-release systems are designed to deliver the drug at a predetermined rate in order to maintain the concentration in the blood or in the target tissue at the therapeutic level for an extended period of time^{226,237,238}. These devices may initially release part of the dose at a fast rate in order to reach the therapeutic concentration in a short time. Then, they provide a maintenance dose at a controlled rate in order to maintain the levels of active agent within the therapeutic window²²⁴. A controlled release is commonly achieved by encapsulating the drug within a polymeric matrix²³⁹. The use of synthetic polymers in the production of these carriers facilitates the modification of the systems in order to modulate the release of the therapeutic agent and obtain the desired release kinetics²⁴⁰. Moreover, these polymeric systems can be functionalised in order to target specific cells and tissues, hence decreasing the incidence of toxic side effects in other parts of the body²⁴¹. While controlled-release systems have proven effective in modulating and extending the release of numerous drugs, some challenges are still faced when employing these devices. Firstly, the use of synthetic polymers can cause cytotoxic responses after injection or implantation. Secondly, the degradation of biodegradable polymers inside the body can result in the generation of harmful byproducts^{233,241}. Another important factor to consider is the method of administration of the drug delivery system to the patient. If this system is produced in the form of an implantable device, a surgical procedure would be necessary for implantation. This would add certain risks and complications to the patient, such as the need for a second operation in order to stop the release of the drug^{234,237,242}. The availability of remotely controlled devices, pre-programmed systems or stimuli-responsive materials would be highly desirable in these cases, since they do not require additional procedures in order to end the delivery of the therapeutic agent^{236,242–244}.

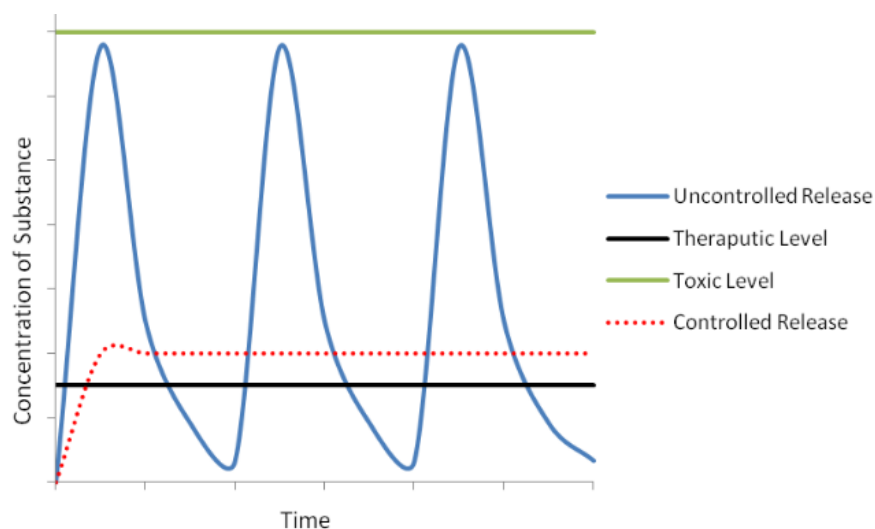


Figure 2.28: Representation of the concentration of substance vs. time, showing the fluctuations in drug concentration that occur upon administration of uncontrolled release systems and the maintenance of a constant level when a controlled release device is administered to the patient²³⁹.

2.6.1 Drug release mechanisms

The release of drug from controlled and sustained release systems can occur via four different mechanisms: diffusion, chemical reaction, solvent activation and stimulated release^{237,245}. These are modulated by the composition of the drug and the carrier and by any physico-chemical interactions that might take place within the system²⁴⁵. Diffusion-controlled release is based on the transport of drug through the carrier by following a concentration gradient, as described by Fick's law of diffusion^{245,246}. Two types of diffusion-controlled systems can be differentiated: reservoir and matrix systems (Figure 2.29). Reservoir systems are formed of a core that contains the drug and a polymeric membrane that controls the release rate of the therapeutic agent^{246–248}. Some examples of reservoir systems include membranes, capsules and liposomes²³³. The other possible configuration for a diffusion-controlled system is the matrix device, in which the drug is evenly distributed throughout the carrier^{237,247}.

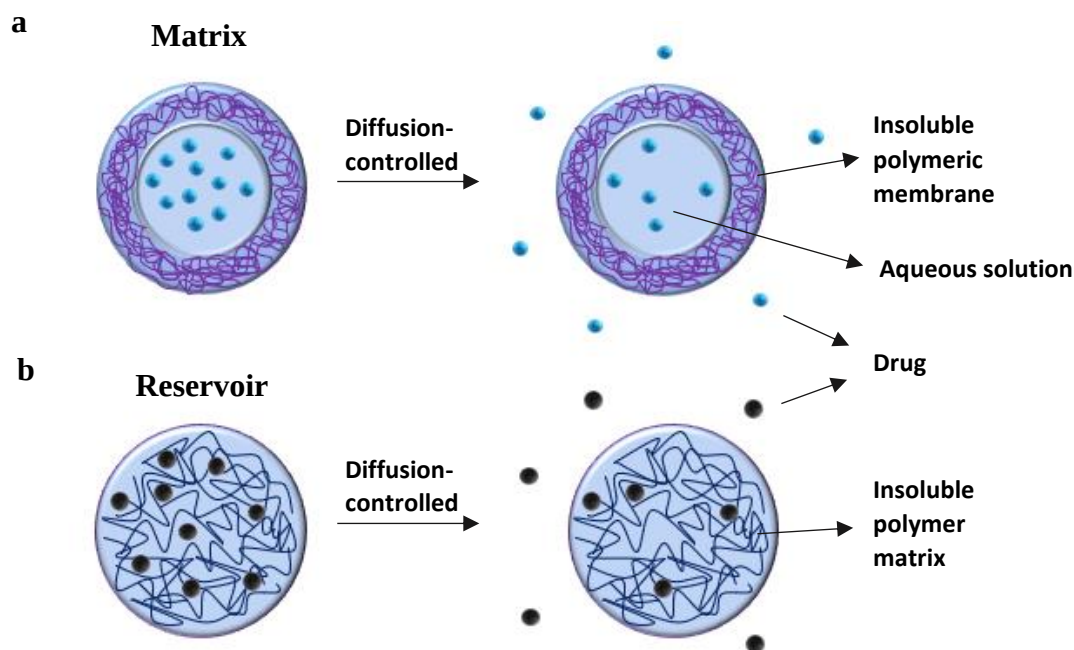


Figure 2.29: Schematic of two diffusion-based delivery systems: reservoir **(a)** and matrix system **(b)**. Adapted from Sun et al., 2014²⁴⁹.

The release of drug via chemical control is regulated by chemical reactions that occur within the system, such as the erosion or biodegradation of the polymer in which the drug is entrapped²³⁷. The release may be dependent on the reaction rate only or on both reaction and diffusion mechanisms that might take place within the polymeric structure²⁴⁰. An advantage of these systems over non-degradable devices is the absorption of the degradation products within the body, hence circumventing the need for a second surgical procedure in order to remove the implant. However, special consideration must be taken with regard to the potential toxicity of these by-products²³³. Another form of chemical control is the covalent conjugation of the drug molecule to the polymer matrix via a cleavable linker, being the release dependent on the rate of cleavage of the covalent bonds established within the system. This opens up the possibility of designing enzyme-degradable matrices that release the drug only when the enzymes which are overexpressed in pathological conditions are present in the tissue, hence increasing the efficiency of the delivery devices^{237,240}.

The third release mechanism is mediated by the uptake of fluids by the polymeric structure. When a polymer swells in water, the glass transition temperature decreases and its constituent chains become more mobile, adopting a relaxed configuration. This facilitates the diffusion of the drug towards the outer surface of the polymeric structure^{233,237,250}. The release rate in these systems is dependent on the rate of diffusion of fluid into the polymer and the subsequent molecular relaxation²⁵¹. An alternative mechanism that can modulate the release of drug is osmosis. Osmotic pumps are formed of a core in which the drug is contained and a perforated membrane that is permeable to water but impermeable to the drug. When water diffuses through the membrane, the therapeutic agent is dissolved and subsequently released at a constant rate as a consequence of the osmotic pressure gradient that exists between the core and the external environment^{237,245,252}.

While these release mechanisms provide a sustained or controlled release of the drug, an improved regulation over the time of release would be desirable in order to increase the efficiency of the system and prevent any toxic side effects derived from an overexposure to the therapeutic agent. An ideal drug delivery device would have the ability to detect any variations that might occur in the physiological conditions as a consequence of a pathology and respond to them by releasing the drug autonomously²⁵³. Furthermore, the system should be able to modulate the release in synchronisation with the progress of the disease and it must show a high specificity towards the affected tissue²⁵⁴. This can be achieved by developing systems in which the release of the drug is only triggered under conditions that are associated with the incidence of a specific syndrome²⁵⁵. Therefore, stimuli-responsive materials that can sense these changes in their environment must be employed to synthesise polymeric carriers that comply with these requirements.

Smart or stimuli-responsive polymers are materials that undergo abrupt changes in their physico-chemical properties when they are subjected to a specific stimulus, such as a change in pH or temperature, the presence of certain enzymes or the application of ultrasounds or electromagnetic fields^{245,248}. The responses that these stimuli might elicit include morphological or conformational changes, variations in the mechanical

properties, aggregation, sol-gel transition and changes in the solubility or permeability of the material^{226,255,256}. When one or more of the aforementioned stimuli are specifically associated with the development of a certain disease, a high degree of control over the release of the therapeutic agent can be attained by selecting or engineering a vehicle that can respond to that signal^{244,257}. Furthermore, if the stimulus is specific to a certain type of cell or tissue, a localised delivery can also be attained. Since all physiological and pathological processes are driven by biological molecules such as hormones or enzymes, the presence of biological domains in the delivery system which can interact with these biomolecules leads to a higher specificity in the responsiveness to specific diseases. An example of this approach is the synthesis of hydrogels with peptide crosslinkers that can be cleaved by a specific enzyme whose levels increase in pathological conditions. If a drug is encapsulated within such a system, it would only be released when the enzyme is present in the environment, and therefore only once the disease has started to develop²⁵⁴. Therefore, this would be accompanied by a significant reduction in the incidence of toxic side effects in other organs or tissues and an increase in the efficacy of the delivery system²⁴².

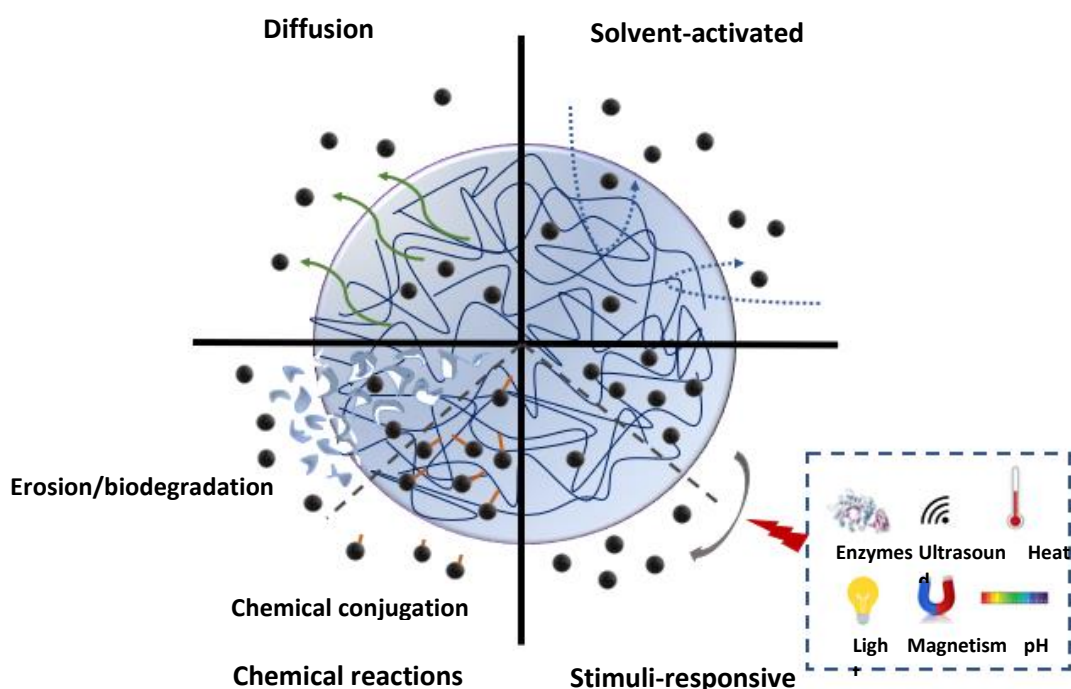


Figure 2.30: Mechanisms of drug release. Adapted from Kumar Patra et al., 2018²⁵⁸.

2.6.2 Drug release kinetics

The release rate of a drug from the carrier is a very important parameter in drug delivery, as it determines the amount of the therapeutic agent that will be available for absorption by the organism²²⁴. As mentioned above, controlled release systems aim at maintaining the drug at its therapeutic concentration for an extended period of time by keeping the release rate at a constant level. Thus, the elution rate of the drug should be independent of its concentration within the system. This regime is known as zero-order release, and the design of a device that can provide it is the main objective of controlled release methods. However, in most of the delivery devices produced so far there is a drop in the release rate when higher amounts of drug are released from the system^{239,259}. Hence, it is essential to make improvements in the design and synthesis of controlled release devices in order to ensure the attainment of an ideal drug release profile upon administration to the patient.

Mathematical models can be employed to predict the release kinetics of a system before it is synthesised, since these equations allow the evaluation of the physical parameters that affect the release rate of the drug. Furthermore, the amounts of therapeutic agent and polymer that are required in order to achieve the desired release profile, as well as the optimal size and shape for the system, can also be determined by making use of these models. It is for this reason that these equations are a very important tool during the design and optimisation of novel drug delivery devices^{224,259,260}. The selection of an appropriate mathematical model is based on the type and geometry of the system and the relationship between the initial concentration of drug and its solubility²⁶¹. Some of the models that have been developed to describe the release profile of controlled release systems are described below.

2.6.2.1 Zero-order release kinetics model

A zero-order process is characterised by a constant rate of release which is independent of the concentration of active agent that is present in the delivery system. This is the ideal release profile for a controlled-release formulation²³⁹. This model is represented by the equation:

$$C_t = C_0 + k_0 t \quad (1)$$

where C_t is the amount of drug dissolved at time t , C_0 is the initial amount of drug in the solution and k_0 is the zero-order release rate constant²⁶². In this release model, the release of drug is expressed as the cumulative amount of released drug versus time. The zero-order release profile is ideal for slow and prolonged drug release and it can be used to describe a variety of dosage forms, such as coated tablets or osmotic systems^{224,245,259}.

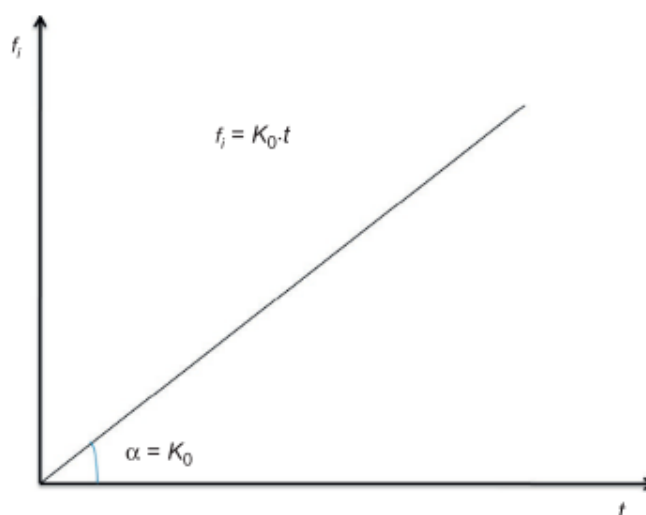


Figure 2.31: Graphical representation of zero-order kinetics; f is the fraction of the drug dissolved during the time t and K_0 is the apparent velocity of dissolution²²⁴.

2.6.2.2 First-order release kinetics model

In a first-order process, the rate of diffusion is proportional to the concentration of drug in the system. As the release of the drug progresses, the concentration of this agent in the device decreases and so does the release rate²³⁹. The first-order kinetic model is expressed by the equation:

$$\log C = \log C_0 + k_1 t / 2.303 \quad (2)$$

where C_0 is the initial concentration of drug, k_1 is the first-order rate constant and t is the time. The release rate is expressed as the cumulative log percentage of remaining drug versus time and a straight line with a slope of value $k_1/2.303$. This release profile is common in systems in which the drug is encapsulated within a porous matrix, since the amount of drug released is dependent on the quantity of drug that remains in the device^{224,245,259,260}.

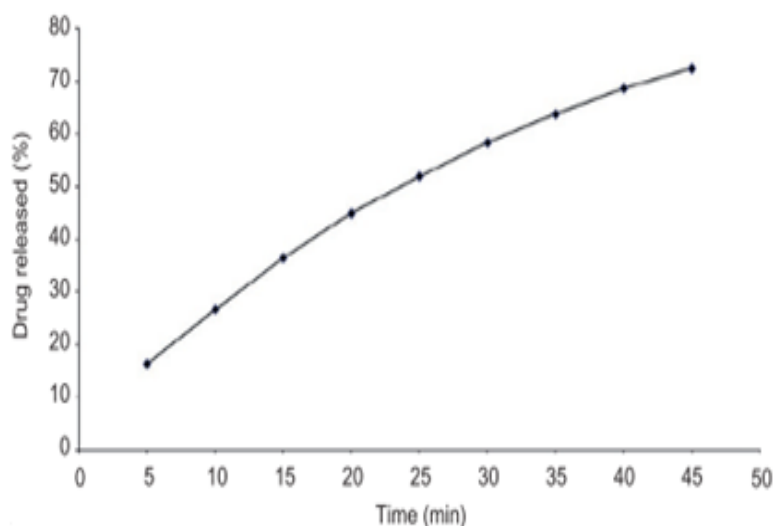


Figure 2.32: Graphical representation of first-order model of drug release: cumulative drug released (%) versus time²²⁴.

2.6.2.3 Higuchi model

The first mathematical model that specifically described the release of a drug from a matrix system was developed by Higuchi in the 1960s²⁶³. This model assumes that the drug is homogeneously distributed throughout an erodible matrix. The diffusion-controlled release is described by the equation:

$$f t = Q t = \sqrt{D(2C - C_s)Ct} \quad (3)$$

where Q_t is the amount of drug released per unit area at time t , C is the initial concentration of the drug, C_s is the solubility of the drug and D is the diffusion coefficient in the matrix²⁴⁵. It is important to note that the application of this model requires the fulfilment of the following assumptions: a pseudo-steady state in which the initial drug concentration is much higher than the solubility of the drug, a constant diffusivity, a negligible matrix swelling and dissolution, and the attainment of sink conditions by ensuring that the total dissolution of the drug in the release medium yields a concentration which is significantly lower than the saturation point^{224,259,261,264,265}.

The development of the Higuchi model led to the formulation of other equations that describe the release from a variety of carriers with a higher accuracy. Some examples of these include the Korsmeyer and Peppas model, which describes the delivery from polymeric systems when more than one phenomenon is involved in the release, or the Brazel and Peppas equation, formulated to model the release from swelling-controlled systems^{224,266}.

2.7 Ophthalmic Drug Delivery Systems

The delivery of drugs into the eye poses a significant challenge due to the numerous barriers that isolate the tissues that form this organ. These include the wash out by tears, the nasolacrimal drainage, the cornea and the blood-aqueous and blood-retina barriers (Figure 2.33). The main delivery routes for administration of drugs into the ocular structures include the topical, systemic and intraocular pathways. The systemic delivery of a drug requires the administration of high doses of the therapeutic agent, which often leads to the occurrence of side effects in the rest of the body²⁶⁷. While the intraocular injection would circumvent this problem, this invasive technique can cause haemorrhages and retinal detachment²⁶⁸. Therefore, topical delivery is the most common technique currently in use for the delivery of drugs into the anterior and posterior chambers of the eye. Due to the simplicity of their application, topical formulations in the form of eyedrops constitute the most common approach for the administration of therapeutic agents into the ocular tissues. However, the low permeability of the cornea and the rapid clearance of the drops by the lacrimal secretions reduce the probability of penetration of the drug into the target tissue down to 1%, approximately²⁶⁷. An additional problem encountered with the use of this delivery route is the inability to deliver drugs into the posterior segment of the eye²⁶⁹. Thus, the use of drug delivery systems that increase the residence time of the therapeutic agent in the eye and which can be inserted into this organ would be highly desirable in order to circumvent the aforementioned issues. Furthermore, these devices would also improve the therapeutic efficiency by targeting the delivery of the agent to the diseased tissue and by controlling the release of the drug in order to ensure a synchronisation with the pathological events that are to be inhibited²⁷⁰.

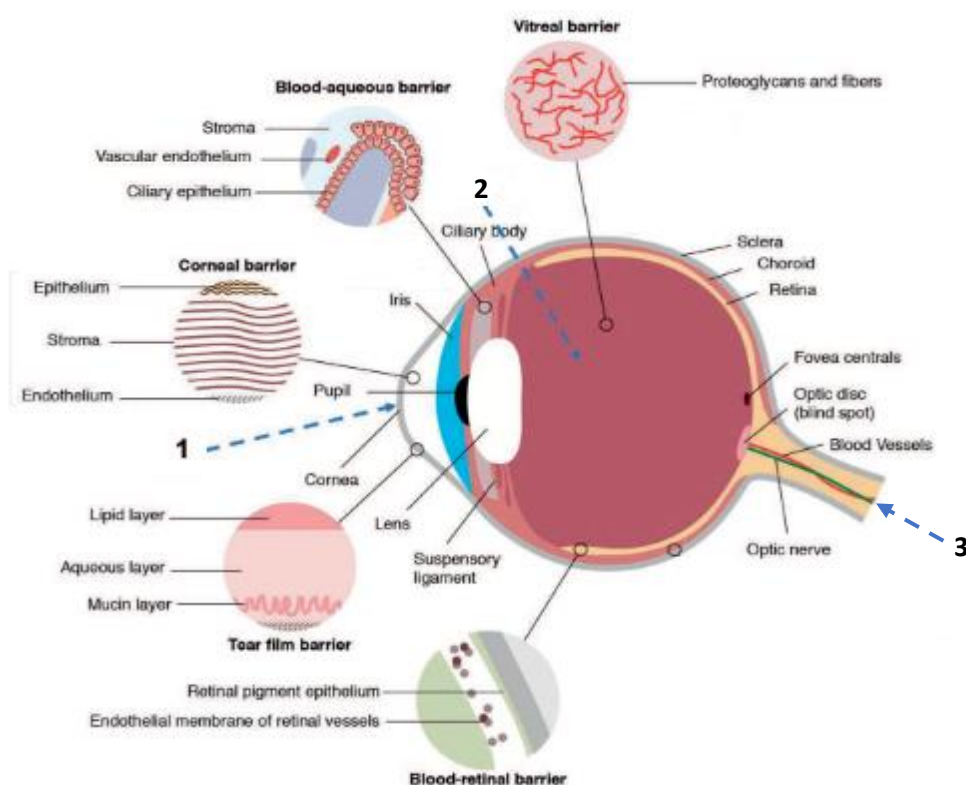


Figure 2.33: Barriers for ocular drug delivery and routes of drug administration to the eye: (1) topical, (2) intravitreal and (3) systemic. Adapted from Santos et al., 2018²⁷¹.

Similar to other delivery systems, ophthalmic drug delivery devices can be classified as matrix or reservoir systems. As previously explained, in matrix-based delivery systems the drug is distributed within a degradable polymer that modulates its release, while reservoir systems contain the drug in the form of a pellet which is dissolved and subsequently delivered to the surrounding tissues. Ocular drug delivery systems can be also classified as implantable or non-implantable. Non-implantable devices, also known as inserts, are placed in the inner side of the eyelid, where the release is assisted by the exposure to lacrimal fluids. Ocusert®, a drug delivery device for the treatment of glaucoma, was the first ocular insert introduced to the market. On the other hand, ocular implants are inserted in the internal structures of the eye via invasive surgical procedures and provide a long-term delivery of the drug. This approach opens up the possibility of using other implants as drug delivery vehicles, hence reducing the

invasiveness of the surgical procedure that is required to insert these devices in the eye^{269,272}.

2.7.1 Drug Delivery Systems for the Prevention of PCO

Cataract surgery can result in a number of complications, such as inflammation and infection, or it can intensify the symptoms of other pre-existing medical conditions. One of the main post-surgical syndromes is PCO, which results in the development of a secondary cataract. This disease can be prevented by administering drugs that interfere with the biological processes which result in the development of these pathologies. The therapeutic agents can be delivered during surgery by dissolving them in the irrigation fluids employed during this procedure. However, this technique is not commonly used in the clinic due to the low therapeutic efficiencies that result from the short exposure times attainable with this approach and the toxic responses that the drugs might elicit on neighbouring intraocular tissues when administered at their therapeutic concentration^{269,273,274}. To overcome these issues, a post-surgical treatment comprising antibiotics and anti-inflammatories is administered during the weeks that follow the surgical procedure. These drugs are often delivered in the form of eye drops, since these liquid formulations can serve as artificial tear substitutes that lubricate the ocular surface^{275,276}. However, the efficiency of this method is compromised by the reduced bioavailability of the formulations which results from blinking and lacrimation. This could be compensated by increasing the frequency of administration, although this is usually accompanied by a poor patient compliance. While systemic drug delivery could be a solution to these issues, this approach does not allow a tissue-specific targeting, thus increasing the incidence of side effects in non-targeted tissues²⁷⁷. Therefore, alternative approaches would be needed in order to increase the efficiency of the post-surgical therapies administered to prevent PCO.

The development of novel drug delivery systems could be the solution to the challenges currently faced in the dispensation of therapeutic agents after cataract surgery. One of the drug delivery devices that has resulted from the research

conducted in this field is Surodex[®], commercialised by Oculex Pharmaceuticals Inc., USA²⁷⁸. This implantable system, composed of PLGA, provides a sustained release of dexamethasone upon implantation in the posterior chamber of the eye, hence causing a reduction in post-operative inflammation^{278,279}. However, the use of this implant increases the invasiveness of the surgical technique, as it must be removed once the drug has been released²⁷⁸. Other drug delivery systems developed for the prevention of PCO include injectable nanoparticles^{273,274}, an intracapsular ring loaded with 5-fluorouracil²¹¹, PLGA implants loaded with EDTA, a diffusion-limiting membrane containing colchicine and a polymeric carrier for the release of heparin^{280–282}. While these devices have shown promising results in the reduction of the incidence of PCO, their utilisation in the clinic could cause problems such as the damage of the lens during the injection of nanoparticles or the loss of vision as a consequence of the displacement of the implants within the capsular bag^{269,283,284}. Therefore, the engineering of novel systems that reduce the invasiveness of post-operative therapies is highly desirable.

The use of intraocular lenses as drug delivery vehicles is being widely researched with the objective of circumventing the problems described above. This method reduces the invasiveness of the post-operative therapy and increases its efficiency by providing a localised delivery that reduces the incidence of systemic side effects²⁷⁶. Numerous studies have reported the delivery of antibiotics, anti-inflammatories and drugs for the prevention of PCO by incorporating these therapeutic agents into implantable IOLs^{276,278,285}. For this approach to be successful, it is essential to ensure that the addition of drugs to the implant does not compromise the optical properties of the lens²⁸³. A wide variety of techniques can be used to load the drugs into these lenses, which are explained in detail as follows.

2.7.1.1 Pendant solvent-cast drug inserts

This type of drug delivery system is created by attaching drug-loaded, capsule-like polymeric inserts in the haptics of the IOL (Figure 2.34). This ensures the preservation of the transparency and composition of the lens^{283,286}. A similar approach was developed by Tan *et al.*, who created a biodegradable drug-loaded, ring-like structure to be attached on the periphery of the IOL without obstructing vision²⁸⁷.

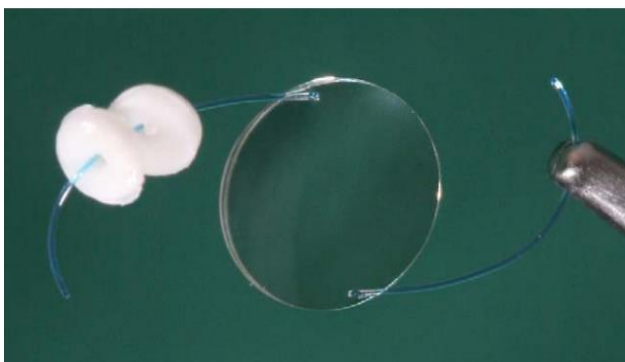


Figure 2.34: IOL combined with two delivery systems inserted in the haptic²⁸³.

2.7.1.2 Drug-soaked IOLs

An IOL can be impregnated with a drug by soaking the implant in an aqueous or alcoholic solution that contains the therapeutic agent. Once the IOL is implanted, the drug is progressively released into the aqueous humour²⁸³. Despite the simplicity of this method, some complications might arise when using hydrophobic IOLs or when loading drugs that show a low affinity towards the lens²⁸⁸. Furthermore, the difficulty to maintain sterile conditions while soaking the implant and the presence of residual solvent within the lens can cause cytotoxic responses once the IOL is implanted in the capsular bag²⁸⁹.

Another technique that results in the incorporation of a drug within the polymeric matrix that forms the IOL is the dissolution of the agent within the monomer solutions used in the manufacture of these lenses²⁹⁰. While this approach ensures a high accuracy and reproducibility in the drug loading, it can also affect the chemical

structure of the loaded agent. Therefore, this must be taken into consideration when choosing a method for the production of drug-loaded IOLs²⁷⁷.

2.7.1.3 Supercritical fluid impregnation

This method allows the incorporation of drugs within the IOL without using any organic solvents. The drug is dissolved in supercritical CO₂ (scCO₂) and the resulting mixture is deposited on the surface of the IOL. The diffusion of the mixture into the pores of the polymeric structure leads to the swelling and plasticisation of the lens, hence enhancing the impregnation of the whole implant with the therapeutic agent²⁸³. When the pressure is released, the scCO₂ leaves the polymer, while the drug remains entrapped within the network^{291–293}. An advantage of this technique with respect to conventional pre-soaking methods is the attainment of greater loading yields as a consequence of the higher solubility of the drug in scCO₂. Furthermore, as opposed to aqueous solvents, the use of CO₂ as a solubilising agent facilitates the loading of hydrophobic IOLs with therapeutic agents and reduces the risks of toxicity caused by organic solvents^{283,292,294}.

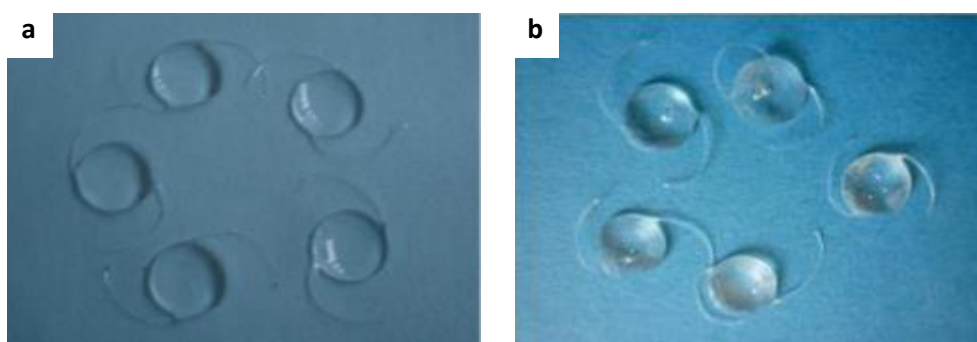


Figure 2.35: IOLs before (a) and after impregnation with cefuroxime sodium via supercritical impregnation (b)²⁹⁵.

2.7.1.4 Deposition of coatings

As previously stated, a variety of methodologies can be employed to functionalise the surface of an IOL. These modifications have been primarily used to increase their biocompatibility. However, the resulting coatings can also serve as vehicles for drug delivery. Various studies have shown that a controlled release can be achieved by incorporating the drug between polymeric layers of opposing charges^{296,297}. One of the advantages of this technique is that the physico-chemical properties of the coating can be finely regulated by controlling the composition²⁹⁶. However, the complexity of this methodology has favoured the use of simpler methods such as spin or spray coating^{283,298–300}. Chemical grafting is another promising technique that results in the formation of stable and durable coatings³⁰¹. Vieira *et al.* have previously reported the plasma-assisted grafting of pHEMA on the surface of lens-like polymeric structures in order to control the release of moxifloxacin for the prevention of endophthalmitis³⁰². The application of this technique for the production of IOLs loaded with drugs which have a cytotoxic or inhibitory effect on LECs would result in the creation of highly efficient delivery systems for the prevention of PCO.

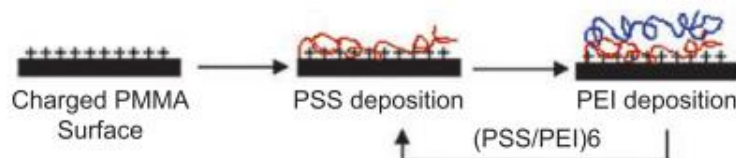


Figure 2.36: Schematic representation of the layer-by-layer deposition of poly (sodium 4-styrenesulfonate) (PSS) and poly (ethylenimine) (PEI)²⁹⁷.

2.7.1.5 Chemically-grafted drugs

The covalent conjugation of the drug onto the surface of an IOL increases the stability of the system, resulting in an enhancement of the tissue-specificity and a reduction in the incidence of side effects²⁸³. Pot *et al.* have reported the grafting of selenocystamine on an IOL made with pHEMA with the objective of preventing PCO. This compound acts as a catalyst for the generation of superoxide radicals ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), which cause apoptosis³⁰³. The use of loading techniques based on covalent

conjugation also enables the immobilisation of drugs through photocleavable linkers that release the agent upon exposure to radiation^{304–306}. Moreover, a novel approach based on the incorporation of silica-coated gold nanorods (Au@SiO₂) on the IOL has proven effective in the elimination of LECs upon irradiation with near infrared light (Figure 2.37)³⁰⁷.

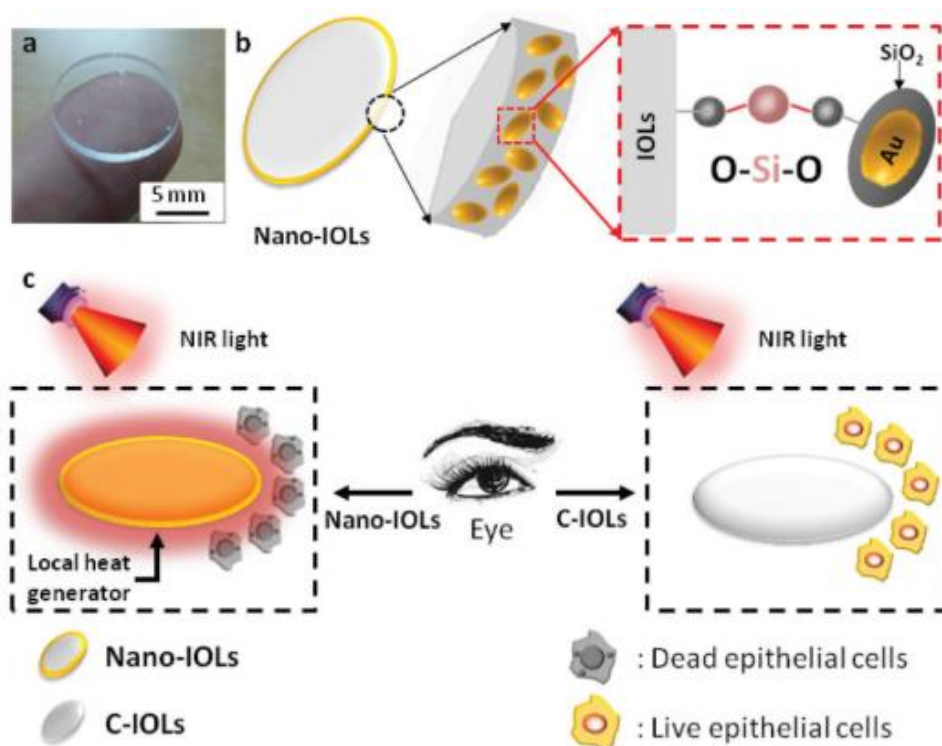


Figure 2.37: Schematic illustration of the working principle of the Nano-IOL, a lens functionalised with Au@SiO₂ for the prevention of PCO via photodynamic therapy³⁰⁷.

2.8 Production of IOLs

2.8.1 General Considerations for the Design and Production of IOLs

There are numerous medical conditions that can only be treated with the implantation of an artificial device. Polymeric biomaterials have been widely employed in the fabrication of implants, scaffolds and other medical devices designed for this purpose³⁰⁸. A crucial characteristic that any biomaterial must possess is a high biocompatibility. The biocompatibility of a material can be defined as its ability to restore a physiological function without eliciting any toxic reaction in the host^{308–311}. This property is mainly determined by the characteristics of the material surface, since this is the area that interacts with the cells and proteins which orchestrate the immune reaction that occurs upon implantation³¹². The physico-chemical properties that influence the biocompatibility of a material include the interfacial free energy, the balance between hydrophilicity and hydrophobicity, the chemical composition, the type and density of surface charges, the topography and roughness of the surface and the molecular weight and conformational flexibility of the polymer³⁰⁸.

The surgical procedure performed for the implantation of a medical device causes an injury in the treated organ or body part. The host reacts to the creation of this wound by triggering an inflammatory reaction that aims at healing the damaged tissue. This biological response is designed to protect the body from external organisms that can pose a threat to the health of the individual. Therefore, once the immune system identifies the implant as a foreign body, the subsequent responses tend to isolate it from the surrounding tissues by creating a collagenous capsule that encloses it and impedes the establishment of further interactions with it (Figure 2.38). This process prevents the integration of the implant with the host tissues, hence thwarting its physiological function. To avoid this, the surface of the implant must be engineered in such a way that the body can recognise it as a biosimilar device, thus making it biocompatible and promoting its ability to restore the functionality of the organ where it is implanted^{310,312–314}.

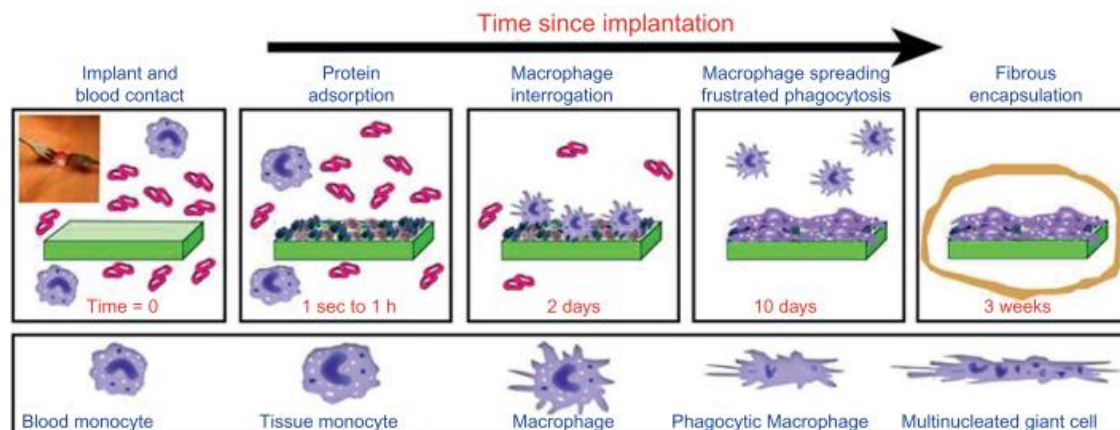


Figure 2.38: Schematic of the processes involved in the foreign body reaction that occurs upon implantation of a medical device³¹⁵.

The responses that a medical implant may elicit in the organism upon implantation is a critical factor to consider during the development of these devices. Therefore, the toxicity, sensitisation, intradermal irritation and acute systemic toxicity of a medical device must be evaluated before it enters human clinical trials^{316,317}. It is for this reason that animal tests must be carried out in order to evaluate the impact that the biomaterial would have in the organism³¹⁸. However, *in vivo* studies are time-consuming, expensive and require complex procedures for ethical approval³¹⁷. Consequently, cytotoxicity testing is a preferable alternative during the first stages of the development of a medical device. These techniques are based on the incubation of the implant in contact with cells cultured *in vitro* and the subsequent evaluation of the effect it may have on the metabolic activity, growth, replication and morphology of the cells. The evaluation of the cytotoxicity *in vitro* is one of the main requirements demanded by the regulatory bodies in order to validate the functionality of a medical device, since this is a quick, sensitive and cost-effective method for the assessment of the biocompatibility of a material. However, more realistic systems such as organ models or animals must be subsequently employed in order to obtain more reliable data^{310,319,320}.

As for any medical device, the biocompatibility of the materials that are used in the manufacture of IOLs is a fundamental requirement for the production of these devices. The absence of an immune reaction after the implantation of an IOL in the capsular

bag, known as uveal biocompatibility, is one of the considerations that must be taken into account when selecting the materials that will be employed for the fabrication of the IOL. An additional aspect to consider is the capsular biocompatibility of the lens, which refers to the interactions that occur between the implant and the residual LECs which remain in the capsular bag after cataract surgery. As the possession of a good capsular biocompatibility is an essential requirement for an IOL to be capable of preventing the development of PCO, these lenses must be made of materials that confer them with this attribute^{106,321}.

The functionality of a medical device is another factor that determines its biocompatibility. For an IOL to be optically functional, the materials used in the manufacture of this device must be transparent and need to maintain their optical clarity when the lens is implanted in the posterior chamber of the eye. Once the IOL is inside the capsular bag, the optical performance of this device can be compromised by the incidence of glistenings and dysphotopsias or the formation of calcium deposits on their surface⁸². Glistenings are caused by the formation of vacuoles within the polymeric structure of the implant upon infiltration of ocular fluids. The polymer-water interfaces subsequently formed lead to light scattering phenomena which causes the appearance of glistenings (Figure 2.39, (a))³²². Dysphotopsias occur as a consequence of the reflection of light from the IOL onto the retina, resulting in the formation of light patterns that are superimposed with the retinal images and which cause visual impairment (Figure 2.39, (b))^{323–325}. A higher incidence of glistenings and dysphotopsias has been reported after the implantation of hydrophobic acrylic IOLs, while the formation of calcium deposits occurs more frequently on hydrophilic lenses⁷⁹. These phenomena can be prevented by introducing improvements in the IOL materials and designs. For example, the lens can be engineered to prevent dysphotopsias by creating a round anterior edge that slopes towards a sharp posterior rim³²⁶. As previously mentioned, the creation of a square posterior edge has the additional advantage of reducing the rates of PCO development by inhibiting the migration of LECs to the posterior capsule³². With regard to the prevention of this syndrome, the materials that have shown the most promising results in the clinic are hydrophobic acrylic polymers^{7,195}. As well as the wettability of the material, other

properties such as the surface roughness or topography have also been shown to affect the cellular responses which lead to the formation of a secondary cataract. Therefore, it is important to consider these factors when designing and manufacturing novel IOLs with improved biocompatibility and functionality.

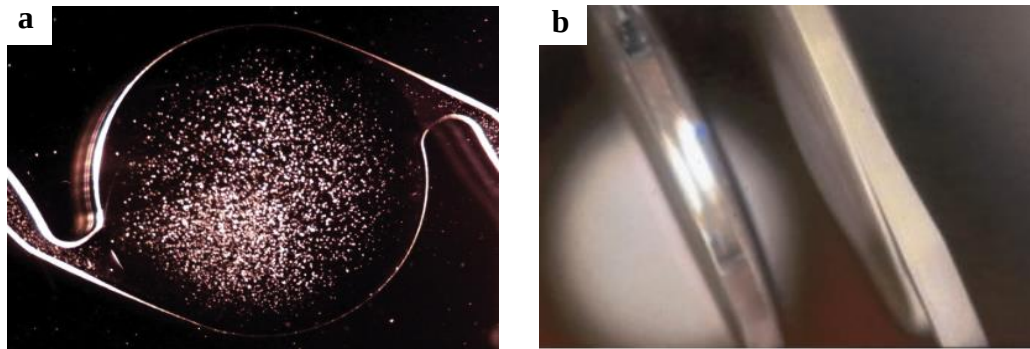


Figure 2.39: (a) IOL with glistenings³²⁷. **(b)** Comparison between the edge of an IOL that causes dysphotopsias (left) and another IOL which prevents this phenomenon (right)³²⁸.

Another factor that influences the functionality of an implant is its applicability in the clinic. As previously discussed, IOLs are implanted in the capsular bag via injection through a small incision that is performed on the anterior side of the lens capsule. The implant needs to be folded in order to facilitate its introduction in the injector and it must unfold in a controlled manner once it is inserted in the capsular bag. Therefore, the material used in the manufacture of these lenses should possess an adequate balance between flexibility and mechanical strength in order to prevent the damage of the implants during this procedure³²⁹.

2.8.2 Techniques currently employed in the production of IOLs

As mentioned in previous sections, IOLs can be made in a single piece or by producing the optic and haptics separately and assembling them afterwards. The techniques currently used for the manufacture of IOLs include moulding and lathe cutting^{330–333}. Moulding-based methods make use of polished stainless-steel moulds, each of them producing a specific design. The polymerisable material is injected or poured into the heated mould and cured by thermopolymerisation until the IOL is formed³³⁰. Moulding is the preferred technique in industry because of the high efficiency of this process³³². On the other hand, lathing involves the production of rods that are divided into disk-shaped structures, known as blanks, which are then machined with a lathe in order to form the lenses (Figure 2.40)^{334–336}. For the manufacture of three-piece IOLs, the haptics are then inserted into a pair of perforations drilled in the periphery of the lens for this purpose³³⁷. The IOLs are then polished by subjecting them to a tumbling process in order to remove any artefacts that may compromise the smoothness of the lenses³³².

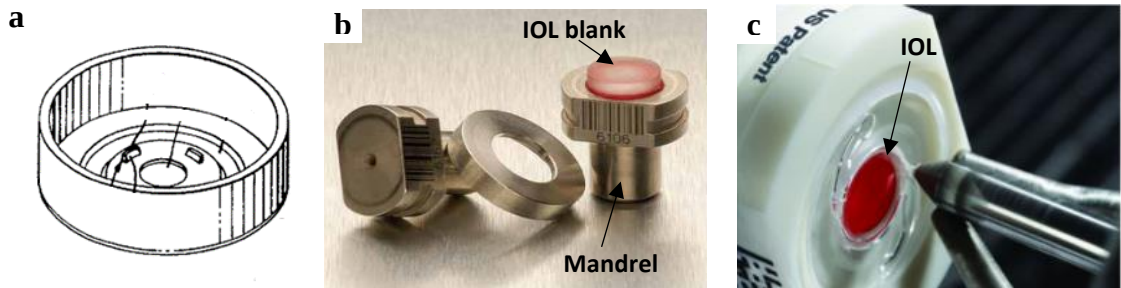


Figure 2.40: (a) Drawing of a mould used for the production of the optic part of an IOL³³⁸. (b) IOL blank mounted on the mandrel prior to lathing³³⁹. (c) lathing of an IOL¹³⁰.

While the utilisation of these techniques results in the production of high-quality IOLs, there are various disadvantages associated with these methods. These include the long times required for thermopolymerisation, the complexity of the process, the high costs of production and the batch-to-batch variation. Another common issue is the spillage of polymer between the two halves of the mould, which results in the presence of unwanted, excess material around the edges of the IOLs. If these artefacts are not removed from the lens, they would potentially cause damage in the

surrounding tissues once the IOLs are implanted in the capsular bag^{330,340}. The elimination of these defects requires a thorough polishing step, which increases the duration and costs of the manufacturing process. Thus, these issues make it desirable to seek faster and more cost-effective techniques for the production of IOLs. Furthermore, it is important to develop a technique that allows the creation of patient-specific implants, as modern healthcare is moving towards the establishment of more personalised medical treatments. This would improve the outcomes of cataract surgery by allowing the production of bioactive IOLs capable of preventing PCO and other post-surgical complications. The introduction of 3D printing technologies in the fabrication of IOLs could be the solution to the issues previously discussed, since it would accelerate the times of production and reduce the associated costs. Moreover, this technique would facilitate the production of personalised lenses, since it circumvents the need for creating a mould for each IOL design by making use of CAD software.

2.9 3D-printing technologies

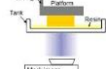
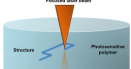
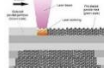
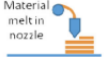
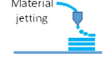
Additive manufacturing, also known as 3D-printing, is a technology that fabricates objects designed on a CAD software by adding the material of choice layer by layer^{341,342}. To achieve this, the model created on the CAD software is converted into a STL format, which is then virtually sliced into bidimensional layers. After that, this information is sent to the printer, which processes the material in order to reproduce the object modelled on the software by sequentially forming and binding the layers created on the STL file³⁴³.

3D-printing was invented in 1986 by Charles Hull, who patented the first stereolithography machine. Since the creation of this system, many other types of additive manufacturing technology have been developed, such as fused deposition modeling (FDM), selective laser sintering (SLS), inkjet printing, extrusion or selective laser melting (SLM)^{10,344,345}. Each of these technologies employ a different

mechanism for the deposition of the layers that form the final object and offer different advantages with respect to the rest of 3D-printing techniques (Table 2.5).

The introduction of 3D-printing technologies into the production lines of numerous industrial sectors has allowed a significant reduction in the cost and time of manufacture of a wide variety of products^{9,346,347}. Biomedical research is one of the fields that is benefiting from this technology, as it is boosting the production of personalised implants and scaffolds for tissue regeneration, patient-specific drug delivery devices and models for surgery that ease the planning of complex surgical procedures^{9–12,344,348}. However, the adoption of these technologies in routine clinical practice still requires the long-term evaluation of the biocompatibility and mechanical performance of the resulting devices³⁴⁹.

Table 2.5: Comparison of various 3D printing technologies^{8,342,350,351}.

Technique	Working principle	Compatible materials	Resolution	Advantages	Disadvantages	Applications
SLA 	Solidification of photopolymers upon curing with UV or visible light	Photopolymers	25 – 100 μm	High surface quality and precision; No problem of nozzle clogging	Single material; Limited mechanical properties; Post-processing required	Biomedical engineering
Projection micro-SLA 	Similar to SLA, but uses dynamic photomasks which are produced by a DMD	Photopolymers	Down to 2 μm	High resolution; No problem of nozzle clogging	Single material; Limited mechanical properties; Post-processing required; High cost	Biomedical engineering; flow sensors
2PP 	Solidification of photopolymers upon curing with a laser focused onto a very small volume	Photopolymers	<100 nm	Very high resolution; No problem of nozzle clogging	Low build speed; Limited materials; High cost	Electromagnetic shielding; biomedical engineering; lockyballs for cell delivery, ear prosthetics, etc.
SLS 	Selective fusion of powdered material using a laser	Thermoplastics, ceramic powders	< 10 μm	No supports required; Large parts; Wide range of materials available; Reduced anisotropy; Good mechanical strength	Post-processing required; High cost; Powdery surface; Shrinkage and warpage due to sintering and cooling	Bone scaffolds; drug delivery devices
SLM 	Spatial-controlled melting of a metallic powder using a laser	Metal powders	20-100 μm	High mechanical strength; High resolution	Post-processing required; Only for metals; High surface roughness	Bone replacements and scaffolds
FDM 	Material extrusion through a heated nozzle	Thermoplastics	100 - 150 μm	Inexpensive equipment and materials; High speed; Deposition of diverse materials	Rough surfaces; High temperature processing; The material should be in a filament form	Customised composites; drug delivery devices; tissue engineering
Inkjet 	A liquid ink is dispensed through a nozzle in the form of droplets	Solutions/ Suspensions	20 -100 μm	Low cost with respect to SLA or SLM; Reduced build times; Allows multimaterial AM; High resolution	Viscous solutions may clog the system; Low mechanical strength	Cell patterning on soft scaffolds; nanocomposites; sensors
LOM 	Sheets are bonded using a heated roller and shaped with a laser	PVC, paper	200 – 300 μm	Compact equipment; Reduced build times	Limited materials; Low resolution	Prototyping; architectural models

2.9.1 Stereolithography

Stereolithography (SLA) is a 3D-printing technology that produces three-dimensional objects through the sequential addition of polymeric layers which are created upon photopolymerisation of a liquid resin^{352,353}. Its main components include a vat that contains the liquid resin, a build platform on which the printed part is adhered and a scanning laser that triggers the photopolymerisation reaction in order to create layers which have the same shape as those created on the STL file^{354,355}. A series of galvanometers and mirrors direct the laser beam onto the desired printing location³⁵⁶. Depending on the configuration of the build platform and the source of radiation, two types of stereolithography systems can be differentiated: top-down and bottom-up³⁴⁶. In the bottom-up approach, the laser is located above the tank that contains the resin. The build platform is completely immersed in the pre-polymer mix and the printed layers are built right under the surface of the liquid. Once each layer is polymerised, the platform moves downwards in order to allow the addition of the following layers from the bottom to the top of the printed object (Figure 2.41, (a)). On the other hand, the top-down set up follows the opposite direction during the fabrication of the object: the resin is irradiated from the bottom of the tank and the build platform is placed on top of the resin, only partially immersed in it (Figure 2.41, (b)). The platform moves upwards after the addition of each of the layers that form the printed object. Before the next layer is built, the previous one is recoated with resin in order to have enough material available at the printing site. This process is repeated until the model is complete^{346,357–359}.

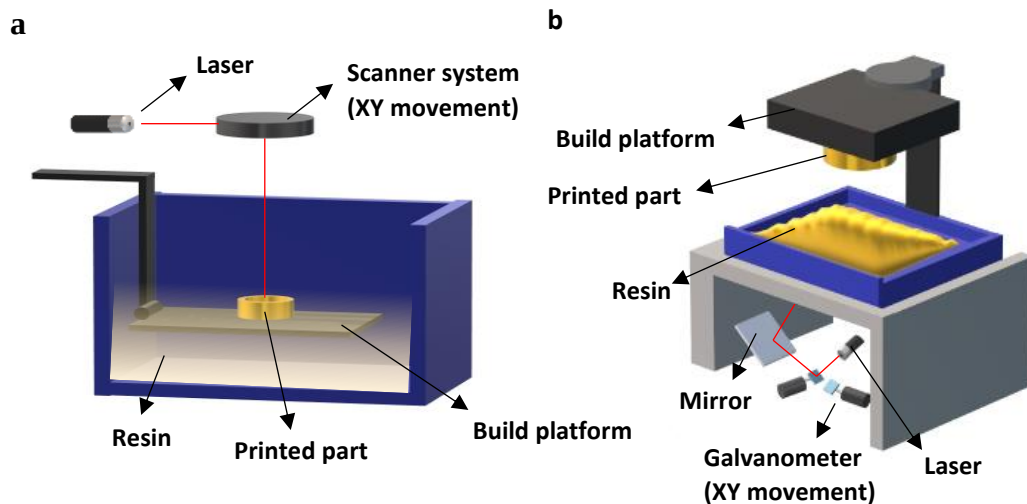


Figure 2.41: Diagram of a bottom-up (a) and a top-down (b) stereolithography system.

The resolution of the system has a significant effect on the quality and functionality of the printed part³⁶⁰. In stereolithography, as in other additive manufacturing techniques, the print quality is directly influenced by the characteristics of the layers that the object is formed of. The production of each of these layers starts with the creation of their outline upon solidification of the resin by the laser beam. Then, the area which is enclosed within this perimeter is filled with a crosshatched pattern formed of “fill vectors”^{8,361,362}. It is important to consider that, as the resin solidifies, a volumetric shrinkage occurs as a consequence of the fluctuations in temperature that take place during polymerisation, since this is an exothermic reaction. These changes in volume cause internal stresses within the printed layers that result in curl distortion and other defects such as delamination or bending, which affect the dimensional accuracy and the mechanical properties of the printed object^{362–365}. Numerous studies have found that the laser scanning pattern, also known as hatching, has a significant influence on the geometrical accuracy of the printed part, with unidirectional patterns resulting in larger errors^{365–367}. The hatch spacing, which is the distance between different printing vectors, is another parameter that has a considerable effect on the printing outcome (Figure 2.42, (a) and (b)). The creation of larger spaces results in the entrapment of resin within the printed part, which would compromise the mechanical properties and spatial accuracy of the object^{362,368}. On the other hand, distances smaller

than the optimal threshold would cause an overlap between the polymerising vectors, leading to an increase in the incidence of dimensional errors (Figure 2.42, (c))³⁶¹. Other factors that affect the geometric accuracy include the cure depth and width, the layer thickness, the power, intensity and width of the laser beam, the critical exposure of the photopolymer resin and the part orientation^{361,368–370}. The depth by which one curing vector protrudes into the adjoining layer, known as hatch over-cure, is essential for the successful adhesion of the layers that form the printed part^{361,371}. However, if the cure depth is much higher than the layer thickness, an excess in the over-curing would occur, resulting in a decrease of the resolution along the Z-axis. The cure depth is influenced by the penetration depth and the exposure of the resin, which is the energy given by the laser beam to the pre-polymer solution during the polymerisation of each layer^{372–374}. While the layer thickness has no effect on the depth of cure³⁷⁵, a reduction in the depth of each layer leads to an increase in the dimension accuracy. This is due to the higher degree of precision that can be achieved when stacking very thin layers, as this allows the reproduction of small design features with a higher accuracy.

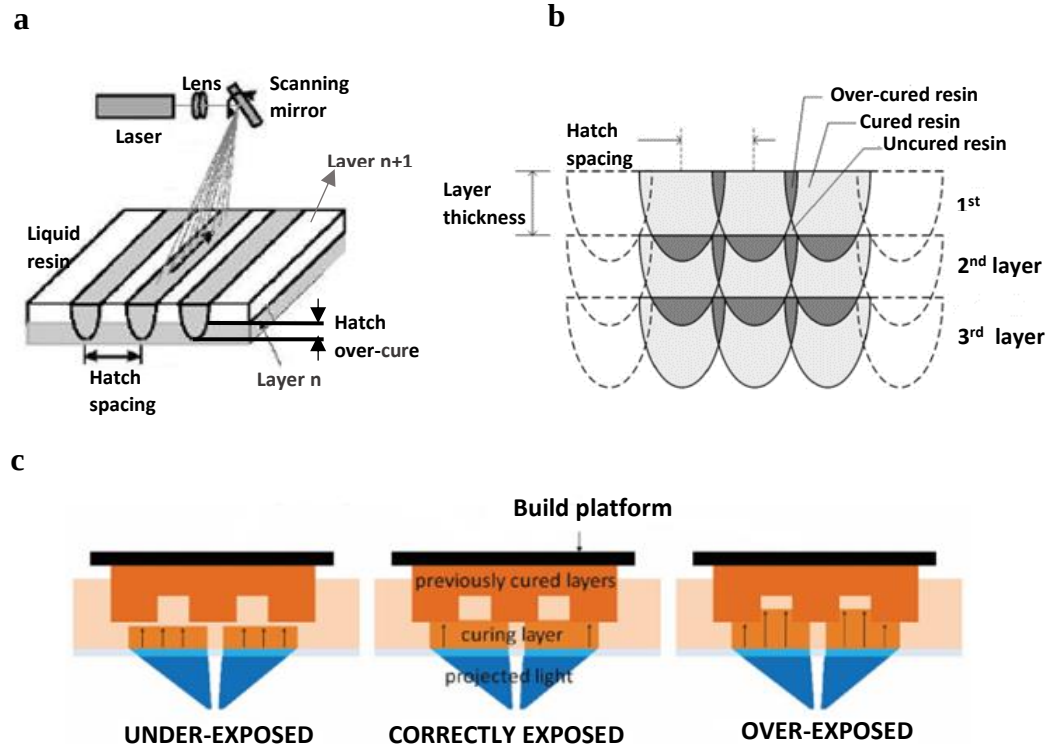


Figure 2.42: Parameters of stereolithography: **(a)** Schematic diagram of the production of two printed layers, showing the hatch spacing and hatch over-cure. Adapted from Khorasani et al., 2013³⁷⁶. **(b)** Schematic diagram of the production of three printed layers, depicting the curing pattern obtained with stereolithography. Adapted from Salmoria et al., 2009³⁷⁷. **(c)** Diagram depicting the importance of achieving the optimum cure depth in stereolithography, since it ensures the adequate attachment of each layer to the previous one. Insufficient cure depth would lead to failure of adhesion of the constitutive layers, while an excessive depth of cure would result in dimensional inaccuracies caused by the curing of resin in spaces that were designed to be empty. Adapted from van Bochove et al., 2016³⁷⁸.

While the optimisation of the parameters described above leads to significant improvements in the resolution of the system, the maximum resolution achievable in stereolithography is not sufficient for certain applications. This has encouraged the development of micro-stereolithography (micro-SLA) technologies, whose increased resolution with respect to conventional stereolithography systems is a direct consequence of the reduction in the laser spot size and the decrease in the cure depth of the resin³⁷⁹. Micro-SLA systems are based on either laser scanning or mask projection and have the ability to produce objects with design features at the microscale³⁷². Mask projection technologies cure each of the layers that comprise the printed object by projecting the whole cross-section at once. This is achieved by

making use of LCD panels or digital light processing (DLP) engines that display a pattern which shapes the incident laser beam to project the desired shape onto the photopolymerisable resin (Figure 2.43, (a))^{380,381}. The advantages of mask projection systems with respect to laser scanning technologies include the reduced fabrication times and the improved dimensional accuracy, since it avoids the introduction of errors caused by the X-Y translation mechanism³⁸¹. While the resolution achieved with these systems is significantly higher than in conventional stereolithography, nanoscale features cannot be produced with these technologies. As this would be fundamental for the creation of novel biomedical, microfluidic and electronic devices, two-photon polymerisation (2PP) has been developed in order to allow the production of objects with nanoscale dimensions³⁸². This additive manufacturing technology is based on the simultaneous absorption of two photons from the light source, as opposed to the single-photon absorption that takes place in stereolithography³⁸³. The result of this process is the polymerisation of a very narrow region of the photopolymerisable resin, hence allowing the achievement of nanoscale resolutions (Figure 2.43, (b))³⁸⁴. Furthermore, this technique has the ability to produce objects without making use of any build platform, since the construction of the printed part is not performed in a layer-by-layer basis³⁸⁵. This is a direct consequence of the mechanism of polymerisation employed by this system, which is highly confined to the focal volume³⁸⁵. Therefore, this eliminates the need for using additional elements that control the volume of resin that will be cured upon radiation with the laser beam.

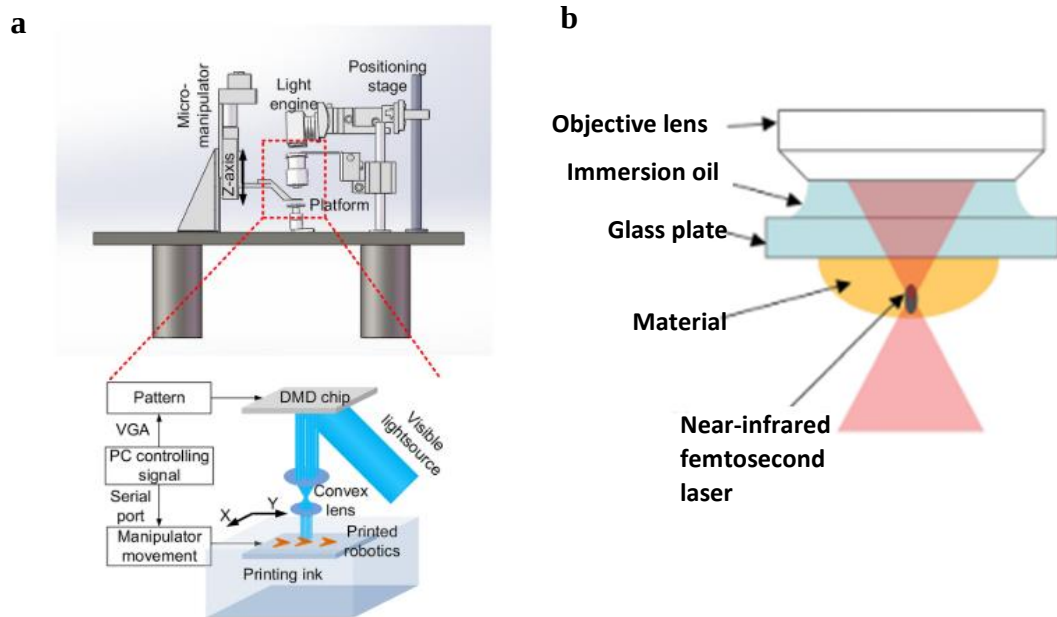


Figure 2.43: (a) Schematic diagram of a DLP-based micro-SLA system containing a digital micromirror device (DMD) which reflects the light onto the photopolymerisable resin³⁸⁶. (b) Schematic illustration of the experimental setup for 2PP. Adapted from Ferreras Paz et al., 2012³⁸⁷.

Once an object has been printed by performing a photopolymerisation-based technique, a post-curing step needs to be performed in order to eliminate any residual monomers left within the structure, since they would compromise the biocompatibility and mechanical strength of the printed part³⁸⁸. This process consists in the irradiation of the object with UV light and in some cases a heating step is applied in order to accelerate the reaction that occurs between the active chains which are entrapped within the polymer. A shrinkage in the volume of the structure is usually observed after this procedure, which might cause alterations in the dimensional accuracy and lead to the formation of voids and cracks that would compromise the mechanical performance of the printed object^{361,389,390}.

Some of the advantages of the application of photopolymerisation-based 3D-printing technologies in biomedical engineering include their high resolution and the wide variety of materials that can be processed with this technology³⁵³. However, there are some issues that still need to be addressed, such as the limitations in the availability of biocompatible resins and the lower mechanical strength of the printed parts in

comparison with other additive manufacturing techniques³⁴⁶. Furthermore, the production of certain medical devices requires the creation of structural elements at nanoscale resolutions, as this ensures the successful interaction of the material with the adhesion proteins located on the surface of the cells which adhere to these devices. As these implants cannot be produced with conventional stereolithography or micro-SLA systems, the use of 2PP technologies is required. However, the employment of this technique leads to a marked increase in the costs of production³⁸². Finally, another issue encountered when using photopolymerisation-based technologies is the detrimental effect of oxygen inhibition on the physico-chemical properties of the printed object, as this phenomenon reduces the yield of the polymerisation reaction³⁹¹. Therefore, further improvements need to be introduced in these systems in order to address this problem.

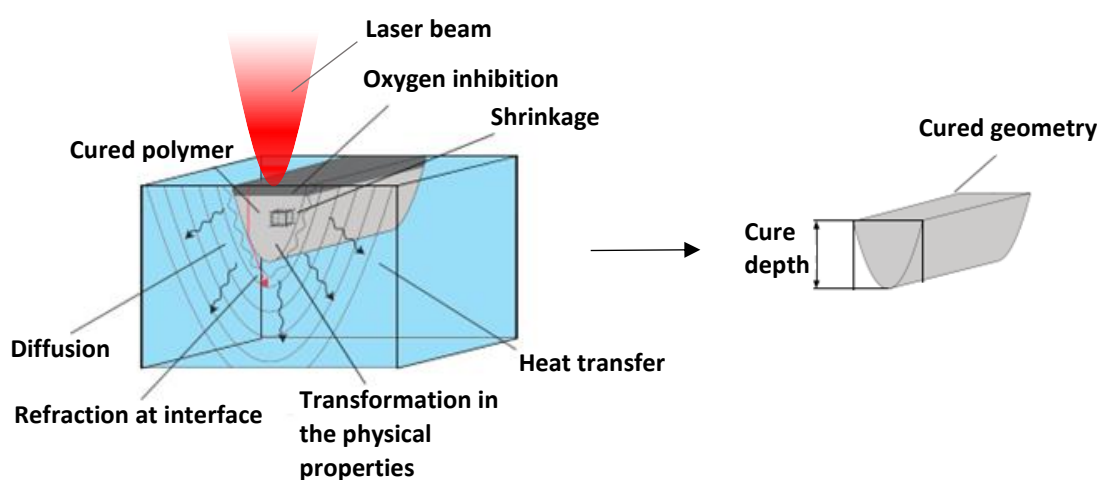
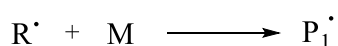
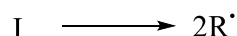


Figure 2.44: Diagram showing the physical phenomena involved in photopolymerisation. The shrinkage of the polymer upon curing and the inhibitory effects exerted by oxygen have detrimental effects on the physico-chemical properties of the printed part. Adapted from Hafkamp et al., 2018³⁹².

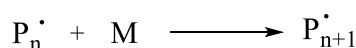
2.10 Free Radical Photopolymerisation

In stereolithography, objects are built by curing a photopolymerisable resin layer by layer. The chemical reaction that occurs during this process is free radical photopolymerisation^{358,393}, in which the building units, or monomers, combine with each other upon excitation with electromagnetic radiation to form polymer chains^{394,395}. Photopolymerisable resins are composed of a photoinitiator, a mixture of monomers and/or oligomers and other compounds such additives or fillers^{390,394,396}. The free radical photopolymerisation reaction starts with a step known as initiation, in which the photoinitiator molecules form primary radicals upon absorption of the radiation emitted by the light source³⁹⁷. These radicals react with the carbon-carbon double bonds from the monomers that are dissolved within the resin³⁹⁸. As a result, these monomers are converted into secondary radicals that will react with other monomer molecules to form active polymer chains, which contain a radical centre that can sequentially react with more monomers, thus increasing the length of the chain. This step is known as propagation, and it will continue until two active radical centres from different molecules react with each other, leading to a depletion of reactive sites during the stage known as termination (Figure 42)^{395,397–401}.

Initiation:



Propagation:



Termination:

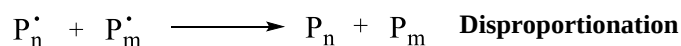
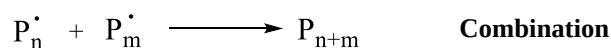


Figure 2.45: Scheme of the free radical polymerisation reaction. I represents the initiator, M is the monomer, $R^{\bullet}$ is a primary radical, $P_n^{\bullet}$ and $P_m^{\bullet}$ are growing radical chains of length n and m, respectively, and P_{n+m} , P_n and P_m represent polymers without any active centre. Adapted from Goodner et al., 1999⁴⁰².

Monomers functionalised with acrylate or methacrylate groups are the most commonly used starting materials for this reaction due to their high reactivity^{391,403}. The polymer networks that result from the polymerisation of these chemical species possess excellent optical and mechanical properties, which has also contributed to their widespread utilisation⁴⁰³. However, the polymerisation of acrylate monomers is particularly sensitive to oxygen inhibition, which causes a reduction in the reaction rate. This results in a substantial decrease in the degree of polymerisation, hence compromising the physico-chemical properties of the resulting polymer⁴⁰⁴. Alternative chemistries that are not inhibited by oxygen, such as thiol-ene or the cationic photoinitiation of epoxy or vinyl ether resins, are available for use in stereolithography. The utilisation of these materials in open-air systems might provide better results than acrylate species^{391,403,405}. Thiol-ene photopolymerisation starts with the excitation of the photoinitiator and the subsequent hydrogen abstraction from a thiol-containing monomer to form a thiyl radical. Then, the thiyl radical reacts with the alkene group from a monomer, leading to the formation of a growing carbon-centred radical. Thiyl radicals are regenerated during this process via chain transfer from the carbon-centred radical to a thiol group. The sequential repetition of these steps results in the formation of a polymeric structure^{405–408}. Thiols reduce the inhibitory effects of oxygen by donating a hydrogen atom to the peroxy radical formed in the process, which results in the formation of a reactive thiyl radical (Figure 2.46)³⁶⁰. Cationic photopolymerisation is based on the generation of acids upon photoexcitation of salts and the subsequent initiation of a polymerisation reaction by these strong acids. As opposed to mechanisms based on the formation of free radicals, this method does not require the continuous illumination of the resin to form the polymer^{409,410}.

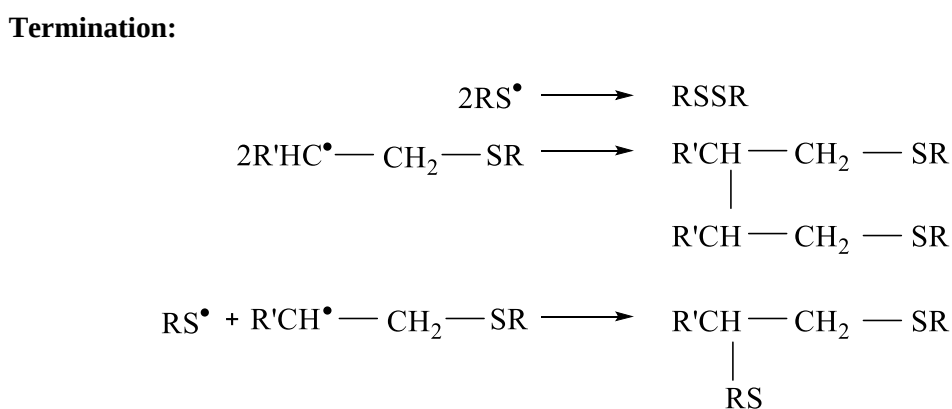
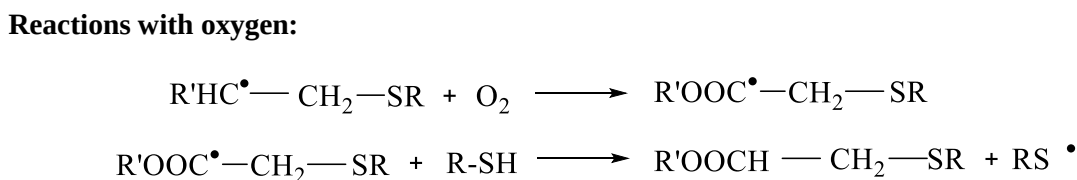
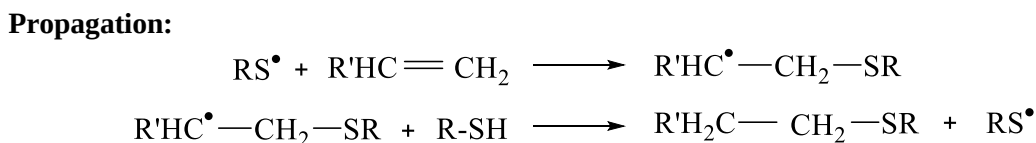
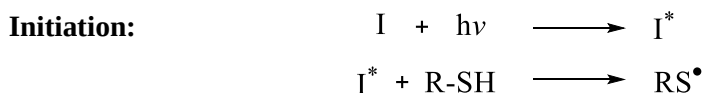


Figure 2.46: Scheme of the thiol-ene reaction and the effects of oxygen on this process. Adapted from Cramer et al., 2001⁴⁰⁷ and Ligon et al., 2017³⁵⁰.

The processes involved in the free radical polymerisation reaction are explained in detail as follows:

2.10.1 Initiation

The free radical photopolymerisation reaction starts when the photoinitiator absorbs the radiation emitted by the light source and is subsequently promoted to an excited state. As a result of this process, primary radicals are generated, which will in turn react with the carbon-carbon π -bonds from the monomer molecules to create secondary radicals. These radicals will react with other monomers dissolved within the polymerisable resin in order to form polymer chains that will continue growing upon addition of more monomers to their active radical centres^{395,397,398,411,412}.

The difference between photopolymerisation and other free radical polymerisation reactions lies in the mechanism by which the primary radicals are formed. In addition to light, heat can also serve as a stimulus to form radicals from an initiator. In comparison with thermal polymerisation, photo-initiated reactions offer several advantages, such as the low energy involved in the process, the fast polymerisation rate and the possibility to utilise solvent-free resins³⁹¹.

Photoinitiators can be classified into two different categories, Norrish Type I and Type II, depending on the mechanism by which they generate radicals (Figure 2.47)³⁵⁰. Type I initiators create primary radicals via α - or β -cleavage after excitation to a triplet state upon the absorption of a photon from the laser beam that irradiates the resin⁴¹³. These initiators usually show the highest efficiencies. The most representative examples of type I photoinitiators include benzoin derivatives, benzyl ketals, hydroxyalkylphenones and derivatives of acetophenone, being the benzoyl radical the major initiating species in all of them^{401,411}. Another class of Type I photoinitiators are acyl phosphine oxides, such as TPO and BAPO. These molecules contain a phosphorous atom adjacent to the carbonyl group, which lowers the energy level of the π^* state, hence shifting the maximum of the $n \rightarrow \pi^*$ transition towards 400 nm. Thus, these initiators can be used in stereolithography systems which operate with visible light³⁶⁰.

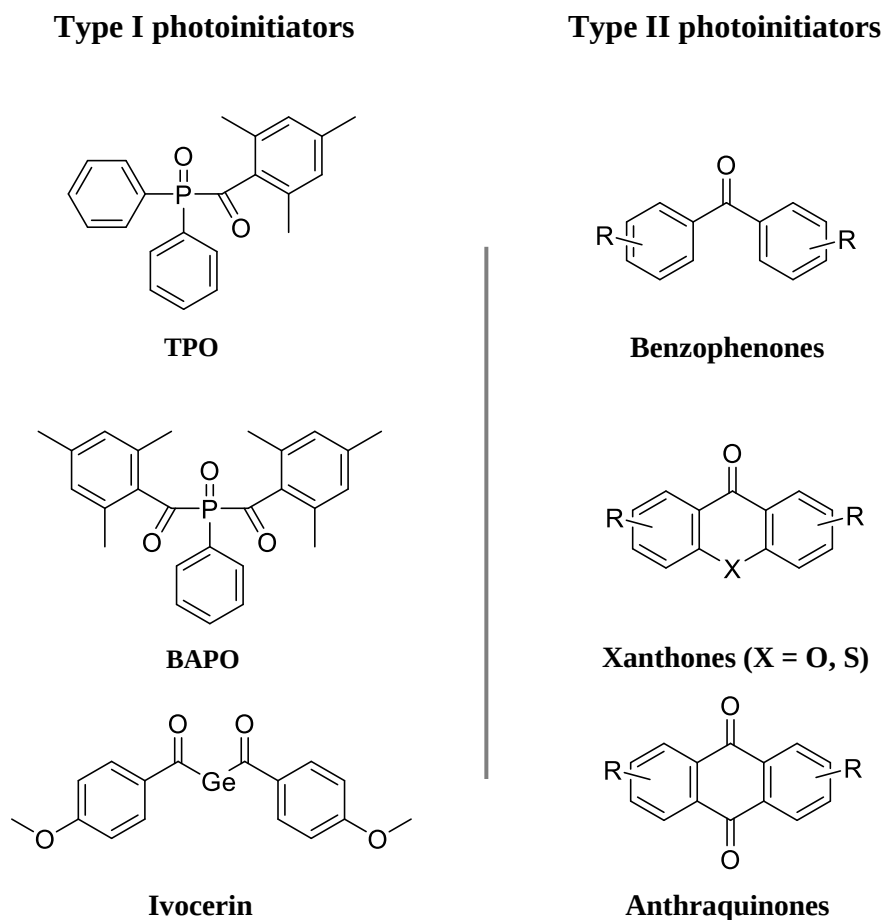
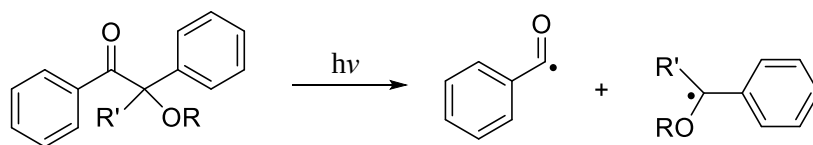


Figure 2.47: Type I and II photoinitiators commonly used in free radical photopolymerisation. Adapted from Ligon et al., 2017³⁵⁰ and Allen, 1996⁴¹⁴.

Type II photoinitiators are two-component systems formed of a photosensitiser and a co-initiator. When the system is irradiated with light, the co-initiator donates a hydrogen atom to the excited sensitiser, thus generating a free radical that initiates the polymerisation reaction^{350,394,413}. The most commonly used co-initiators include amines, ethers and alcohols⁴¹¹. The main advantage of Type II initiators with respect to those classified as Type I is the lower amount of energy that they require for the formation of primary radicals, since the cleavage of a bond needs high-energy light sources. Thus, the use of Type II initiators enables the utilisation of longer wavelengths of light for the production of polymers, which is advantageous in biomedical applications⁴¹³.

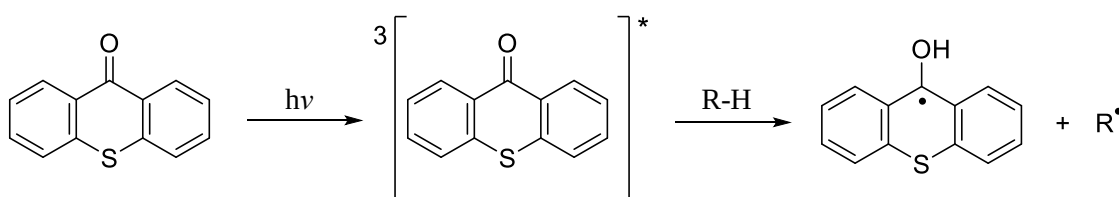
Type I photoinitiation



R: H, alkyl, substituted alkyl

R': H, alkyl, substituted alkyl

Type II photoinitiation



R-H (H-donor): amines, alcohols, ethers, etc.

Figure 2.48: Schematic of the mechanism of generation of free radicals with Type I and Type II photoinitiators. Adapted from Dadashi-Silab et al., 2015⁴¹³.

The initiation rate is determined by the efficiency of the photoinitiator and the intensity of the light absorbed by this molecule. The efficiency can be defined as the fraction of primary radicals that initiate the growth of a polymer chain⁴¹⁵. This parameter is influenced by the number of radicals produced per photon of light absorbed, or quantum yield, the reactivity of these radicals and the molar absorptivity of the initiator^{394,416,417}. Moreover, for a photoinitiator to be effective in a specific system, its absorption spectrum should match the wavelengths of emission of the light source⁴¹⁸. The selection of an appropriate photoinitiating system is of paramount importance in photopolymerisation, since its efficiency has a direct effect on the cure depth and therefore on the physico-chemical properties of the polymer^{395,398,419,420}.

2.10.2 Propagation

The chains that form the polymer are generated during this step through the sequential reaction between the unpaired electron from a secondary radical and the carbon-carbon double bond from a monomer unit. The resulting kinetic chains have an active radical centre at one of their ends, which is where the monomers will bind to these growing macromolecules^{398–400,411,412}. The rate of the propagation reaction is expressed as:

$$R_p = k_p [M] [M_n\bullet] \quad (4)$$

where k_p is the propagation rate constant, $[M]$ is the concentration of double bonds from monomers and $[M_n\bullet]$ is the concentration of propagating radicals³⁹⁸.

As well as the growth of the kinetic chains through the addition of monomers to their active centres, other reactions can take place during the propagation phase which may affect the polymerisation rate. For example, chain transfer occurs when a compound that contains a labile atom, usually a hydrogen or halogen, is present in the polymerisation medium. A macroradical can abstract an atom from this molecule to yield a terminated polymer chain and a new radical, which reacts with the monomer to form a new propagating macromolecule in a step known as re-initiation. As a result, the active centre is transferred between different chains and the length of these molecules decreases substantially^{398,421–423}. Another reaction that can affect the propagation rate and the structure of the polymer is the addition of a radical centre to a pendant double bond in systems that contain multifunctional monomers. Polymer chains can undergo cyclisation if this reaction occurs between a radical and a double bond from the same macromolecule. This leads to the formation of microgels, which cause a decrease in both the homogeneity of the polymer structure and the degree of crosslinking. As a result, the mechanical strength of the polymer can be considerably compromised^{411,424}.

The presence of oxygen in the polymerisation medium is another factor that has a detrimental effect on the photopolymerisation reaction. Oxygen can quench the excited triplet state of the photoinitiator, leading to a reduction in the quantum yield. In addition, this element reacts with primary and secondary radicals to form peroxy radicals, which do not undergo addition to double bonds and tend to terminate the polymerisation reaction through recombination. Peroxy radicals show a high tendency to abstract hydrogen from other molecules, resulting in the formation of hydroperoxides and new radicals (Figure 2.49). These radicals have a low reactivity towards acrylate functionalities and are thus incapable of reinitiating the polymerisation reaction^{391,405,411,425}. A partial or total inhibition of the polymerisation reaction results from the occurrence of these processes. Therefore, it is essential to adopt strategies to prevent oxygen inhibition when carrying out a free radical polymerisation reaction. These are based on the regulation of the parameters that affect the extent of the inhibitory effects exerted by oxygen, which include the efficiency of the photoinitiator, the viscosity of the resin, the thickness of the polymer and the intensity of the light⁴²⁵. Other methods that are commonly employed to achieve this objective include the elimination of oxygen from the polymerisation medium by purging it with inert gases and the addition of hydrogen donors, such as thiols and amines, which reduce the unfavourable effects of oxygen by forming active radicals via chain transfer reactions^{391,405}.

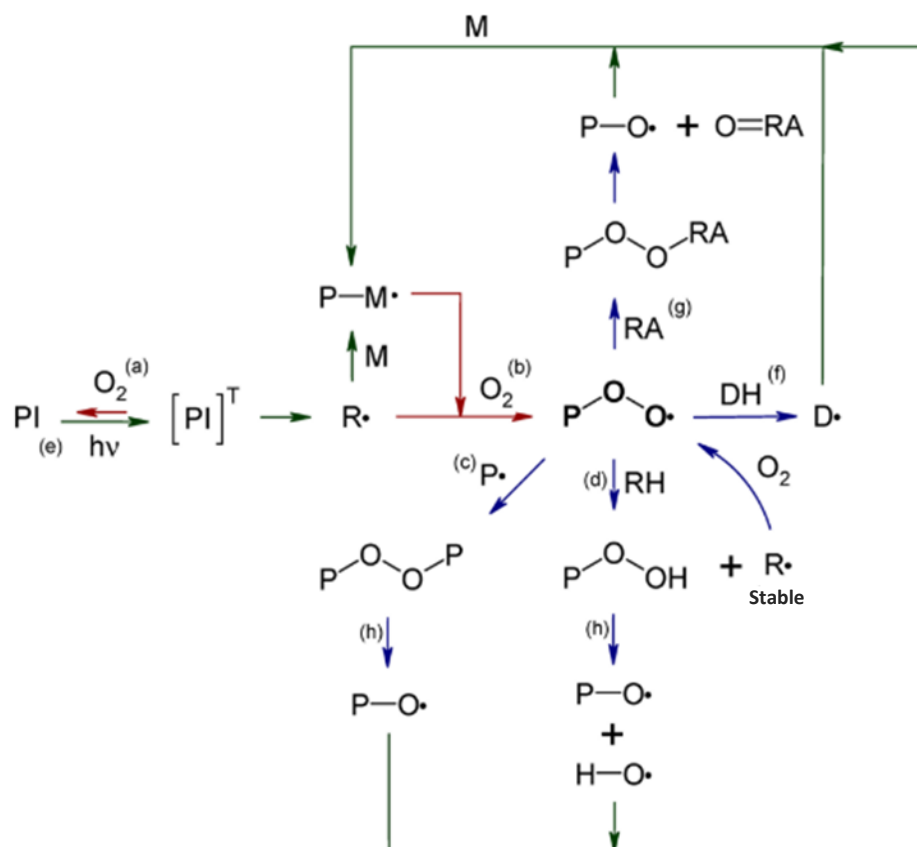


Figure 2.49: Schematic diagram of the mechanistic pathways for the inhibition of polymerisation by oxygen and the strategies that can be adopted to prevent this phenomenon. The green arrows represent the processes involved in the polymerisation reaction. The red arrows highlight the mechanisms of oxygen inhibition: **(a)** quenching of the excited state of the photoinitiator (PI), **(b)** formation of inactive peroxy radicals from initiating or propagating radicals. The blue arrows indicate the mitigation strategies: **(c)** termination via radical-radical recombination; **(d)** termination via hydrogen abstraction; **(e)** initiation stage strategies (inerting, light source, photoinitiators, singlet oxygen scavengers); **(f)** hydrogen donors; **(g)** reducing agents (RA) and **(h)** peroxide decomposition³⁹¹. “P” denotes the polymer chain, while “M” denotes the monomer.

The kinetics of photopolymerisation are not only governed by chemical reactions. Physical processes such as the diffusion of the molecules that contain active radical centres have a considerable effect on the kinetic coefficients of this reaction^{400,411}. As the polymerisation reaction progresses, the formation of increasingly larger molecules leads to a dramatic increase in the viscosity of the medium, which restricts the mobility of the macroradicals. At conversions within the range of 30-50% (intermediate levels of conversion), the reduced mobility of the polymer chains starts to hinder the combination of macroradicals that results in the termination of the

reaction. Thus, the radical centres stay active for longer times. This results in a phenomenon known as autoacceleration, or gel effect, which can be defined as a temporary increase in the propagation rate at relatively high conversions despite monomer depletion^{398,411,412,426,427}. As the reaction continues and a higher degree of polymerisation is attained, the viscosity of the medium increases to such an extent that even the reactions that take place between shorter macroradicals become hindered by diffusion. This causes a decrease in the propagation rate that results in the onset of the autodeceleration stage and eventually in the termination of the reaction even in the presence of active radicals and monomers³⁹⁸. The processes herein described have been observed in both linear and crosslinked systems, although the gel effect occurs in earlier stages of the reaction when multifunctional monomers are present in the photopolymerisable resin^{411,426}.

2.10.3 Termination

The propagation of the kinetic chains continues until the active radical centres are depleted during a process known as termination. This can occur via bimolecular combination of two radicals from two different active molecules or through atomic transfer between active chains in a process known as disproportionation. An additional mechanism that can result in the formation of a dormant polymer molecule is chain transfer to a monomer unit, a polymer chain, the solvent, the initiator or to an additive^{399,411,428}.

For a termination reaction to occur, the radical centres from the molecules involved in the process must be in close proximity with each other. Since this is dependent on the mobility of these chemical species, termination can be considered a diffusion-controlled process^{400,429}. While the propagation stage is also affected by diffusion during some stages of the reaction, these effects are more prominent in the termination step³⁹⁸. Translational diffusion, which is the movement of the centre of mass of a molecule, is the predominant mechanism that enables termination in small radicals and some polymer chains. When the size of the macroradicals increases, translation

becomes more difficult and other diffusive processes such as the segmental reorientation of the chains or reaction diffusion via propagation of the active centre become the main routes towards termination^{411,429}.

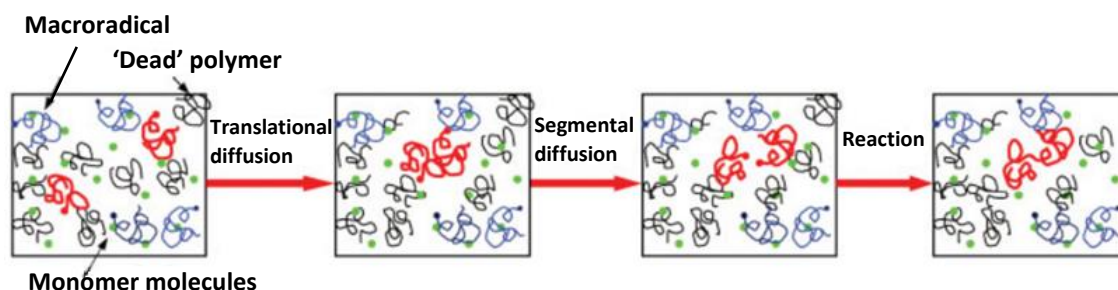


Figure 2.50: Diffusion and reaction steps during termination between two macroradicals (red)⁴⁰⁰.

2.11 Controlled Radical Polymerisation

The main limitation of free radical polymerisation is that it is impossible to control the molecular weight, polydispersity, composition and chain architecture of the resulting polymers. The solution to this issue is the use of controlled radical polymerisation techniques. These methods allow the production of polymers with specific structures and compositions, hence resulting in a higher degree of control over the functionality of these constructs^{399,430}.

Some of the controlled radical polymerisation techniques that have been developed to overcome the issues described above include atom transfer radical polymerisation (ATRP) and reversible addition-fragmentation chain transfer polymerisation (RAFT). The rationale of these methods is based on the extension of the lifetime of the propagating chains by establishing a dynamic equilibrium between a low concentration of active chains and a higher amount of dormant molecules that cannot undergo termination⁴³⁰. However, these dormant chains can be briefly activated to form a propagating radical before returning to their inactive state⁴³¹. As a result, the end of the polymerisation reaction is determined by the depletion of monomers in the system, as opposed to being controlled by termination. Furthermore, all chains grow

at nearly the same propagation rate, resulting in a highly predictable molecular weight and a narrow molecular weight distribution⁴³¹.

ATRP is based on the reversible transfer of halogen atoms between dormant chains and a transition metal catalyst via redox chemistry³⁹⁹. The initiator is an alkyl halide or pseudohalide which interacts with the catalyst, a low-oxidation state transition metal complex, to form a propagating radical. This radical is rapidly deactivated by a higher-oxidation state transition metal complex, resulting in the creation of a dormant chain. The most commonly used catalysts are based on copper. The main disadvantage of this technique is the relatively high amount of residual metal present in the product⁴³¹.

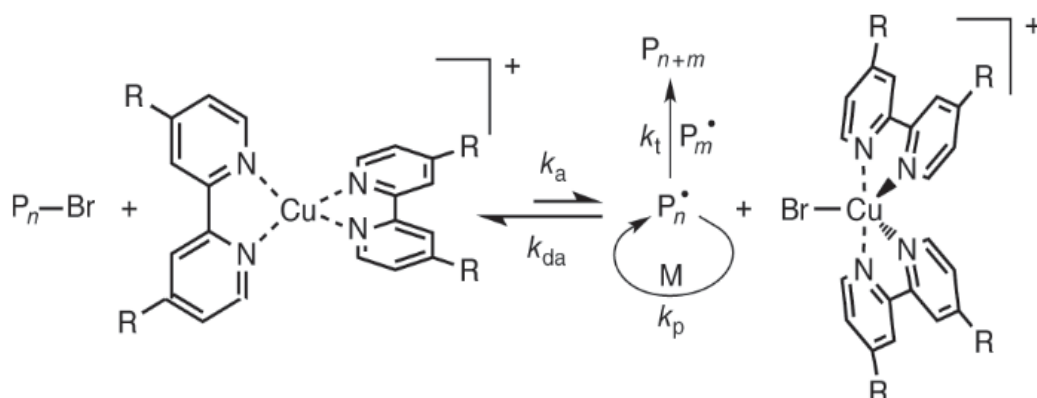


Figure 2.51: Equilibrium between active and dormant species in ATRP catalysed by Cu/2,2'-bipyridine complex⁴³¹.

Another technique that allows the achievement of a controlled radical polymerisation is RAFT polymerisation. This method is based on the use of reagents that react with the propagating macroradicals via reversible chain transfer in order to achieve an equilibrium between active and dormant chains⁴³². Thiocarbonylthio compounds are the most commonly used RAFT agents. During this process, the activated radical species undergo a sequential addition to the C=S bond of the RAFT agent and the growing macroradicals are reversibly deactivated by a degenerate chain transfer reaction. The term degenerate implies that the overall number of radicals is kept

constant during the process. This reaction results in an equilibrium between active and dormant species and a similar rate of growth in all polymer chains^{432–434}.

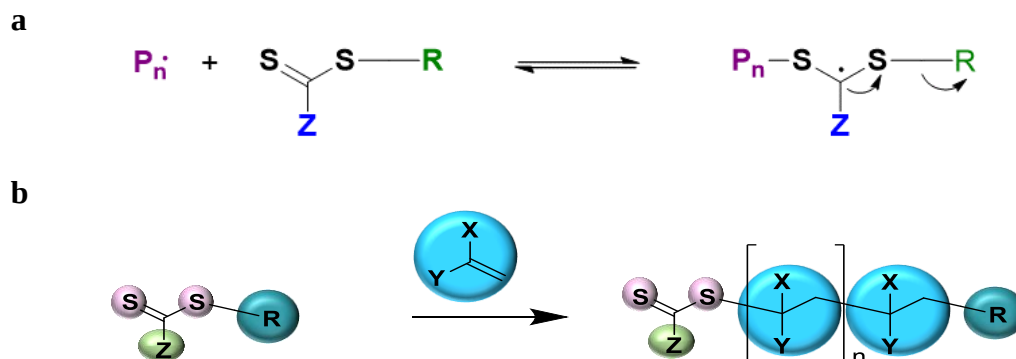
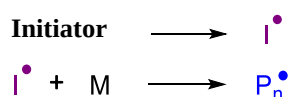


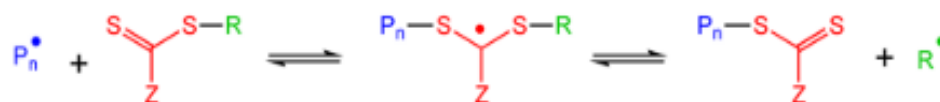
Figure 2.52: (a) Mechanism of action of RAFT agents: the reactive S=C bond in the RAFT agent reacts with the growing polymer chain $P_n\cdot$ to form an intermediate from which the leaving group (R) can reinitiate polymerisation. The curly arrows depict the movement of the radical centre towards the leaving group R. **(b)** Schematic diagram of the RAFT process, where the monomer reacts with the RAFT agent to form the polymer chain. Adapted from Moad et al., 2013⁴³⁵.

The success of the RAFT polymerisation reaction depends on the selection of the adequate RAFT agent for the monomers present in the system and the appropriate reaction conditions⁴³³. The choice of the suitable RAFT agent is based on the selection of a Z group that confers the thiocarbonyl double bond with the adequate reactivity and an R group that is simultaneously an effective initiating radical and a good leaving group⁴³⁵. Another requirement for a successful control over the molecular weight of a polymer synthesised by this mechanism is that the rate of addition/fragmentation must be higher than that of propagation. Therefore, all chains would have a similar degree of polymerisation at any time, which would result in a narrow molecular weight distribution^{432,436}.

Initiation



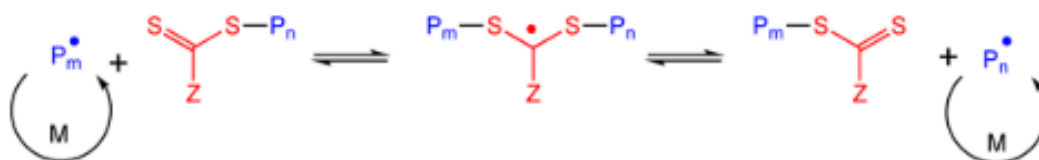
Reversible chain transfer



Reinitiation



Main equilibria



Termination



Combination



Disproportionation

Figure 2.53: Mechanism of the RAFT polymerisation reaction. Following initiation, the radicals add to the RAFT agent and an equilibrium is established between active and dormant species. The chain transfer steps involve a reversible transfer of the chain end-group ($\text{Z}-\text{C}(=\text{S})\text{S}-\text{R}$) between the dormant chains and the propagating radicals. The rate of addition/fragmentation should be higher than that of the propagation reaction in order for all chains to have a similar degree of polymerisation. Adapted from Perrier, 2017⁴³².

2.12 Reactivity of Radicals

The production of polymers with specific properties requires a thorough understanding of the relationship between the structure of the constituent monomers and the reactivity of the radicals that are formed during the initiation step^{437,438}. This facilitates the selection of the optimal reaction conditions that lead to the highest monomer conversion possible. If the reaction does not proceed to completion, unreacted molecules would remain within the polymer structure that results from this process. This would compromise the physico-chemical properties of the polymer due to the incomplete polymerisation of the macroradicals and monomer units of which it is formed. Therefore, it is essential to control both the polymer composition and the rate of polymerisation in order for the product to possess the properties required for its intended application^{437,439}.

Polymers which are to be employed as tissue replacements must be biocompatible and should possess an optimum mechanical strength, which will vary depending on the tissue where they will be implanted^{440,441}. The fulfilment of these requirements depends on the efficiency of the photopolymerisation reaction, since a homogeneous composition and a high degree of conversion are essential for the material to possess the appropriate mechanical properties and biocompatibility^{442–445}. The presence of unreacted monomers has been shown to cause cytotoxic responses and allergic or even mutagenic reactions, so the implantation of a device that does not fulfil the aforementioned conditions would pose a serious health risk^{439,446}. Thus, it is fundamental to optimise the photopolymerisation system in order to maximise the reaction yield. As mentioned previously, the reactivity of the radicals involved in the polymerisation reaction has a significant effect on the kinetics of the process⁴⁴⁷. Therefore, it is vital to consider the various factors that can affect the activity of these molecules before establishing the conditions in which the reaction will be performed.

Free radicals are molecules that have an unpaired electron in a *p* orbital (Figure 2.54, (a)). The reactivity of these chemical species is inversely proportional to the stability of this electron. Any alkyl group attached to the carbon atom that bears the radical centre leads to the stabilisation of the unpaired electron as a consequence of the

donation of electron density to this unstable entity. Tertiary radicals, i.e., those which have three alkyl substituents bound to the carbon atom that contains the radical centre, are therefore less reactive than secondary radicals, which are in turn less reactive than primary radicals (Figure 2.54, (b))⁴⁴⁸.

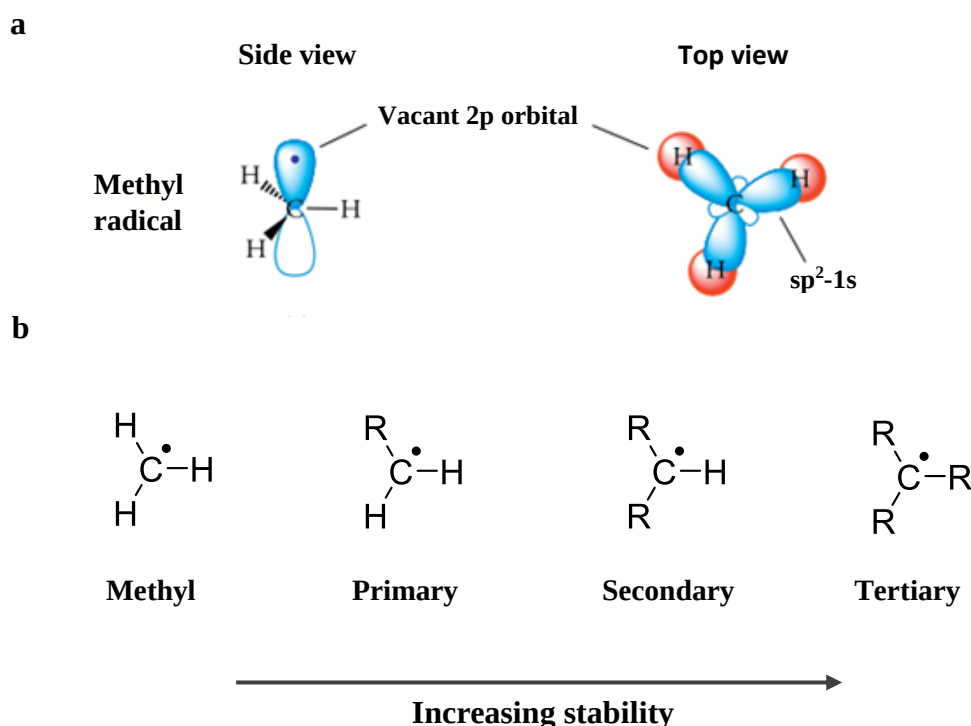


Figure 2.54: (a) Molecular orbital diagram of the methyl radical. (b) Diagram showing the trend of increasing radical stability with increasing degree of substitution. Adapted from Ouellette et al., 2018⁴⁴⁹.

The chemical structure of the substituents is another factor that influences the reactivity of the active centre. The presence of aromatic or alkene groups in the proximity of the radical results in the occurrence of resonance effects, which have a stabilising effect that leads to a decrease in the reactivity of the free radical^{450–452}. Furthermore, the chemical composition of these substituents can also cause variations in the reactivity. For example, the presence of electronegative groups in the proximity of the radical can result in an increase in the reactivity due to the electron-withdrawing effects of these functionalities^{453,454}.

The formation of hydrogen bonds between different polymer chains has also been reported to affect the polymerisation rate. The reason for this lies in the fact that hydrogen bonding leads to a higher order in the arrangement of the radical molecules, which increases the efficiency of radical propagation through the aligned double bonds. In addition, these intermolecular interactions increase the viscosity of the resin, which results in a decrease in the termination rate as a consequence of a reduction in the diffusivity of the propagating radicals⁴⁵⁴.

An additional factor that contributes to the increase in the reactivity of a radical is the number of polymerisable functionalities that the monomer contains. The reaction of radicals which have higher numbers of these functional groups leads to a more rapid increase in the viscosity of the media, which in turn results in an earlier onset of the autoacceleration effect^{455,456}. The steric effects exerted by the substituents attached to the radical centre is another factor that should be taken into consideration when predicting the reactivity of the radicals present in the system, since they reduce the accessibility of the reactive sites^{454,457,458}. The differences in reactivity between acrylate and methacrylate polymers can be attributed to this phenomenon, as the extra methyl group in methacrylate monomers causes a higher steric hindrance. Moreover, the difference in the number of alkyl substituents between acrylates and methacrylates is another factor that contributes to these disparities⁸⁵.

2.13 Influence of the Polymer Structure on its Physico-Chemical Properties

It is of great importance that the polymers employed in the manufacture of medical devices possess the mechanical properties that are required for their purpose^{440,441,459}. The characteristics of a polymer are determined by the chemical composition, chain conformation and the spatial arrangement of the molecules that it is composed of^{442,460}. Thus, the mechanical strength of a polymer can be adjusted by tuning its composition and crosslinking density. This opens up the possibility of engineering polymers that

mimic the physico-chemical properties of the natural tissues in order to elicit a variety of biological responses^{85,442,443}.

The physical properties of amorphous and semi-crystalline polymers are strongly influenced by their glass transition temperature, or T_g , which is the average of the range of temperatures at which an amorphous polymeric structure undergoes a gradual transformation from a glass-like, rigid state to a rubbery form, or vice versa^{85,461,462}. Below the T_g , the polymer chains have a lower mobility, limited to low-amplitude atomic vibrations and the reorientation of some groups of atoms. This causes the characteristic stiffness and brittleness observed in polymers that are subjected to these conditions⁴⁶³. As the temperature increases, the consequent rise in the energy of the system causes an increase in the amplitude of the atomic vibrations. This results in a reduction of the strength of the intermolecular bonding forces, hence leading to an increase in the mobility of the polymer chains. When the T_g is reached, the polymer chains absorb the amount of energy that is required for the disruption of these intermolecular forces and they start experiencing greater rotational and translational motions^{85,464}. Therefore, the T_g can be considered as the starting point of a process in which the molecules gain enough freedom of movement to cause a transition from a rigid polymer to a ductile material. In semi-crystalline polymers, this process occurs only in the amorphous regions of the structure, while the crystalline domains remain unchanged and contribute to the reinforcement of the system. However, if the temperature continues to rise and the melting temperature of the crystalline regions is reached, the polymer structure will change dramatically as a consequence of the degradation of the crystalline lattice⁸⁵.

Given that the changes that occur in a polymer during the glass transition are directly related with the mobility of its constituent molecules, any structural features or external conditions that alter the freedom of movement of these chains will cause variations in the T_g . A detailed description of the effects that these factors exert on the T_g , and therefore on the mechanical properties of a polymer, is provided below.

2.13.1 Molecular weight of the polymer chains

An increase in the molecular weight of a polymer is often accompanied by a rise in the T_g of the material. This is due to the increase in the number of chain ends as the molecular weight decreases. As these segments are more mobile than the internal sections of the macromolecule, an increase in their proportion leads to a reduction in the rigidity of the polymer structure⁸⁵.

Synthesising a polymer with a predetermined molecular weight poses a challenge in free radical polymerisation due to the high reactivity of the molecules involved in this reaction⁴⁶⁵. The addition of a chain transfer agent is an effective method to control the structural and mechanical properties of a polymeric material. As previously explained, these chemical species have labile atoms that are readily abstracted by the growing macroradicals. This results in the formation of a terminated polymer chain and a new radical, which can reinitiate the polymerisation reaction (Figure 2.55). Thiols and phenols are among the most widely employed chain transfer agents, since they possess easily abstractable hydrogen atoms in the S-H and O-H bonds, respectively. The addition of a low concentration of these compounds leads to a significant reduction in the molecular weight of the polymer chains^{421,423,466}. This principle has led to the development of more sophisticated reactions, such as RAFT polymerisation, which allow for a higher degree of control over the molecular weight and mechanical properties of a polymer⁴³².

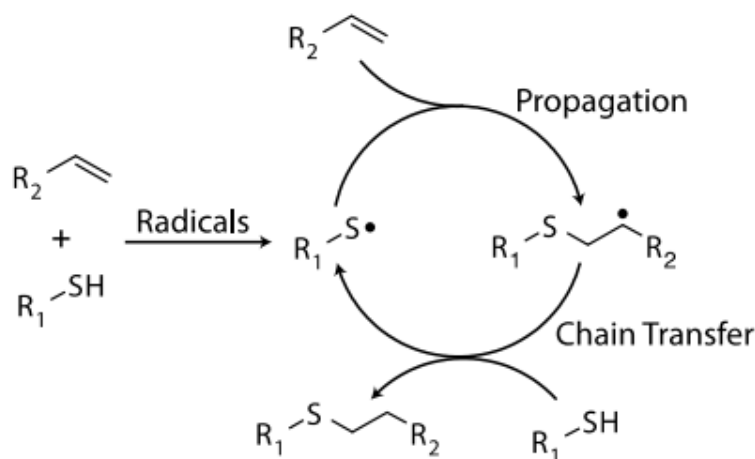
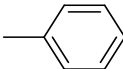
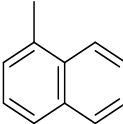


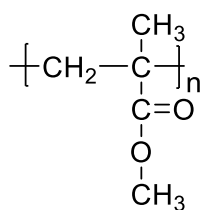
Figure 2.55: Schematic diagram depicting the polymerisation reaction between alkene and thiol groups. The thiol groups can act as chain transfer agents that reduce the molecular weight of the resulting polymer chains⁴⁶⁷.

2.13.2 Side chains

The stiffness of a polymer increases with the presence of bulky and rigid side chains attached to the backbone. These chains impose a restriction on the molecular translation and rotation of the macromolecules that form the material, thus leading to an increase in the T_g (Table 2.6)^{85,468}. The introduction of smaller side groups on the chains, such as methyl substituents, has a similar effect on the rigidity of the polymer. It is for this reason that methacrylate polymers show a higher mechanical strength than their acrylate counterparts. As the length and flexibility of the side chains increases, a slight decrease in the T_g is observed due to the disruption of the van der Waals interactions established between the main polymer chains (Figure 2.56)^{85,469}. Moreover, the chemical composition of the substituents can also affect the T_g , since some functional groups can lead to the creation of dipole-dipole interactions that reduce the mobility of the molecules that form the polymer^{468,470}.

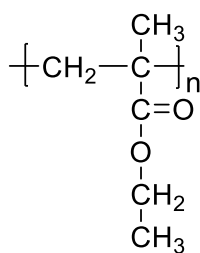
Table 2.6: Effect of the steric effects exerted by bulky side groups on the T_g of a polymer.

Polymer $\text{+CH}_2\text{-CHR+}$	R	T_g
Poly(ethylene)	-H	$-93\text{ }^\circ\text{C}$
Poly(propylene)	-CH_3	$-20\text{ }^\circ\text{C}$
Poly(styrene)		$100\text{ }^\circ\text{C}$
Poly(α -vinyl naphthalene)		$135\text{ }^\circ\text{C}$



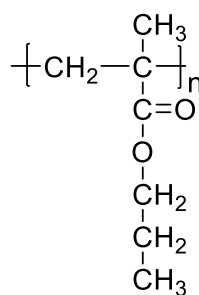
PMMA

$T_g = 105\text{ }^\circ\text{C}$



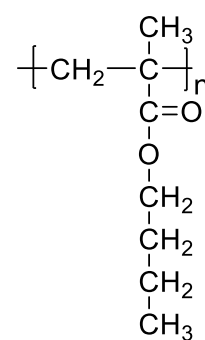
PEMA

$T_g = 65\text{ }^\circ\text{C}$



PPMA

$T_g = 43\text{ }^\circ\text{C}$



PBMA

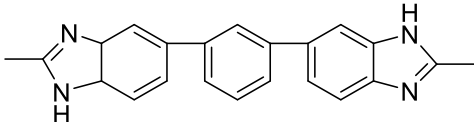
$T_g = 20\text{ }^\circ\text{C}$

Figure 2.56: Effect of the increase in the length of the side chains on the T_g of the polymer.

2.13.3 Characteristics of the polymer backbone

The chemical structure of the main chain is the factor that exerts the greatest influence on the flexibility, and thus on the T_g , of the polymer structure. Polymer chains are formed of a sequence of carbon-carbon sigma bonds that can easily rotate, leading to an increase in the flexibility of the chains and a subsequent decrease in the T_g ^{85,471,472}. In contrast, the presence of unsaturated double bonds, aromatic functionalities and cyclic structures in the main chain increases the rigidity of the polymer, hence causing a rise in the T_g (Table 2.7)^{85,473,474}.

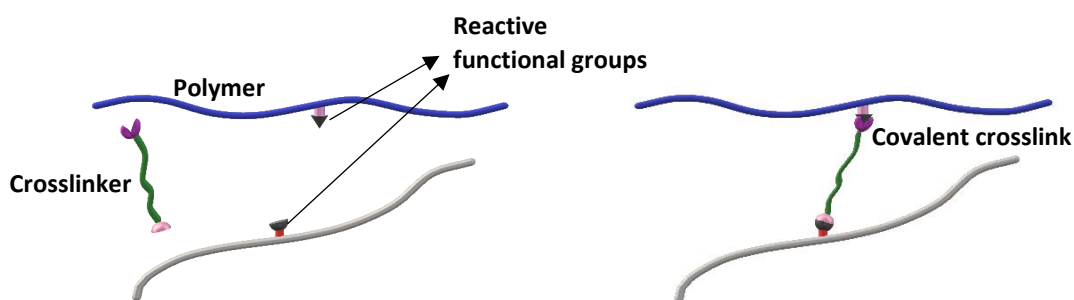
Table 2.7: Effect of the structure of the polymer backbone on the T_g of the material.

Polymer	Repeat unit	T_g
Aliphatic polyester	$-\text{C}(=\text{O})-(\text{CH}_2)_4-\text{C}(=\text{O})-(\text{CH}_2)_2-\text{O}-$	-63 °C
PET	$-\text{C}(=\text{O})-\text{C}_6\text{H}_4-\text{C}(=\text{O})-\text{O}-(\text{CH}_2)_2-\text{O}-$	80 °C
Poly(benzimidazole)		429 °C

2.13.4 Crosslinking

A crosslink can be defined as a connection between different polymer chains through chemical bonds or physical interactions (Figure 2.57)⁴⁷⁵. The creation of crosslinks imposes a restriction on the mobility of the polymer chains which leads to an increase in the mechanical strength of the material^{185,476}. This effect becomes more pronounced with higher degree of crosslinking, although this is also accompanied by a decrease in the strain capacity of the material^{470,477,478}.

Covalent crosslinking:



Physical crosslinking:

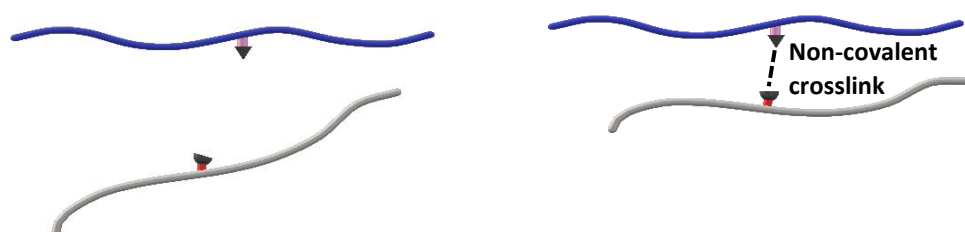


Figure 2.57: Schematic representation of the formation of chemical and physical crosslinks between polymer chains.

2.13.5 Intermolecular forces

The establishment of intermolecular interactions between polymer chains occurs via secondary attractive forces such as hydrophobic interactions, π - π stacking, dipolar connectivity, van der Waals forces and hydrogen bonds. While these forces are present in all molecular systems, their effects become more prominent in polymers that are not highly crosslinked, since the properties of these systems will be primarily determined by these bonds. A representative example of the effects that these interactions have on the mechanical properties of the material is the synthetic polymer known as Kevlar®. This fibrous material is a type of aromatic polyamide formed of rigid polymer chains that lock together via hydrogen bonds and aromatic interactions (Figure 2.58)⁴⁷⁹. These forces reduce the mobility of the polymer chains and cause a substantial increase in the T_g of the material^{460,470}.

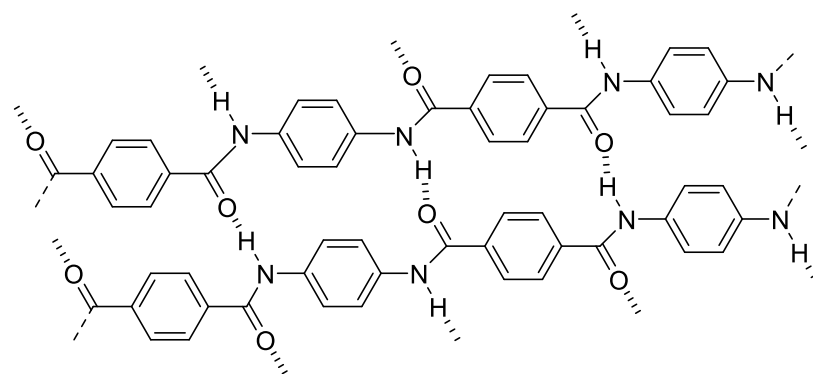


Figure 2.58: Chemical structure of Kevlar®. Adapted from Kabir et al., 2013⁴⁷⁹.

2.13.6 Polarity of the polymer chains

The presence of polar functional groups in the molecules that form a polymer results in an increase in the T_g . This is due to the increase in the strength of the intermolecular bonds as a consequence of the establishment of polar interactions. As discussed previously, a direct consequence of this phenomenon is the reduction in the mobility of the polymer chains, causing an increase in the stiffness of the material⁸⁵.



CHAPTER 3

Development of 3D-Printable Hydrophilic Formulations



3. Development of 3D-Printable Hydrophilic Formulations

3.1 Introduction

Since their invention by Sir Harold Ridley in 1949, IOLs have been manufactured with a wide variety of materials. Poly(methyl methacrylate), or PMMA, was the first biomaterial used in this application⁸². Due to its high rigidity, PMMA became obsolete with the advent of microincision surgery in favour of flexible polymers that allowed the production of foldable IOLs⁹³. The advantage that these implants offer in the context of modern surgical practice is the possibility of being folded in order to facilitate the implantation through a small incision. Silicones and acrylic materials have been used in the production of foldable IOLs, acrylates being the most popular choice currently^{18,32,82}. The first acrylic polymers utilised in the manufacture of IOLs were hydrophilic¹⁰⁴. These three-dimensional polymeric structures, known as hydrogels, are capable of absorbing large amounts of water due to the hydrophilic nature of their constituent monomers⁹⁹. However, the solubilisation of the polymer chains is prevented by the presence of crosslinks that lead to the formation of a stable network¹⁰¹. Their soft consistency, flexibility, porosity and high biocompatibility make them ideal materials for biomedical applications, as they resemble the physical properties of the extracellular matrix very closely⁹⁹. In the particular case of the production of intraocular lenses, hydrogels offer numerous advantages over other materials. These include the possession of a refractive index that is similar to that of the natural crystalline lens, their high biocompatibility and the low levels of abrasion exerted towards neighbouring tissues as a consequence of their softness⁷⁹. As the mechanical strength of these materials can be compromised by the uptake of substantial amounts of water, the hydrogels used in the manufacture of IOLs are usually copolymerised with hydrophobic monomers in order to reduce the equilibrium water content of the polymer. Hydrophilic IOLs have a higher uveal biocompatibility than their hydrophobic counterparts. Therefore, lower levels of inflammation are elicited after the implantation of hydrophilic lenses⁸⁴. However, higher rates of PCO development have been reported with these IOLs. This could be due to the increase in

lens cell adhesion and migration on hydrophilic surfaces or the decrease in the sharpness of the square edge when the hydrophilic IOLs swell in an aqueous environment^{82,107}. In addition to PCO, the deposition of calcium phosphates on the surface of hydrophilic acrylic IOLs is another factor that contributes to the opacification of these lenses upon implantation in the capsular bag (Figure 3.1). The formation of these calcium deposits may be related with the deposition of cellular debris on the surface of the implants and the infiltration of aqueous fluids within the polymer structure. However, further studies are required in order to elucidate the exact causes of this phenomenon^{7,79,480}.



Figure 3.1: IOL opacification due to the formation of calcium deposits on its surface⁸².

While hydrophilic IOLs elicit higher rates of PCO than their hydrophobic counterparts, and despite the reported opacities associated with calcium deposits, their use in the clinic can still be beneficial in certain scenarios. For example, patients with uveitis benefit from the implantation of hydrophilic IOLs due to their superior uveal biocompatibility⁴⁸¹. Moreover, the implantation of hydrophilic lenses has been associated with a reduction in the incidence of glistenings and dysphotopsias, since these phenomena have been more widely observed in IOLs made of silicone and hydrophobic acrylic materials^{82,323}. As each type of polymer offers various advantages and disadvantages that might affect each patient differently, a universal material has not been found for the manufacture of these implants. Instead, the surgeon chooses the IOL that is most suitable for each patient, based on their age and medical history⁸⁹.

Given the circumstances described above, it is highly desirable to make improvements in the materials used for the manufacture of IOLs in order to prevent any issues that might deteriorate the quality of vision of patients. Furthermore, the design of the implants plays a fundamental role in the prevention of post-surgical complications such as PCO. Therefore, this is another factor that must be considered when developing IOLs with improved biocompatibility^{82,114}. However, the techniques currently employed in the IOL manufacturing industry are not easily accessible for researchers as a consequence of the elevated costs associated with the utilisation of the equipment required for this endeavour. Thus, this poses a challenge on the creation of novel bioactive IOLs with the capability to improve the outcomes of cataract surgery.

Additive manufacturing technologies allow the production of devices with a wide variety of materials, in relatively short times and at a low cost⁹. Thus, it was hypothesised that the introduction of this technology in the manufacture of IOLs could be the solution to the issues currently encountered in research and clinical settings. While recent studies have reported the production of IOLs with this technology, the optical performance of these lenses was not comparable to that of commercial IOLs and the low resolution achieved with the systems employed resulted in the occurrence of dimensional inaccuracies in the implants^{13,14}. Moreover, in one of these studies the IOLs could not be produced layer by layer, which meant that it was not possible to accurately control the thickness of the implant. Therefore, the work described in this project aims at addressing these problems by developing novel printable formulations that would result in the production of lenses with improved optical and mechanical properties and a high fidelity with respect to the design of the implants that are currently available in the market. Within this context, the production of hydrophilic acrylic IOLs using additive manufacturing technologies is described in this chapter. The 3D-printing technology of choice for the manufacture of IOLs was stereolithography. The selection of this technique was based on the high resolution achievable with this equipment and the possibility of producing acrylic hydrogels with this system.

Once a medical device is produced, it must be subjected to a meticulous series of characterisation techniques that ensure its safety and functionality. Therefore, the chemical, physical and biological properties of IOLs are evaluated before considering their application in a clinical setting. The chemical characterisation involves the analysis of the composition of the polymer and the potential release of leachable materials from the implant. The physical evaluation encompasses the assessment of the optical and mechanical properties of the lenses. With regard to the optical performance, the FDA states that the dioptric power, spectral transmittance and imaging quality of the lenses should be evaluated before considering the approval of these devices. The mechanical properties of the lenses are evaluated by measuring the loop pull strength, dynamic fatigue durability and by performing an injection test. All IOL haptics should be able to withstand a force of 0.25 N before becoming detached from the optic. Moreover, the haptics of IOLs that will be folded during implantation should withstand 250,000 cycles of near-sinusoidal deformation of ± 0.25 mm at a frequency between 1 and 10 Hz without being substantially damaged⁴⁸². It is also fundamental that no changes occur in the physical and optical properties of the IOLs after performing the pertinent injection tests. Finally, the biocompatibility of the IOLs is investigated by means of cytotoxicity experiments, genotoxicity testing and implantation tests *in vivo*⁴⁸².

Based on the requirements described above, the chemical, optical and mechanical properties of the implants produced in this project were thoroughly evaluated. The chemical composition and release of leachables were assessed by means of ATR-FTIR spectroscopy, while the physical properties were evaluated via thermal analysis. The optical quality was assessed by evaluating the transparency after incubation in an aqueous medium. As further post-processing and polishing treatments would be required before considering the translation of these lenses into further stages of testing and development, the dioptric power was not measured at this stage of the process. Finally, the mechanical strength was assessed by performing injection tests.

3.2 Aim and Objectives

The aim of this work was to produce hydrophilic acrylic IOLs by additive manufacturing. These IOLs had the same design as the Alcon AcrySof[®] single-piece IOL. This design was chosen due to the high success rate of this model in preventing PCO. The haptic-to-haptic length of this implant is 13 mm and the optic diameter is 6 mm.

The first objective was to develop a printable hydrophilic formulation for the production of these lenses. A review of the literature was performed in order to gain a greater understanding of the composition of hydrophilic acrylic IOLs. These implants are usually composed of HEMA crosslinked with PEG-DMA and, in some cases, copolymerised with MMA or vinyl pyrrolidone^{483,484}. As the printer used in the present project did not allow the polymerisation of methacrylate resins due to the relatively low energy of the laser, acrylate monomers were used instead. After testing a number of formulations, a copolymer of HEA and MA was chosen as the optimum material for the production of the IOLs.

Once the implants were produced, the next objective was to evaluate the physico-chemical properties of these lenses. The techniques utilised in this endeavour included TGA, DSC, ATR-FTIR, SEM, swelling tests and optical imaging.

3.3 Materials and Methods

3.3.1 Manufacture of IOLs

Intraocular lenses with the same design as AcrySof[®] (Alcon, Texas, USA) were produced by additive manufacturing. The first step was to create the CAD model of the IOL, which was generated by using Blender 2.79b (Amsterdam, The Netherlands) as a creation suite. This single-piece IOL is formed of two haptics and an optic portion. The haptic-to-haptic length is 13 mm and the optic diameter is 6 mm. The thickness was set at 0.9 mm and all the edges were sharp. The lens was made convex in order to evaluate the ability of the printer to produce structures with this design feature.

Customised supports were created along with the IOL in order to produce a structure that protected the printed lenses from the suction forces created during the printer's peel process. Once the CAD model was created, this was converted into a STL format and transferred to the PreForm software (Formlabs, Massachusetts, USA) in order to upload it to the printer (Figure 3.2).

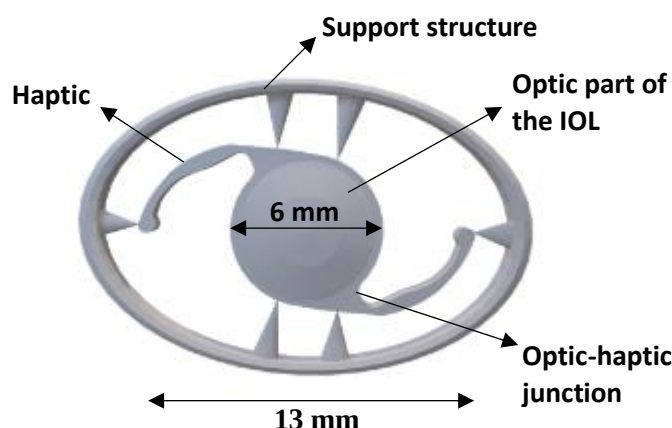


Figure 3.2: CAD model of the AcrySof® IOL and the support structure created in order to protect the lens from the forces created by the movement of the resin during the printer's peel process.

The second step in the production of IOLs was the preparation of the photopolymerisable resin used for printing these lenses. This pre-polymer mixture was produced by mixing the monomers and crosslinker with a photoinitiator. All the chemicals used in this procedure were from Sigma-Aldrich and were used without further purification, unless otherwise stated. The hydrophilic IOLs were printed with a resin composed of 82% (w/w) 2-hydroxyethyl acrylate (96% purity), 16% (w/w) methyl acrylate (99% purity), 1.5% (w/w) poly(ethylene glycol) diacrylate (M_n 700 g/mol) and 0.5% (w/w) diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide (97% purity) (Figure 3.3). The resin was prepared by mixing these components in a round-bottom flask and stirring under a nitrogen atmosphere until dissolution was complete. The IOLs were then 3D-printed using a bottom-up stereolithography system (Form1+, Formlabs, Massachusetts, USA) (Figure 3.4). One hundred grams of

photopolymerisable resin were poured into the printer vat and the CAD model was transferred to the PreForm software as a STL file, as previously mentioned. The laser was set in the “Black laser” mode and the layer thickness of choice was 0.05 mm. The model was printed without adding any of the supports generated by the software, as these had been previously created on the CAD software.

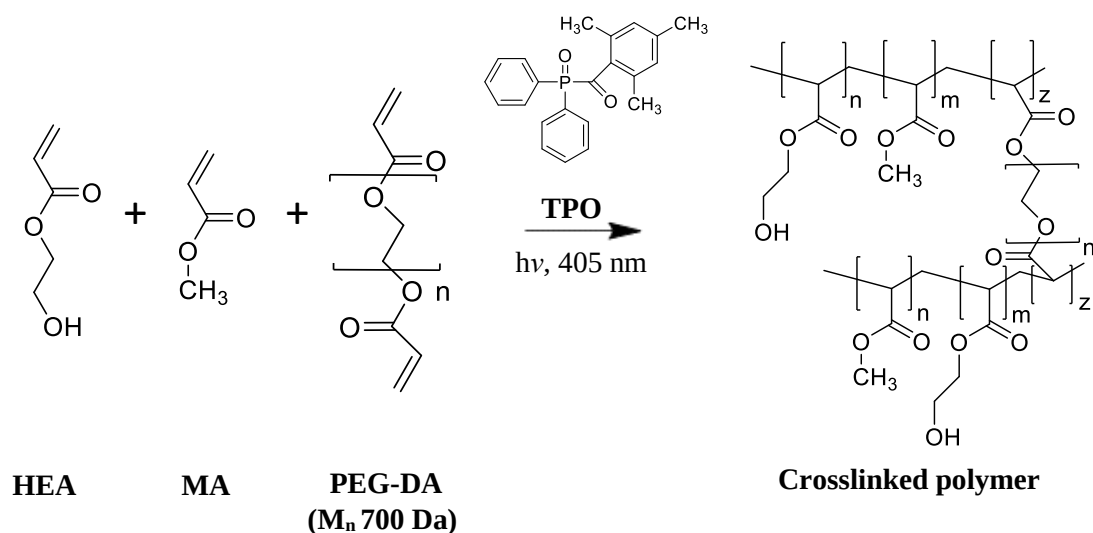


Figure 3.3: Reaction scheme detailing the photopolymerisation reaction of HEA, MA and PEG-DA.



Figure 3.4: Form1+ stereolithography system.

Once the IOLs were printed, these implants were incubated in isopropanol for 20 minutes with agitation at 300 rpm in order to wash away the resin that remained on their surface. After that, the lenses were post-cured by irradiating them with UV light for one hour and incubating them overnight at 50 °C in a round-bottom flask that had been purged with nitrogen. The rationale for this procedure is that the irradiation with UV light and the application of heat would contribute to the completion of the reaction between the unreacted monomers and radicals that remained within the printed polymers, thus eliminating these chemical species. This is a fundamental step in the production of implants via stereolithography, as the presence of leachable monomers reduces the biocompatibility of these devices. Furthermore, the mechanical properties of the resulting polymeric structures might be compromised if the conversion of the polymerisation reaction is not high enough. Therefore, it is of paramount importance to ensure that these issues are circumvented by optimising the manufacturing process in order to produce functional IOLs that could potentially be translated into a clinical setting.

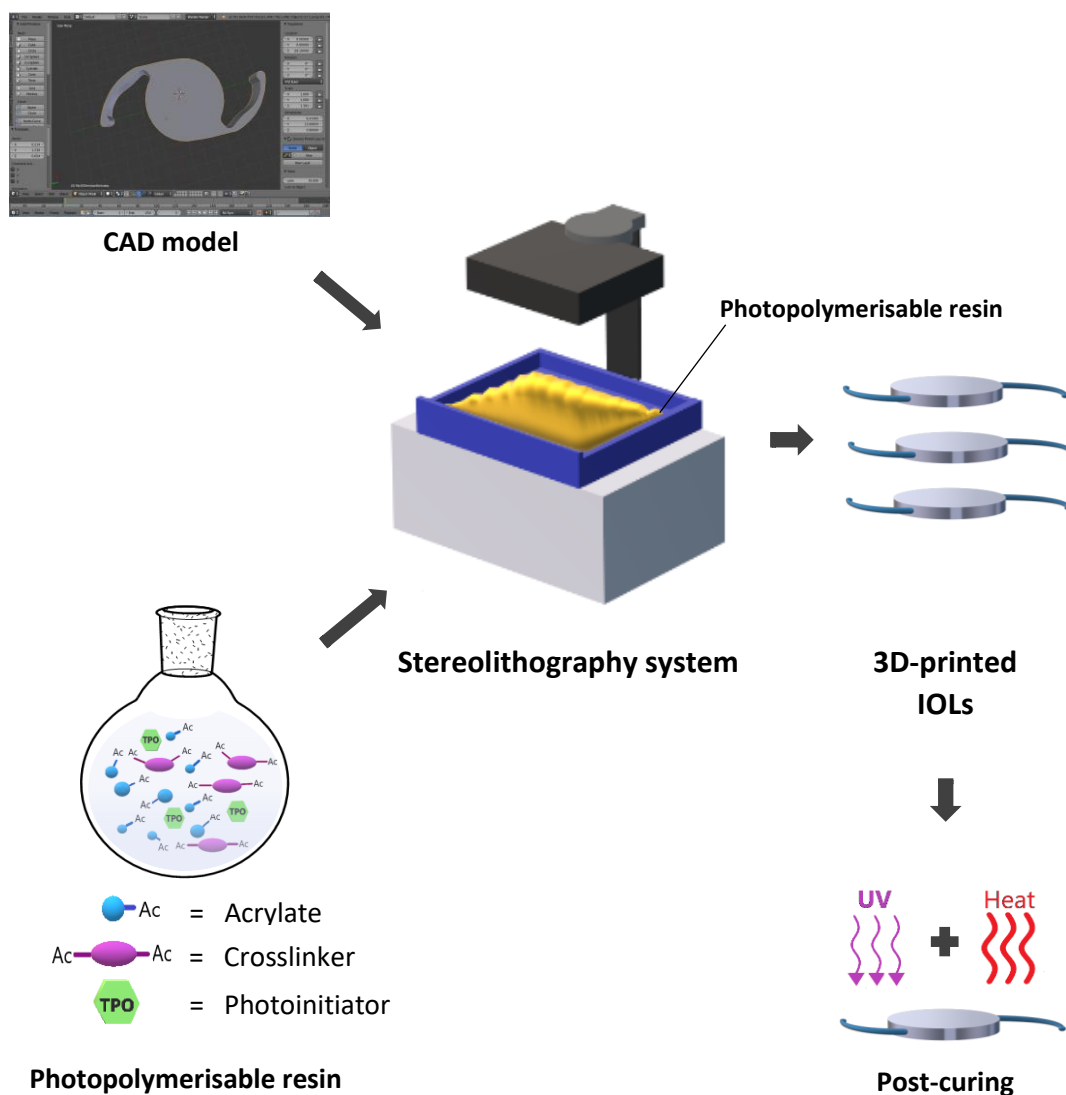


Figure 3.5: Diagram depicting the production of IOLs by stereolithography. The process involves the creation of the CAD model and the preparation of the formulation which is used for the manufacture of the IOLs. Once these are produced, a post-curing process is performed in order to eliminate the unreacted monomers that remain within the polymer after printing and which compromise the biocompatibility and mechanical properties of the implants.

3.3.2 ATR-FTIR

The effectiveness of the resin curing and post-curing protocols was determined by evaluating the depletion of the unreacted monomers after carrying out each of these procedures. The technique of choice for this analysis was infrared spectroscopy, for which a Bruker Vertex 70 FTIR spectrophotometer (Bruker Corporation, Massachusetts, USA) was employed. Resin and polymer samples were analysed for

comparison of the signal produced by the alkene groups from the monomers. The spectra were obtained by running 32 scans between 4,000 and 500 cm^{-1} at a resolution of 4 cm^{-1} .

3.3.3 Differential Scanning Calorimetry

The T_g of the polymer used in the construction of the IOLs was evaluated by DSC using a DSC Q2000 V24 system (TA Instruments, New Castle, USA). Samples with a mass of 1-3 mg were pressed into aluminium DSC pans that were introduced into the equipment used to perform the analysis. The samples were subjected to a heating cycle from -50 to 150 $^{\circ}\text{C}$, followed by a cooling step from 150 to -50 $^{\circ}\text{C}$ and a second heating ramp back to 150 $^{\circ}\text{C}$. The scan rate for all cycles was 10 $^{\circ}\text{C}/\text{min}$ and the nitrogen flow was maintained constant at 50 mL/min. The resulting data was analysed using TRIOS software v4. 3. 1. 39215. The T_g value was obtained from the thermogram generated during the second heating cycle as the temperature at the midpoint of the decline in the heat capacity that takes place during this thermal event.

3.3.4 Thermogravimetric analysis

The thermal stability and the composition of the IOLs were evaluated by TGA. This analysis was performed in a TGA Q5000 V23 system (TA Instruments, New Castle, USA). Samples with a mass of 1-3 mg were placed in platinum pans and heated from 25 to 500 $^{\circ}\text{C}$ at a heating rate of 10 $^{\circ}\text{C}/\text{min}$, under a nitrogen atmosphere. The experiment was performed in triplicate. The resulting data was analysed using TRIOS software v4. 3. 1. 39215.

3.3.5 Swelling tests

The water uptake of the 3D-printed IOLs was evaluated by performing a swelling test in an artificial aqueous humour composed of 130 mM NaCl, 5 mM KCl, 5 mM NaHCO₃, 1 mM CaCl₂, 0.5 mM MgCl₂, 5 mM glucose and 20 mM HEPES, adjusted to pH 7.25 with NaOH . Six IOLs were incubated at 35 °C in 0.5 mL of this buffer, their weights being recorded before the experiment and after 10, 30, 60, 120 min and 24 hours. The dimensions of these IOLs were also measured at these time points and a digital photographic record was obtained with a Canon EOS 1200D camera in order to evaluate the change in the size of the lenses.

3.3.6 Scanning Electron Microscopy

SEM images of the printed IOLs were generated before and after post-curing using a JEOL JSM 5900 LV microscope (Jeol Ltd., Tokyo, Japan) with a 20-kV accelerating voltage. The samples were mounted on aluminium stubs and coated in an argon-filled sputter gold coater (Polaron SC7640, Quorum Technologies, UK) for 30 seconds before acquiring the images.

3.4 Results

3.4.1 3D printing IOLs

The experiments described herein resulted in the successful production of hydrophilic acrylic IOLs via stereolithography. These IOLs were replicas of the commercial model Alcon AcrySof[®] and possessed sufficient mechanical strength to resist both the detachment from the build platform and the post-processing protocols. However, several aspects of these lenses should be optimised in order to obtain functional IOLs. Firstly, the low resolution achieved with the printer used in this project did not allow the replication of the commercial IOL model with a high accuracy. The high depth of cure obtained with the resin used for the production of the IOLs posed a challenge when printing these lenses with or without the supports generated by the PreForm

software, as the excessive over-curing caused significant alterations in the shape of the implants (Figure 3.6). Therefore, an optimisation in the support structures, printing parameters and the composition of the formulation was carried out in order to improve the morphology and physical properties of the lenses. This resulted in the production of IOLs with a higher fidelity respect to the commercial model, although a perfect replication of the AcrySof® design could not be achieved in any case. As can be seen in Figure 3.7, (a) and (c)), the optic-haptic junction from the original model could not be reproduced in the printed lenses, and the haptics were in most cases wider than in the AcrySof® IOL. With regard to the dimensional accuracy, the first batches of IOLs produced in this project had larger dimensions along the X-Y plane than the CAD model. However, the optimisation of the process parameters, support structures and formulation led to the production of lenses that had an optic diameter of approximately 6.5 mm, 0.5 mm larger than that of the IOL generated on the CAD software (Figures 3.2 and 3.7). Despite the increase in the accuracy on the X-Y plane, the Z resolution could not be improved. Thus, the 3D-printed IOLs were always significantly thicker than the lens created on the CAD software, possessing an average dimensional error of $+53.21 \pm 4.2\%$ (Appendix 1). It was hypothesised that the low Z resolution was a consequence of the high cure depth of the photopolymerisable resin, and that this could be solved by adding a light absorber to the formulation.

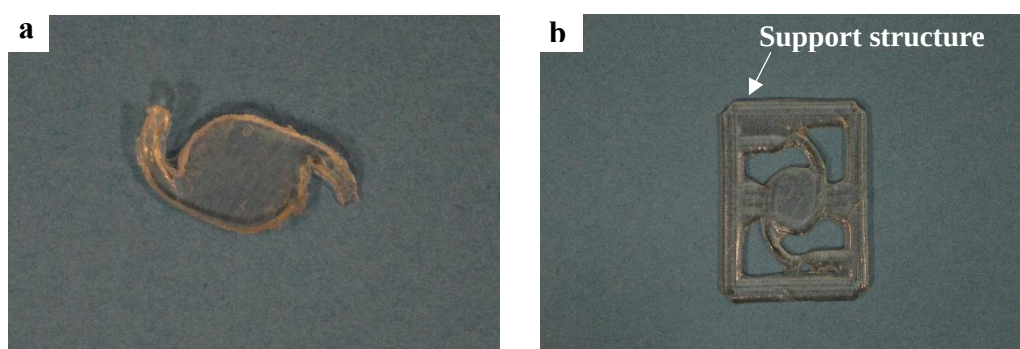


Figure 3.6: Optical images of some of the first 3D printed hydrophilic IOLs that resulted from the experiments described in this chapter. **(a)** Replica of the AcrySof® IOL printed without any support structures. **(b)** Same IOL design printed with a custom-made, frame-like support. A distortion in the shape with respect to the original model can be seen in both cases.

Other characteristics of the printed IOLs that should be improved include the transparency and mechanical strength. The lenses were translucent rather than transparent when dry, and they became opaque when incubated in an aqueous environment (see section Swelling tests, Figure 3.13). Furthermore, while the lenses were quite robust after printing and post-curing, their mechanical strength was significantly compromised upon uptake of water. Therefore, these IOLs were not considered valid for implantation in the intraocular environment.

Table 3.1: Formulations tested during the optimisation of the composition of the 3D printed hydrophilic IOLs. The formulation composed of 82% HEA, 16% MA, 1.5% PEG-DA and 0.5% TPO yielded the best results when printing IOLs with it.

HEA (%)	MA (%)	PEG-DA (%)	TPO (%)	Results
89.7	8	2	0.3	Flexible and transparent, although quite hard.
82	16	1.5	0.5	The haptics and optic part were formed with a good definition. Very flexible.
85	15	0.5	0.5	Low resolution, very flexible.
50	50	0.5	0.5	The IOLs were printed but they disintegrated when detaching them from the platform.

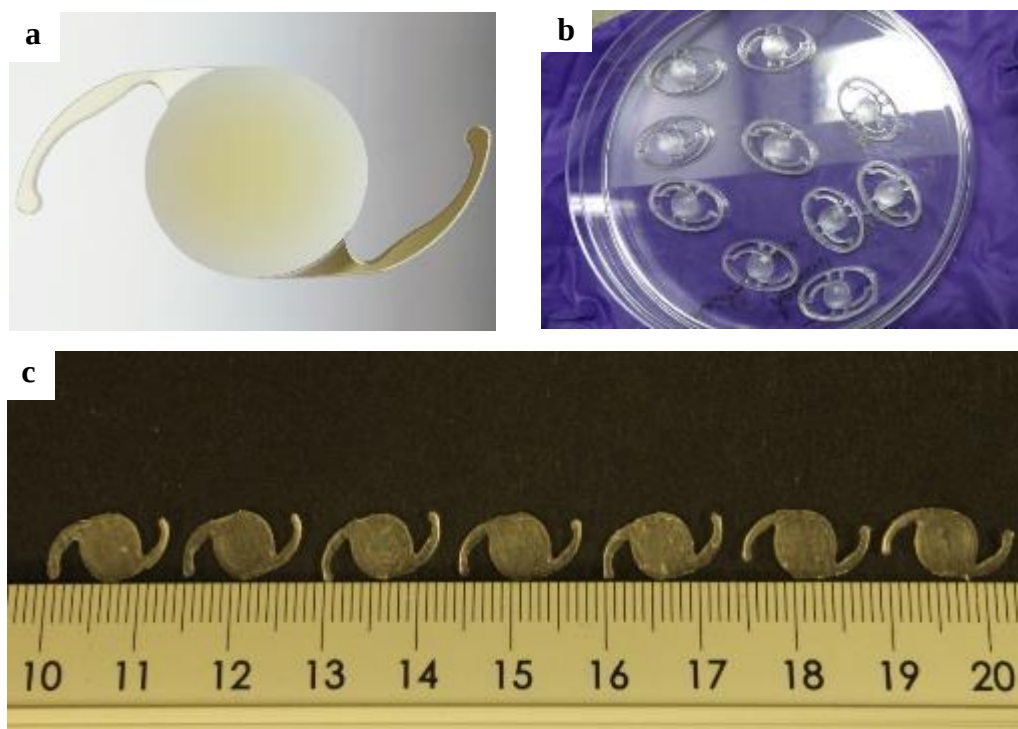


Figure 3.7: (a) Image of the AcrySof® IOL. (b) 3D printed hydrophilic acrylic IOLs with the support structure still attached to them. (c) 3D printed hydrophilic acrylic IOLs after detaching the supports, with their haptic-to-haptic length measured in centimetres.

3.4.2 ATR-FTIR

The effectiveness of the curing and post-curing processes was evaluated via ATR-FTIR by analysing both the resin used for printing the IOLs and samples of printed lenses before and after being post-cured. This analysis was based on the detection of the signal produced by the carbon-carbon double bond from the acrylate group of the monomers present in the resin and polymer samples. This signal appeared at a frequency of $1,630\text{ cm}^{-1}$ and, as seen on the resulting spectra (Figure 3.8), its intensity was considerably higher in the spectrum generated from the analysis of the resin than in the printed polymers. Furthermore, the intensity of this signal was lower in the post-cured samples than in the not post-cured counterparts, hence confirming the efficiency of the post-curing process for the elimination of unreacted monomers from the polymer. The intensity of the peak observed at approximately 1410 cm^{-1} had also decreased after curing and post-curing, since this signal was generated by the $=\text{CH}-\text{H}$

bonds. With regard to the rest of the peaks observed on the spectra, the presence of a broad signal at approximately 3400 cm^{-1} indicated the presence of OH groups, most likely from HEA, in the resin and polymers. The peaks at 1725 cm^{-1} and 1150 cm^{-1} were generated by carbonyl and ether groups, respectively. These bands corroborated the presence of acrylate groups in both the monomer mix and polymeric materials.

Overall, this analysis confirmed the effectiveness of the curing process performed in the 3D printer and the efficiency of the post-curing protocol for the elimination of the unreacted monomers that remained within the polymer after printing.

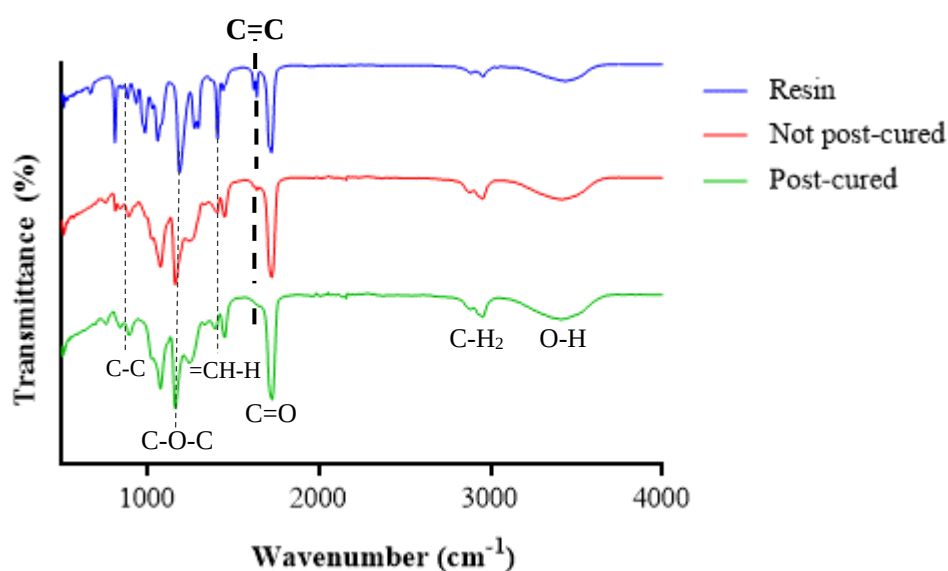


Figure 3.8: ATR-FTIR spectra obtained from the analysis of the resin used for the production of the IOLs (blue) and from printed IOLs before and after being post-cured (red and green, respectively). A reduction in the intensity of the signal generated by the C=C functional groups from acrylate monomers was observed after curing and post-curing.

3.4.3 Differential Scanning Calorimetry

The T_g of the printed IOL material was determined by DSC analysis. As seen on the thermogram generated from this analysis, the T_g of the printed polymer was 4.97 °C (Figure 3.9). This value is lower than the T_g of the AcrySof® IOL, which is approximately 15 °C¹⁸⁸. Therefore, the lenses produced with the hydrophilic formulation developed in this project were more flexible at both room and human body temperature than the commercial model used as a reference. The flexibility of an IOL is an essential requirement for it to be implanted through a microincision, as the lens has to be folded in order to facilitate the insertion in the capsular bag. Therefore, the 3D printed IOLs were, in theory, implantable by performing minimally invasive surgical procedures.

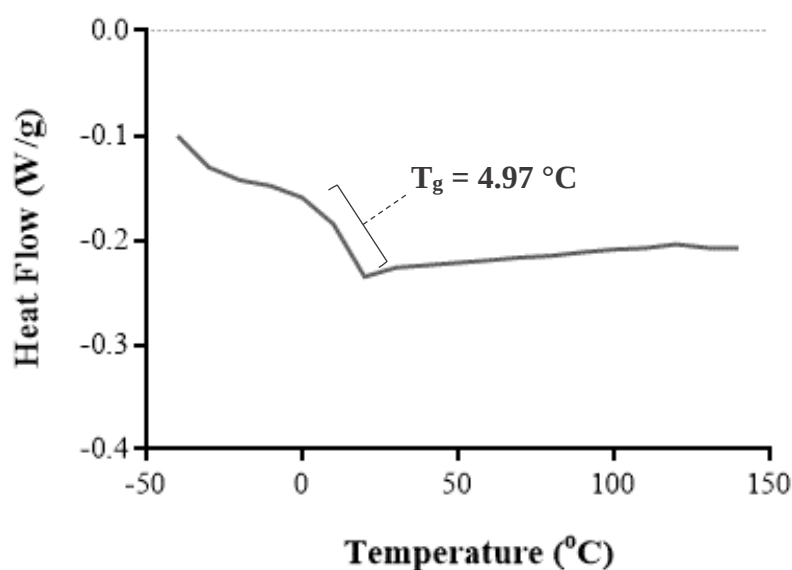


Figure 3.9: Thermogram obtained from the DSC analysis of the polymer that forms the printed IOLs. This graph was generated by the second heating cycle. The value of the T_g is 4.97 °C.

3.4.4 Thermogravimetric analysis

The thermal stability and the composition of the 3D-printed IOLs were analysed by TGA. The thermogram obtained from this analysis showed that the initial degradation

temperature (IDT) of the polymer was 415.9 ± 18.9 °C (Figure 3.10 and Table 3.3). This indicated that the material had a high thermal stability. Furthermore, the results showed that approximately 15% of the polymer had degraded before reaching 200 °C. This is most probably due to the decomposition of pMA, as the degradation profile of this polymer shows a similar trend at this temperature (Appendix 2)⁴⁸⁵. The TGA thermogram of pHEA shows that this polymer is completely stable until it reaches a temperature of approximately 330 °C (Appendix 2). This supports the idea that the first degradation events may have occurred in the fraction of the polymer that was composed of pMA. The rest of the material degraded at a higher temperature (400-500 °C) and it seemed to be composed of the remnants of pMA and all the pHEA present in the copolymer, as inferred by comparison with the thermal curves of these polymers^{485,486}. Therefore, the thermal degradation profile of the copolymer used in the production of the hydrophilic acrylic IOLs corroborated the presence of two different polymers in this material. The decomposition of the crosslinker was not detected, which may be due to the presence of a relatively low concentration of this component in the printed polymers.

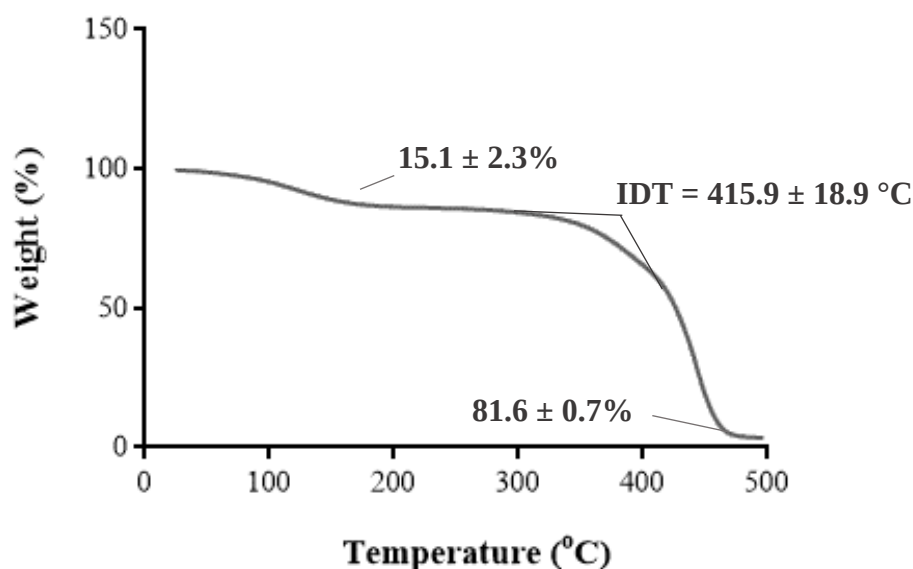


Figure 3.10: Thermogram obtained from the thermogravimetric analysis of the polymer that forms the printed IOLs. The initial degradation temperature (IDT) of this material is 415.19 ± 18.9 °C. Two different phases can be discerned in this graph: one that corresponds to the degradation of approximately 15% of the polymer and another one that results from the degradation of the remaining 81% of material. These values match those of the theoretical composition of the polymer (82% HEA and 16% MA), hence confirming that the IOLs have the desired composition.

Table 3.2: Initial decomposition temperature (IDT) of the three IOL samples that were analysed by TGA and the average value of this parameter.

Sample	IDT (°C)
1	437.58
2	403.52
3	406.46
Mean	415.9 ± 18.9

3.4.5 Swelling tests

A swelling test was performed in order to evaluate the water uptake of the printed IOLs when they were incubated under conditions comparable to those found in the posterior chamber of the eye. Six IOLs were incubated in an artificial aqueous humour at 35 °C and the water uptake of the polymer was monitored by recording the weight at specific time points, ranging from one to 24 hours. As seen on the graphs obtained by plotting the increase in the weight of the IOLs (Figure 3.11) and the swelling percentage (Figure 3.12), the mass of the implants increased by more than 100% after one day of incubation. The weight of a dry IOL was approximately 42 mg, while that of an IOL that had been incubated for 24 hours in an aqueous medium was 100 mg. This is considerably higher than the water content of commercial hydrophilic IOLs composed of pHEMA and PMMA, which is approximately 26%. Furthermore, as the polymer that formed the 3D-printed IOLs had not reached the equilibrium water content after one day of incubation, the swelling capacity should be evaluated over a longer period of time in order to obtain the value for this parameter.

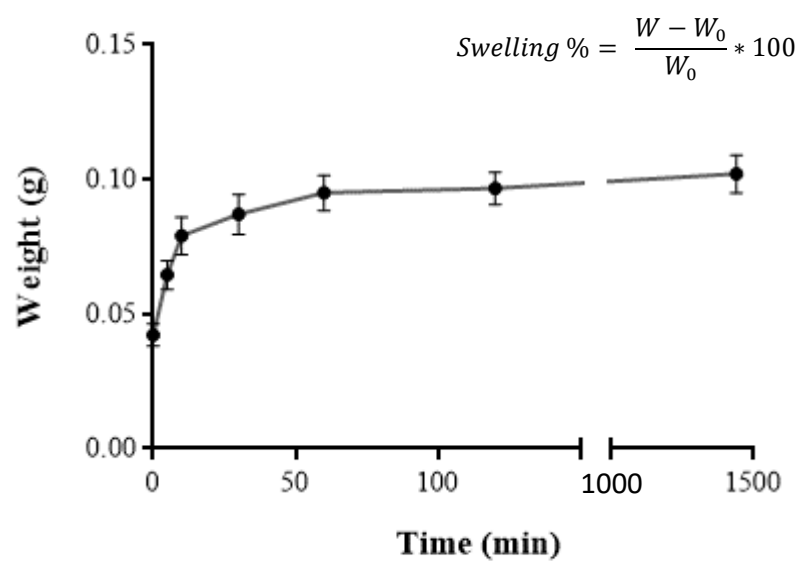


Figure 3.11: Increase in the weight of the 3D-printed hydrophilic IOLs incubated in artificial aqueous humour for one day.

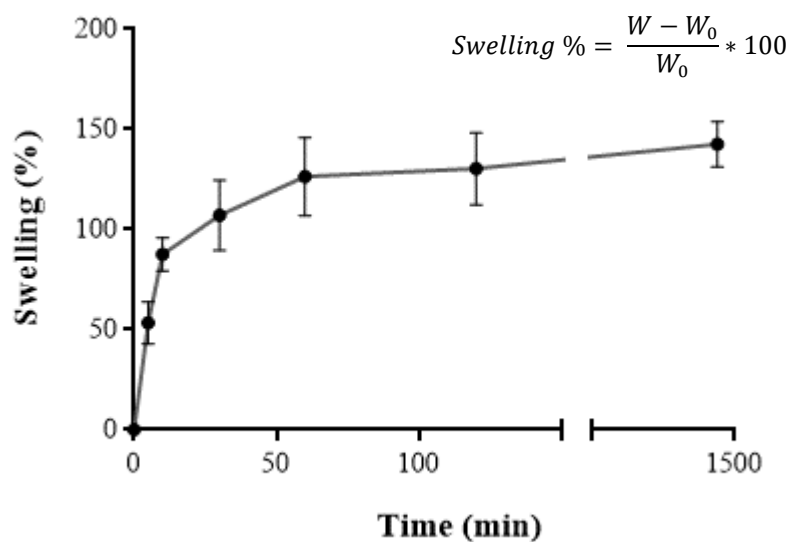


Figure 3.12: Increase in the degree of swelling of the hydrophilic IOLs during the incubation in artificial aqueous humour for one day. The equation used for the calculation of the swelling percentages is shown on the graph, where W is the weight of the polymer after swelling and W_0 is the weight of the material before the swelling test.

The increase in the size of the IOLs upon swelling was also evaluated by measuring the change in the haptic-to-haptic length of the IOLs. As seen in Figure 3.13, the length of a dry IOL was approximately 13 mm, while that of an IOL that had been incubated in the artificial aqueous humour for one day had increased to almost 20 mm. This would compromise the suitability of these lenses for implantation in the capsular bag, as such an increase in the size of the IOL once it is implanted within this structure would cause a stretch in the bag that could result in significant damage of the surrounding tissues. Furthermore, the transparency of the lenses was also affected by the absorption of water. As shown in Figure 3.13, the IOL was translucent when dry and it became more opaque as the incubation time increased. This has been previously reported during the incubation of hydrophilic IOLs in phosphate buffers⁴⁸⁷ or after the implantation of the lenses in the capsular bag, and it could be due to the deposition of salts on the polymer⁴⁸⁸ or the occurrence of phase separation phenomena between the polymer chains and the water. A closer examination of the IOLs via SEM imaging would have been beneficial for the determination of the cause of the opacification of the lenses, as mineral deposits are observable with this technique⁴⁸⁹. Finally, another factor that contributed to the failure of these implants was the significant decrease in mechanical strength when the lenses were swollen in water. The IOLs used in the clinic have the ability to resist the high levels of mechanical stress that they are subjected to during implantation, even when they have been incubated in an aqueous environment. However, the 3D-printed IOLs described in this chapter failed to resist the mechanical strain that was applied on them when they were inserted in a surgical injector during the implantation tests (data not shown). Therefore, it was concluded that these lenses were not functional and, consequently, not transferrable to a clinical setting.

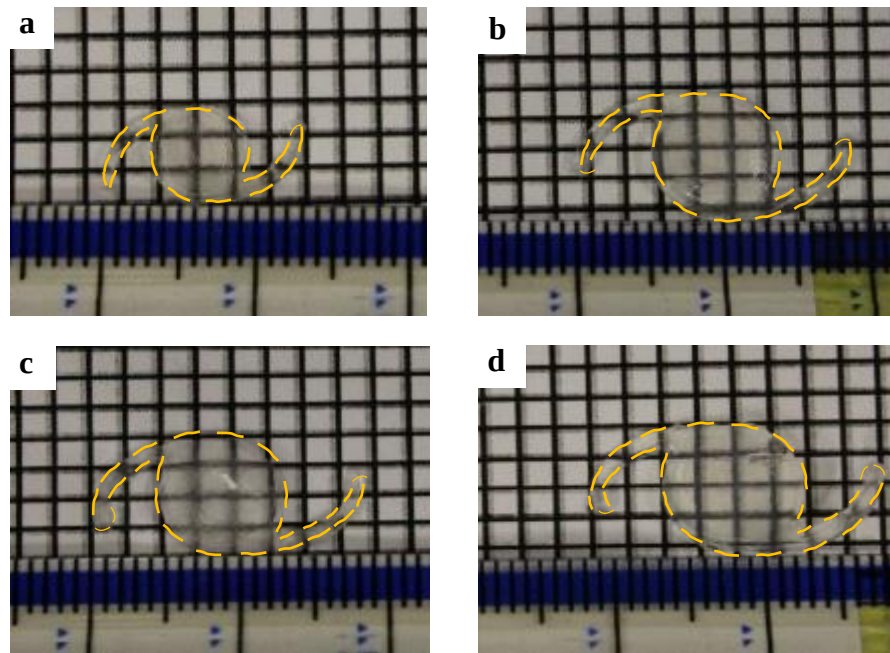


Figure 3.13: Optical images of a hydrophilic IOL subjected to a swelling test, at each of the stages of this experiment: dry **(a)** and after 1 h **(b)**, 2 h **(c)** or 24 h of incubation in artificial aqueous humour **(d)**.

3.4.6 Scanning Electron Microscopy

SEM images were taken before and after post-processing and post-curing in order to compare the effect of these processes on the surface smoothness of the lenses (Figure 3.15). The images were focused on the optic and optic-haptic junction of the implants, as the sharpness of the optic edge is critical for the prevention of PCO and the characteristics of the optic portion determine the optical quality of the lenses. When compared with the AcrySof® IOL (Figure 3.14), it can be observed that the surface roughness of the printed lenses was higher and that the optic edge was blunter. This may be due to the spatial resolution of the printer and the fact that the printed IOLs had not been polished. Moreover, while the morphology of the haptics and optic-haptic junctions of the 3D-printed IOLs were comparable to those of the commercial implant, the edges of these structures were also sharper in the AcrySof® IOL. Other features that can be observed on the images are the residual pieces of the constructs which connected the support structure with the IOL. The presence of these artifacts on the surface of a lens would cause abrasion on the surrounding tissues once it is

implanted in the capsular bag. Therefore, the IOLs should be polished before proceeding to animal experimentation or clinical trials had these lenses been suitable for implantation in the capsular bag.

With regard to the effect of post-processing on the smoothness of the surface of the lenses, the images shown in Figure 3.15 demonstrate that this treatment contributed to the removal of artefacts that could compromise the visual quality of the IOLs. However, further post-manufacturing treatments such as polishing should be performed in order to improve the optical performance of the lenses.

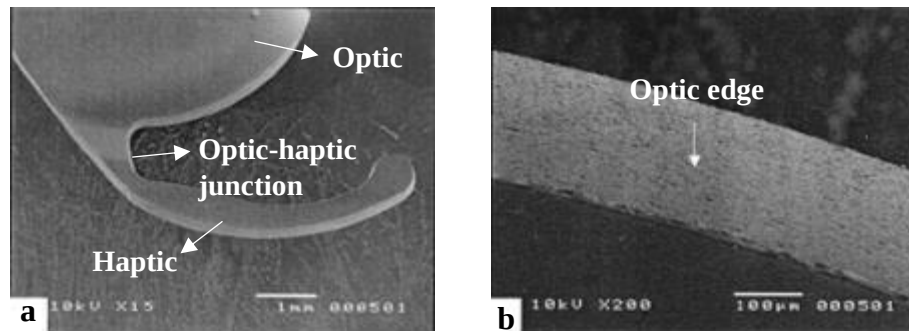


Figure 3.14: SEM micrographs of the AcrySof® SA30AL IOL. **(a)** shows the optic-haptic junction and one of the haptics of the implant (original magnification X15). **(b)** shows the sharpness of the optic edge (original magnification X200). Adapted from Escobar-Gomez et al⁴⁰⁷.

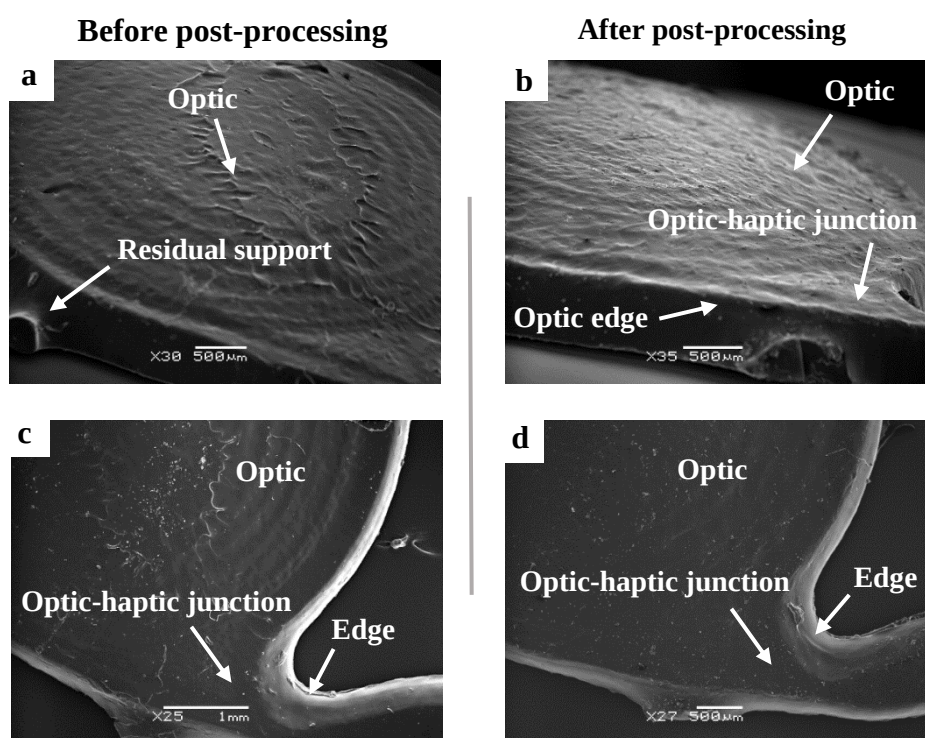


Figure 3.15: SEM images of the optic part (a, b) and optic-haptic junction (c, d) before (a, c) and after post-processing and post-curing (b, d). An increase in the smoothness is noticeable after these procedures.

3.5 Discussion

The experiments described in this chapter demonstrated that hydrophilic acrylic IOLs with the same design as the commercial implant Alcon AcrySof® could be manufactured via stereolithography. The formulation used in the production of these implants was based on a material reported on a patent that describes the production of foldable IOLs⁴⁸⁴. The patented material was composed of 82% HEMA, 16% MMA and 0.5% PEG-DMA. However, this composition could not be replicated with the printer used in this project due to the slow polymerisation rates that the methacrylate monomers show in this system, which impaired the printability of the IOLs when using this material. Therefore, HEA and MA were used instead of their methacrylate counterparts. The optimisation of the proportion of each monomer in the photopolymerisable resin and the addition of PEG-DA as a crosslinker resulted in the production of foldable IOLs with a relatively high mechanical strength, which is

required for the implantation of these devices in the capsular bag. However, these lenses became soft and fragile when incubated in an aqueous environment, hence compromising their suitability for implantation in the posterior chamber of the eye. The use of methacrylate monomers could possibly have prevented this issue, as the presence of an additional methyl group in the polymer chains would restrict the molecular mobility and lead to the formation of materials with a higher mechanical strength.

Once the IOLs were produced, a post-curing process was performed in order to eliminate any unreacted monomers from the printed polymer network. The efficiency of this technique was confirmed by FTIR spectroscopy analysis. The post-curing treatment promotes the reaction between any active polymer chains or free radicals that may remain within the polymer after printing. By completing the polymerisation of these molecules, the mechanical strength of 3D-printed structures increases and they become capable of tolerating relatively high mechanical stresses. This is fundamental for the implantation of these IOLs via phacomulsification, as the lenses need to be folded in order to facilitate their injection into the capsular bag through a microincision.

After post-curing, the IOLs were subjected to a physico-chemical characterisation in order to validate their functionality as medical implants. The techniques employed for the evaluation of the printed lenses included TGA, DSC, FTIR, swelling tests and SEM. The results obtained from the thermogravimetric analysis confirmed that the IOLs possessed a high thermal stability and indicated that the copolymer was composed of pHEMA and pMA. The DSC results indicated that the T_g of the polymer was low enough for the lenses to be foldable at room temperature and under physiological conditions. As mentioned above, this facilitates the implantation of these implants via microincision surgery.

The images obtained by SEM showed that the morphology of the printed IOLs was comparable to that of the commercial model used as a reference in this project. However, the optic edges of the 3D-printed lenses were blunter than those of

AcrySof®. This could affect the performance of the printed IOLs if these were implanted in the capsular bag, as the square edge of the IOL promotes the formation of a capsular bend which prevents the migration of lens cells to the posterior capsule and therefore reduces the incidence of PCO. Moreover, these images proved that the post-processing of the IOLs contributed to the increase of the smoothness of their surface. This is important as any artefacts present on the surface of the lenses can lead to light scattering and, consequently, cause a reduction in the transparency of the implants. The visualisation of the IOLs by SEM also revealed the presence of residual pieces of the structural supports used during printing. As these artefacts would cause abrasion on the surrounding tissues upon implantation in the posterior chamber of the eye, the removal of these structures would be a priority if these lenses were to be tested *in vivo* or translated to the clinic.

Finally, a swelling test was performed by incubating the IOLs in an artificial aqueous humour for 24 hours. After this period of time, the IOLs had doubled their weight as a consequence of the water uptake. This resulted in a significant increase in the size of the lenses, which would cause over-stretching of the capsular bag if these IOLs were implanted in the eye. Moreover, the transparency and mechanical strength of these devices were also compromised after immersion in aqueous solution. The reduction in the mechanical resistance of the polymer caused the failure of the implants to endure the strain that they were subjected to when the implantability of these lenses was tested with a surgical injector. Therefore, these IOLs were deemed not suitable for use in cataract surgery.

3.6 Conclusions

The experiments described herein demonstrated that additive manufacturing technologies can be employed to produce IOLs that could potentially be used in cataract surgery. While the 3D-printed implants contained all the design features of the commercial IOL that was used as a model, their functionality was compromised by the poor optical and mechanical properties that the material exhibited when swollen in water. This indicated that the lenses that resulted from this work were not

suitable for implantation in the posterior chamber of the eye, as the IOLs would be immersed in an aqueous medium when inserted in the capsular bag. Therefore, further improvements in the formulation would be required in order to obtain functional IOLs whose optical and mechanical properties would not be significantly compromised when incubated or implanted in an aqueous environment. Since the use of methacrylate monomers was not possible with the printer used in this project, other strategies should be undertaken in order to optimise the physical properties of the lenses. For example, the use of acrylate monomers with phenyl groups in their structure would increase the T_g of the resulting polymer. Moreover, the production of hydrophobic IOLs would reduce the equilibrium water content of the material, thus preventing the occurrence of substantial changes in the size of the IOLs after implantation. Finally, the printing resolution is another aspect that should be improved. While the optimisation of the formulation and printing parameters led to an improvement in the dimensional accuracy of the printed implants, further optimisation would be required in order to increase the resemblance of the replicas to the original AcrySof[®] IOL, as well as the reproducibility of the results. The following chapters describe the experimental work that was performed in order to optimise the physical and morphological characteristics of the 3D-printed IOLs.



CHAPTER 4

Development of 3D-Printable Hydrophobic Formulations



4. Development of 3D-Printable Hydrophobic Formulations

4.1 Introduction

Soft hydrophobic acrylic materials were introduced in the manufacture of IOLs by Alcon with the launch of AcrySof^{®82}. This IOL went on to become the most successful implant in terms of PCO prevention^{104,107,490}. The presence of sharp edges and the hydrophobicity of its surface are the attributes that confer this IOL with the ability to reduce the incidence of secondary cataract^{32,104}. Generally, foldable hydrophobic acrylic materials are copolymers of acrylate and methacrylate monomers derived from PMMA⁸². The presence of acrylate monomer units adds flexibility to the material, as the extra methyl groups present in methacrylate monomers reduce the degree of freedom of the polymer chains⁸⁵. Therefore, IOLs made of these biomaterials are foldable at room temperature and easy to implant through small incisions. Furthermore, the monomers used in the manufacture of these polymers often contain phenyl groups in their structure. This is the case for AcrySof[®], which is composed of a copolymer of 2-phenylethyl acrylate (PhEA) and 2-phenylethyl methacrylate (PhEMA)¹⁸⁸. The presence of phenyl rings results in an increase in the refractive index of the material, thus allowing the production of thinner lenses²⁰². Moreover, the hydrophobicity of the aromatic rings leads to a reduction in the equilibrium water content of the polymer. This moderates the decrease in the mechanical strength of the materials upon implantation in an aqueous environment, hence making them less fragile than hydrogels.

With regard to tissue interactions, hydrophobic acrylic IOLs have lower uveal biocompatibility than their hydrophilic counterparts. However, their capsular compatibility, i.e., the reaction of the LECs and capsular tissue to the IOL, is significantly better. Consequently, the implantation of hydrophobic IOLs leads to a reduction in the incidence of PCO^{153,202,323}. To explain this, Linnola *et al.* suggested that fibronectin adhered more strongly to hydrophobic materials, leading to the establishment of a physical connection between the IOL and the capsular bag mediated by fibronectin-collagen binding. Consequently, the migration of LECs from

the anterior to the posterior capsule would be hindered by the formation of this assembly¹⁹². An additional explanation provided for this phenomenon is the prevention of cell migration by the creation of a barrier effect that results from the interaction between the sharp posterior optic edge of the IOL and the capsular bag (Figure 4.1). This leads to the establishment of a tight shrink-wrap effect upon insertion of the IOL in the capsular bag, which reduces the space available for cell movement¹⁷⁰. Since hydrophobic IOLs maintain their sharpness to a greater extent than hydrophilic lenses upon implantation in an aqueous medium, this effect is enhanced in implants that are made with water-repellent polymers¹⁶⁰.

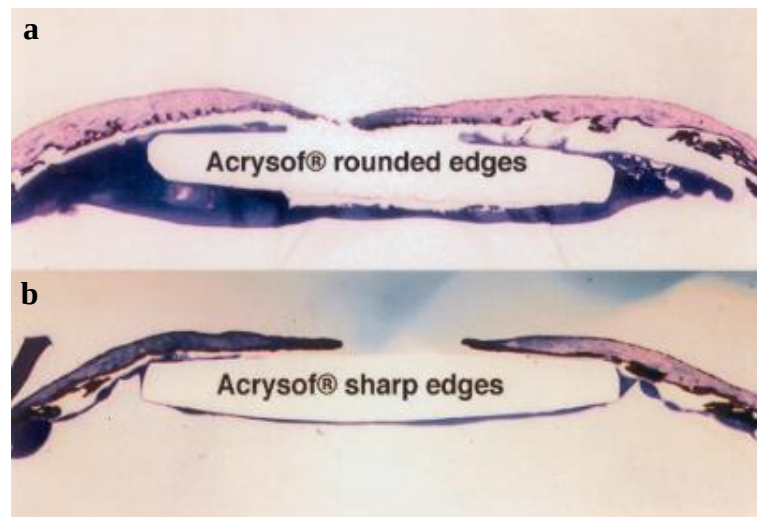


Figure 4.1: Histopathological examination of the interactions between a round-edge AcrySof® IOL **(a)** and a sharp-edge analogue **(b)**. The latter induces a tight wrapping of the capsular bag around the IOL, which prevents the migration of LECs to the posterior capsule⁴⁹¹.

Despite the positive results observed after the implantation of foldable hydrophobic IOLs, some issues have also been reported in these cases. The formation of glistenings and the occurrence of dysphotopsias are the main problems encountered in the clinic^{82,323}. As explained in chapter two, glistenings are vacuoles that form within the IOL material upon infiltration of ocular fluid and accumulation of this liquid in the micro-voids of the polymer. The difference in refractive index between the aqueous humour and the material that forms the IOL causes the refraction of light at the polymer-liquid interface. This is manifested as glare effects observed in the locations

where vacuoles have formed^{86,492}. Numerous studies have evaluated the effect of glistenings on visual acuity. Whilst some of them argued that this phenomenon has a negative effect on vision, most investigations concluded that the quality of vision is not significantly affected by the presence of glistenings^{86,322,327}. The formation of these vacuoles has been reported in all the materials used in the manufacture of IOLs, although the highest incidence has been detected in hydrophobic acrylic lenses⁸⁶. Another major problem associated with the implantation of these IOLs, known as dysphotopsia, occurs as a consequence of the reflection of light from the IOL onto the retina. Unwanted light patterns are thus superimposed with the retinal image and cause visual impairment. This phenomenon is related with the implantation of IOLs that possess high refractive indices and square edge designs³²⁵. Furthermore, a higher incidence of dysphotopsias has been reported in IOLs with straight optic edges in comparison with those that have slanted profiles⁴⁹³.

Given the challenges currently faced in the production of hydrophobic acrylic IOLs with design features that prevent PCO while maintaining a high quality of vision, further research needs to be conducted in order to develop IOLs that satisfy these requirements. As mentioned in previous chapters, current moulding- and lathing-based manufacturing techniques are difficult to implement in research environments, hindering the development of novel bioactive implants with improved optical quality. Thus, the high accessibility of 3D-printing technologies would be advantageous in this endeavour. With this motivation, and given the benefits provided by the implantation of hydrophobic acrylic IOLs in terms of PCO prevention, the work described in this chapter focused on the production of hydrophobic lenses by additive manufacturing.

The first step towards the production of IOLs by stereolithography was the development of the photopolymerisable resins. Firstly, a formulation composed of phenylethyl acrylate was produced in an attempt to reproduce the physico-chemical properties of the AcrySof[®] IOL. However, the fragility of the resulting polymers rendered them unsuitable for the production of these lenses (data not shown). Thus, alternative formulations were developed with the aim of increasing the mechanical

strength of these materials while mimicking the characteristics of AcrySof®. Two hydrophobic acrylic resins resulted from this process. The main monomer in both formulations was 2-phenoxyethyl acrylate, which was selected as the major constituent molecule because of its high refractive index and optimum value of glass transition temperature, which is almost 10 °C higher than that of poly(phenylethyl acrylate). The presence of a phenyl group in this monomer is the primary cause of these properties. The delocalised electrons from this functional group cause an increase in the refractive index, while the bulkiness of the ring and the establishment of π - π interactions reduce the mobility of the polymer chains, thus increasing the rigidity of the material. The difference between the two formulations developed in this project lied in the choice of crosslinker. The first resin, identified as composition 1 (C1), used poly(ethylene glycol) dimethacrylate as a crosslinking agent, while the second formulation, C2, employed an aromatic urethane diacrylate macromolecule instead. A methacrylate functionality was selected for the first composition because it would add a higher rigidity than an acrylate-based molecule. It was hypothesised that this would compensate for the lack of phenyl groups in the crosslinker. The second formulation, C2, contained phenyl groups in both the crosslinker and the main monomer in order to obtain a polymer with a higher refractive index and therefore a better transparency. Both formulations contained TPO as a photoinitiator and this was added at the same concentration in both of them. They also contained the same proportion of crosslinker, which had been optimised in order to achieve an optimal balance between mechanical strength and flexibility.

As stated in chapter three, IOLs are subjected to a series of physico-chemical and biological characterisation techniques in order to assess their biocompatibility and biofunctionality. These procedures include the evaluation of the chemical composition, release of leachable materials, mechanical properties, optical performance, cytotoxicity and implantability⁴⁸². With regard to the latter, a capsular bag *in vitro* model is employed to perform this assessment. These systems are developed by performing a sham cataract surgery in human donor eyes. This procedure involves the creation of an incision on the anterior capsule by performing a continuous curvilinear capsulorhexis and the subsequent removal of the lens fibres

via hydrodissection. An intraocular lens can then be implanted in the capsular bag that is generated during this process, allowing the study of the interactions that take place between this artificial device and the natural tissue (Figure 4.2). To facilitate these investigations, the capsular bag is usually isolated from the eye by excising the zonules and is then transferred to a dish for culture².

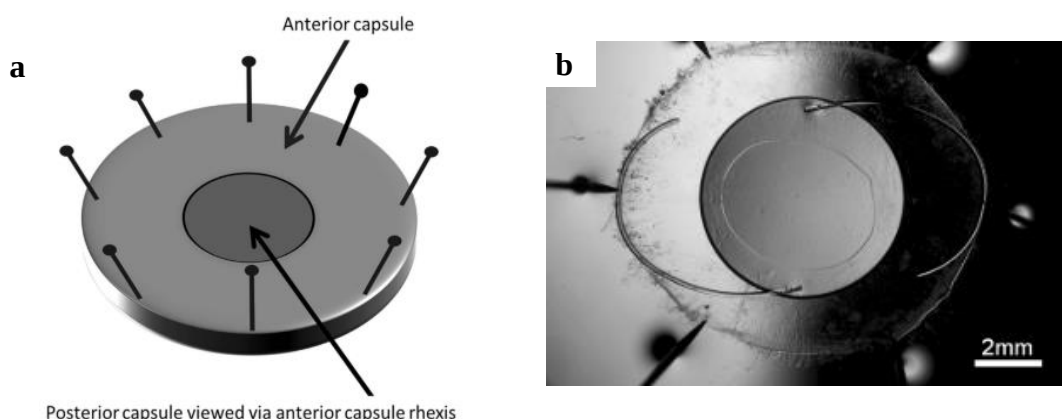


Figure 4.2: (a) Diagram of a capsular bag preparation from a human donor lens¹⁴¹. (b) Modified dark-field image of a capsular bag implanted with a three-piece AcrySof® MA60BM IOL⁴⁹⁴.

4.2 Aim and Objectives

The aim of this work was to produce hydrophobic IOLs by additive manufacturing. These implants were replicas of the single-piece AcrySof® IOL commercialised by Alcon. The first objective was to develop a printable hydrophobic formulation that allowed the production of transparent IOLs with an optimal balance between flexibility and mechanical strength. To achieve this, a monomer selection was carried out based on the T_g and optical properties of the resulting polymer. Then, the physico-chemical properties of the material were optimised by adjusting the concentration of crosslinker in the polymer.

The second objective was to evaluate the functionality of the resulting IOLs by performing a series of characterisation techniques that included thermal analysis, ATR-FTIR, ¹H-NMR, SEM, optical imaging, swelling tests and the evaluation of the

cytotoxicity of the material. Finally, the implantability of the IOLs was assessed by carrying out an implantation test using a human capsular bag *in vitro* model.

4.3 Materials and Methods

4.3.1 Manufacture of IOLs

Replicas of the AcrySof® IOL were produced by additive manufacturing. The CAD model used in these experiments was the same as for the manufacture of the implants described in the previous chapter. However, no support structures were added this time. As previously mentioned, the haptic-to-haptic length of the IOL created on the CAD software was 13 mm and the optic diameter was 6 mm (Figure 4.3). The thickness was maintained at 0.9 mm. Once the CAD model was created, the file was converted into a STL format and transferred to the PreForm software (Formlabs, Massachusetts, USA) in order to upload it to the printer.

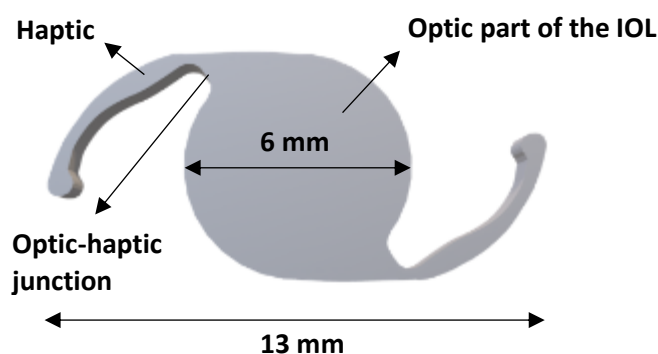


Figure 4.3: CAD model of the AcrySof® IOL.

The next step in the process was the preparation of the photopolymerisable resins. All the chemicals used in this procedure were used without further purification, unless otherwise stated. Both formulations were composed of 93% (w/w) 2-phenoxyethyl acrylate (>93% purity, TCI Chemicals, Tokyo, Japan), 7% (w/w) crosslinker and

additional 1% (w/w) of TPO (>98% purity, TCI Chemicals, Tokyo, Japan) in proportion to the total mass of the pre-polymer mix. Each resin contained a different crosslinker. In the first formulation, named C1, poly(ethylene glycol) dimethacrylate (M_n 750 Da, Sigma-Aldrich, UK) was used as a crosslinking reagent, whilst in the second formulation, C2, an aromatic urethane diacrylate (M_n 1,500 Da, kindly provided by Sartomer) was used instead (Figures 4.4 and 4.5). The chemical composition of this reagent is 4,4'-isopropylidenediphenol, ethoxylated, esters with acrylic acid and isononanoic acid. The resin was prepared by mixing these components in a round-bottom flask and stirring under an atmosphere of nitrogen until dissolution was complete. The IOLs were then manufactured using the bottom-up stereolithography system Form 2 (Formlabs, Massachusetts, USA) (Figure 4.6). Approximately 10-20 g of resin, depending on the number of IOLs to print, were poured into an optically clear PDMS substrate. Then, the CAD model was converted into a STL file which was processed on the PreForm software in order to set the printing parameters. The layer thickness was set at 0.05 mm/layer and the “Black laser” was chosen. The print was carried out without the build platform. Instead, a glass slide was placed on top of the first layer of the printed IOLs and the print was left to continue until the polymerisation of the sixth layer.

The process was stopped before completion in order to avoid over-curing. The function of the glass slide was to ensure that the surface of the lenses was smooth. The resulting IOLs were washed with isopropanol and post-cured by irradiating them with UV light for one hour in a UV curing chamber (Meccatronicore, Trento, Italy) and heating them overnight in a vacuum oven at 70 °C.

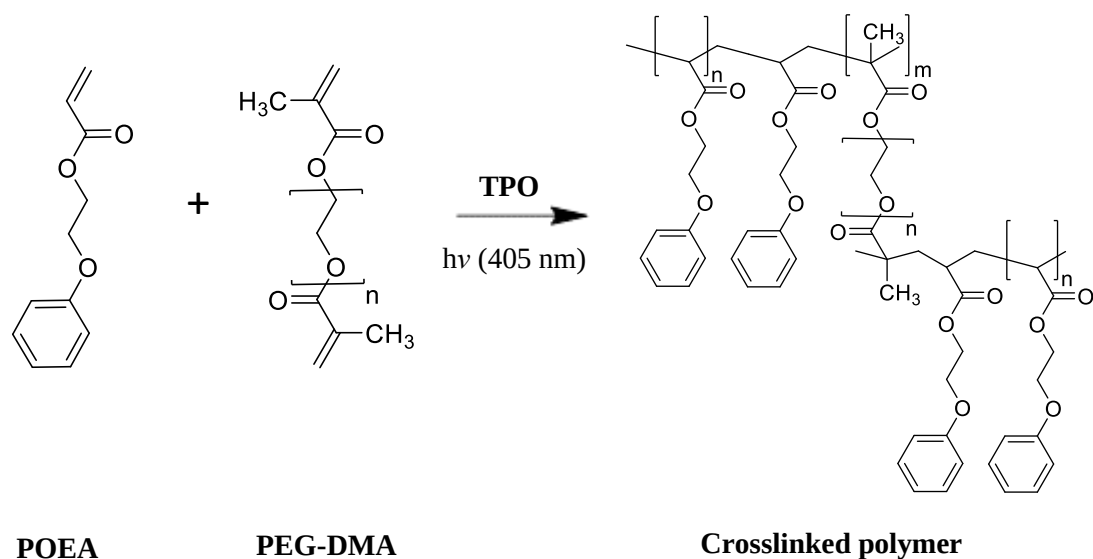


Figure 4.4: Reaction scheme detailing the photopolymerisation reaction of 2-phenoxyethyl acrylate (POEA) and poly(ethylene glycol) dimethacrylate (PEG-DMA) to form the 3D-printed crosslinked polymer structure that the IOLs are made of.

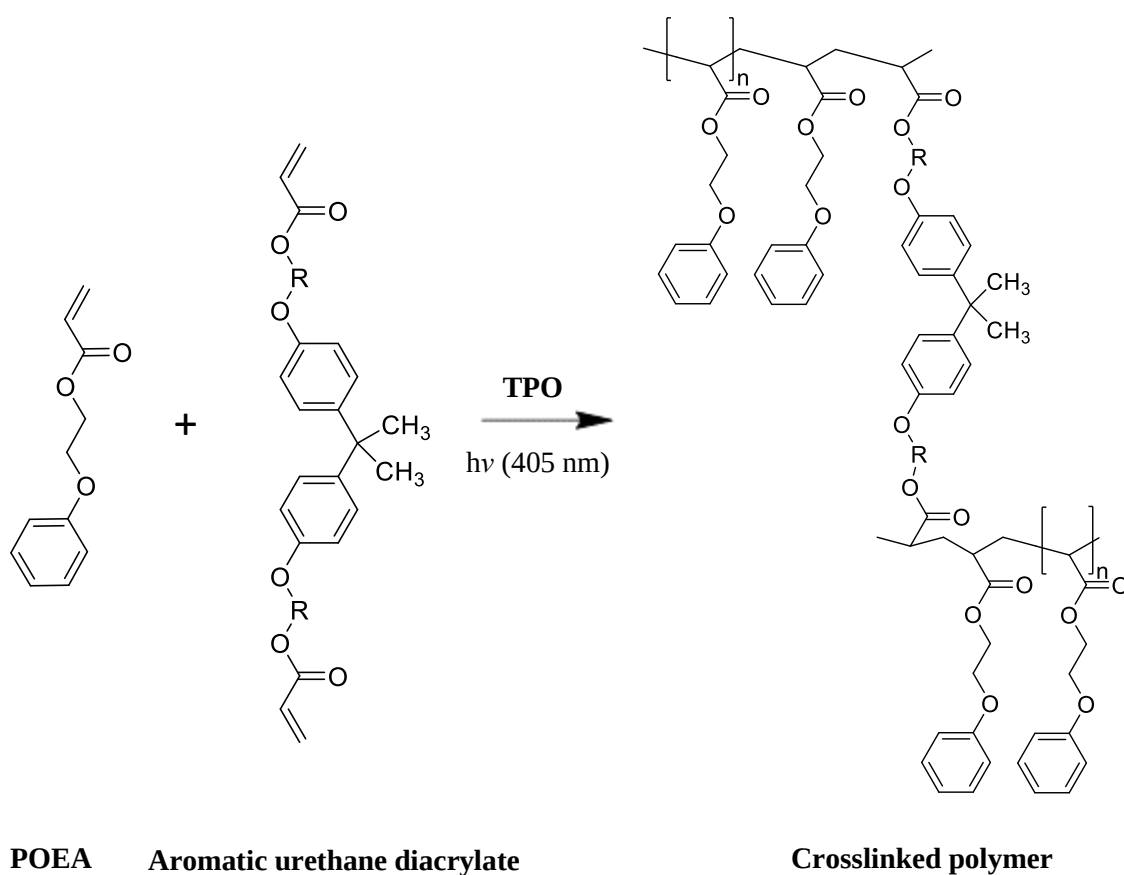


Figure 4.5: Reaction scheme detailing the photopolymerisation reaction of 2-phenoxyethyl acrylate and the aromatic urethane diacrylate crosslinker to form the 3D-printed polymer structure that the IOLs are made of.

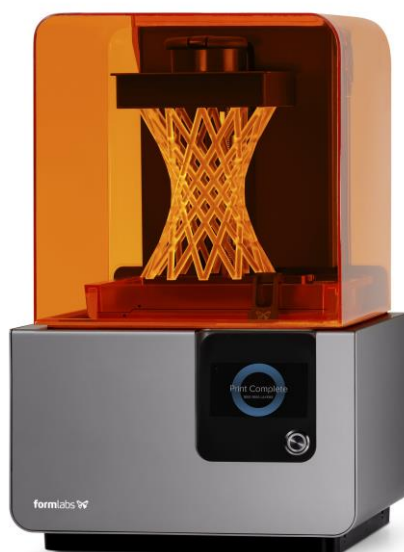


Figure 4.6: Form 2 stereolithography system.

4.3.2 Optical microscopy

Optical microscopy images of the printed IOLs were obtained using a Leica MZ7.5 stereomicroscope (Leica Camera Inc., Wetzlar, Germany). The accuracy of the dimensions respect to the CAD model was evaluated by measuring the thickness, optic diameter and haptic-to-haptic length of three IOLs made with each formulation.

4.3.3 Analysis of the efficiency of the post-curing process

The efficiency of the post-curing procedure in the elimination of the unreacted monomers that remained within the polymer after printing was evaluated by $^1\text{H-NMR}$. The analysis was performed after different treatment times in order to determine the minimum duration that was required to ensure that all the monomers had been eliminated. Thus, the experimental groups evaluated in this analysis included IOLs that had not been post-cured, lenses which had been irradiated with UV light for either one or two hours, and implants that had been subjected to thermal post-curing for either three, four, eight hours or overnight after a one-hour treatment with UV light.

Each of the samples were incubated in 800 μ L chloroform-d (99.96 atom% D, Sigma Aldrich) for one hour. After that, the incubation medium was filtered and the eluent was analysed by ^1H -NMR for detection of any unreacted monomers that might have leached from the polymer structure that forms the IOLs. The spectra were acquired at 20 °C on a Bruker 400 spectrometer at 400 MHz (Bruker Corporation, Massachusetts, USA). All chemical shifts (δ) reported are in parts per million (ppm) and they were referenced to the residual protons (^1H) of the deuterated solvent. The spectral area investigated was the region between 5.84 and 6.47 ppm, which corresponds to the signal produced by the protons attached to the alkene groups that are present in acrylate monomers. The data was processed using Topspin 3.5 pl6 software (Bruker Corporation) and Mnova v6.0.2-5475 (Mestrelab Research, Santiago de Compostela, Spain).

4.3.4 ATR-FTIR

The effectiveness of the resin curing and post-curing protocols was determined via ATR-FTIR by evaluating the depletion of the unreacted monomers after carrying out each of these procedures. Resin and polymer samples were analysed for comparison of the signal produced by the alkene groups from the monomers. The spectra were recorded using a Bruker Vertex 70 FTIR spectrophotometer (Bruker Corporation, Massachusetts, USA) by running 32 scans between 4000 and 500 cm^{-1} at a resolution of 4 cm^{-1} .

4.3.5 Water Contact Angle

The hydrophobicity of the printed IOLs was evaluated by performing water contact angle measurements using a goniometer built in-house. This device was comprised of a red LED light source, a Hamilton needle and an imaging source video camera (1080 x 768 pixel) connected to image capture software. Three IOLs made with formulation C1 and three printed with formulation C2 were analysed for statistical significance. Then, the water contact angle was measured using ImageJ (NIH, Maryland, USA).

4.3.6 Differential Scanning Calorimetry

The T_g of the polymer that forms the IOLs printed with formulations C1 and C2 was evaluated by DSC using a DSC Q2500 system (TA Instruments, New Castle, USA). Samples with a mass of 1-3 mg were pressed into aluminium DSC pans that were introduced into the equipment used to perform the analysis. The samples were subjected to a heating cycle from -50 to 150 °C, followed by a cooling step from 150 to -50 °C and a second heating ramp back to 150 °C. The experiment was performed in triplicate for dry samples and polymers that had been incubated in PBS at 35 °C for one month in order to evaluate the stability of the materials in these conditions. The scan rate for all cycles was 10 °C/min and the nitrogen flow was maintained constant at 50 mL/min. The resulting data was analysed using TRIOS software v4. 3. 1. 39215. The T_g value was obtained from the thermogram generated during the second heating cycle as the temperature at the midpoint of the decline in the heat capacity that takes place during this thermal event.

4.3.7 Thermogravimetric analysis

The thermal stability of the IOLs printed with formulations C1 and C2 was evaluated by TGA. The analysis was carried out using a TGA 5500 system (TA Instruments, New Castle, USA). Samples with a mass of 1-3 mg were placed in platinum pans and heated from 25 to 500 °C at a heating rate of 10 °C/min, under a nitrogen atmosphere. The experiment was performed in triplicate for dry samples and polymers that had been incubated in PBS at 35 °C for one month in order to evaluate the stability of the materials in these conditions. The resulting data was analysed using TRIOS software v4. 3. 1. 39215.

4.3.8 Swelling tests

The water uptake of the IOLs fabricated with formulations C1 and C2 was evaluated by performing a swelling test. Four IOLs of each formulation were incubated in 0.5 mL PBS 1X (pH 7.4) at 35 °C for one month. The weights were recorded before the

incubation and they were measured after 4, 24 and 48 hours, one week, two weeks and one month.

4.3.9 Scanning Electron Microscopy

SEM images of a post-cured IOL made with formulation C1 were taken in order to evaluate the morphology of the implants and the sharpness of the edges. The samples were mounted on aluminium stubs and coated in an argon-filled sputter gold coater (Polaron SC7640, Quorum Technologies, UK) for 30 seconds before acquiring the images in a JEOL JSM 5900 LV microscope (Jeol Ltd., Tokyo, Japan) with a 20-kV accelerating voltage.

4.3.10 Cytotoxicity assays

The toxicity of the materials used in the manufacture of the IOLs was evaluated by performing a cytotoxicity test. The efficiency of the post-curing protocol in the reduction of the toxicity of the material was also assessed by monitoring the changes in the biocompatibility of the polymers that occurred after each of the stages that comprised this treatment. Therefore, the experimental groups evaluated in this assay included IOLs that had not been post-cured, implants that had been treated with UV light for one hour and lenses that, as well as having been post-cured with UV light, had also been heated in a vacuum oven for either three, four, eight hours or overnight. A negative control group in which the cells were grown in the absence of an IOL was also included in the experiment. Three tests were carried out for each of the formulations, C1 and C2, described in this chapter.

FHL-124 cells were grown in 5% FCS-supplemented EMEM (PAN Biotech Ltd., Aidenbach, Germany) until they reached confluency. This media had been further supplemented with L-glutamine and gentamicin. The 5% FCS-supplemented media was then removed and replaced with serum-free (SF) EMEM for 24 hours. Following the period of serum starvation, the printed IOLs were pinned on top of the cells and the media was replaced with fresh SF EMEM in order to eliminate any cells that might

have died as a consequence of the mechanical stresses exerted during the introduction of the lenses. The dishes were then incubated at 35 °C in a 5% CO₂ incubator. After 48 hours, the media was collected and transferred to 1.5-mL Eppendorf tubes. A lactate dehydrogenase (LDH) analysis was performed on this media by following the protocol provided by the supplier (Roche, Basel, Switzerland). Briefly, 100 µL of media from each of the 1.5-mL tubes were transferred to a 96-well plate. This was repeated four times for each study group. After that, 100 µL of the reaction mixture were added to each well and the plate was incubated at room temperature for ten minutes in darkness. The reaction was then stopped by adding 50 µL of stop solution (1N HCl) and the absorbance was measured at 490 nm using a FLUOstar® Omega plate reader (BMG Labtech, Aylesbury, UK). Before carrying out the LDH test, the cell survival in each experimental group had been visually assessed using a Nikon Eclipse TE200 inverted microscope (Nikon, Tokyo, Japan).

4.3.11 Statistical analysis

The significance of the variations observed between the experimental groups analysed in the cytotoxicity tests was evaluated by performing a one-way ANOVA analysis with a post-hoc Tukey test. The mean and standard deviation were calculated using Microsoft Excel® 2016 software. The statistical package GraphPad Prism v.6.01 was used for the statistical analysis. The level of significance was set at $p < 0.05$.

The significance of the differences in the dimensions of the IOLs, as well as of the variations in the T_g and IDT after incubation in an aqueous environment, were analysed by performing a Mann-Whitney test (non-parametric). GraphPad Prism v.6.01 was also used in these analyses, and the level of significance was set at $p < 0.05$.

4.3.12 Implantability test

A suspended human capsular bag system was used to evaluate the IOL implantation into a capsular bag. Donor human eye globes were obtained with UK National

Research Ethics Committee approval (REC 04/Q0102/57) and used in accordance with the tenets of the Declaration of Helsinki. The donor eyes were received with the cornea removed and they were placed on a dissecting platform, where the iris was removed carefully using surgical forceps. The ciliary body was then dissected free of the surrounding tissues using curved corneal scissors. The ciliary body remains attached to the lens by the zonules of Zinn. The ciliary-zonule-lens complex was then transferred to a Petri dish and secured to a silicone support ring through the ciliary body using entomological pins. This allows the lens to suspend in the lumen of the ring. A continuous curvilinear capsulorhexis, approximately 5mm in diameter, was introduced on the anterior surface of the lens capsule. This creates an opening to allow the central fibre mass to be removed via hydrodissection, leaving the capsular bag in the dissecting platform. Viscoelastic was introduced to the capsular bag in order to inflate it, hence facilitating the implantation of the IOL. Then, the 3D-printed IOL was folded and inserted into a DualTec injector (Ophtec, Groningen, The Netherlands), which was used to implant the IOL into the capsular bag. The author would like to thank Prof. Michael Wormstone for carrying out this experiment.

4.4 Results

4.4.1 Manufacture of IOLs

Hydrophobic acrylic IOLs which had the same design as Alcon AcrySof® were successfully produced by stereolithography. This process involved the development of two novel printable resins which contained 2-phenoxyethyl acrylate as the main monomer. As previously mentioned in the Materials and Methods section, the formulation C1 contained PEG-DMA as a crosslinker, while for the preparation of C2 an aromatic urethane diacrylate crosslinking agent was used instead. During the development of these resins, the monomer and crosslinker were mixed at different ratios and the composition that showed an optimum balance between mechanical strength and flexibility was chosen as the definitive formulation for the production of the IOLs (Table 4.1). The optimal concentration of crosslinker was 7% (w/w) for both formulations. The photoinitiator was maintained at an additional 1% (w/w) in

proportion to the total mass of the resin in all the compositions tested in these experiments.

Table 4.1: Formulations tested during the development of the printable formulations C1 and C2. The crosslinker used for formulation C1 was PEG-DMA (M_n 750 Da), while for C2 an aromatic urethane diacrylate crosslinker (M_n 1,500 Da) was used instead.

Monomer (%)	Crosslinker (%)	Results
96	4	Very poor mechanical strength. Over-curing around the edges.
94	6	Poor mechanical strength
93	7	Optimal flexibility and mechanical strength

While all the printed lenses resembled the AcrySof® IOL, some issues were observed which would affect their suitability for use as implantable devices. Firstly, it was not possible to control the thickness of the lenses, as the absence of the build platform while printing did not allow for a layer-by-layer production with control of the cure depth. Moreover, the diameter of the optic portion was larger than in the original model, which could have a detrimental effect on the performance of the IOL when implanted in the capsular bag. An inward tapering of the edges of the lenses was also detected, which resulted in a larger optic diameter at the bottom than at the top of the IOLs (Figure 4.7). This was a consequence of the non-straight shape of laser beams, which leads to the formation of tapered structures. While this effect is not noticeable in very thin constructs, it becomes more perceptible as the thickness increases. This was the case for the IOLs that resulted from the manufacturing process herein described, since they were printed without the build platform that controls the thickness of the constitutive layers. The low reproducibility of the results was an additional issue encountered in these experiments. Some IOLs presented some over-curing effects around the edges which led to a decrease in the dimensional accuracy, while this effect was not perceptible in other lenses.

An evaluation of the dimensions of the printed IOLs was carried out in order to evaluate the dimensional accuracy obtained with the stereolithography system employed in these experiments. The thickness and the optic diameter of both top and bottom surfaces of the lenses were measured in three IOLs printed with formulation C1 and other three printed with C2. The results from the Mann-Whitney tests showed that the differences between the optic diameter measured at the top and bottom of the lenses were not statistically significant for either C1 or C2 IOLs ($p = 0.1$ in both cases). However, larger sample sizes would be required in order to increase the reliability of this data. It is important to note that average errors of $+5.5 \pm 0.42\%$ and $+4.1 \pm 1.9\%$ for C1 and C2, respectively, were calculated with respect to the diameter of the IOL created on the CAD software (6 mm) (see dimensions in Figure 4.7 and Appendix 3). The haptic-to-haptic length was also measured in triplicates, being 11.26 ± 0.5 mm for C1 and 12.97 ± 0.26 mm for C2. However, these measurements were not used for the calculation of the dimensional error due to the bias added by the different haptic-optic angles that each IOL adopted. With regard to the thickness of the lenses, the dimensional errors calculated with respect to the CAD model (0.9 mm) were $+64.5 \pm 7.98\%$ and $+99 \pm 3.9\%$ for C1 and C2, respectively. The high variability in this parameter between both formulations may be a consequence of the differences in the cure depth and the lack of control over the thickness of the printed parts due to the absence of the build platform during the printing process.

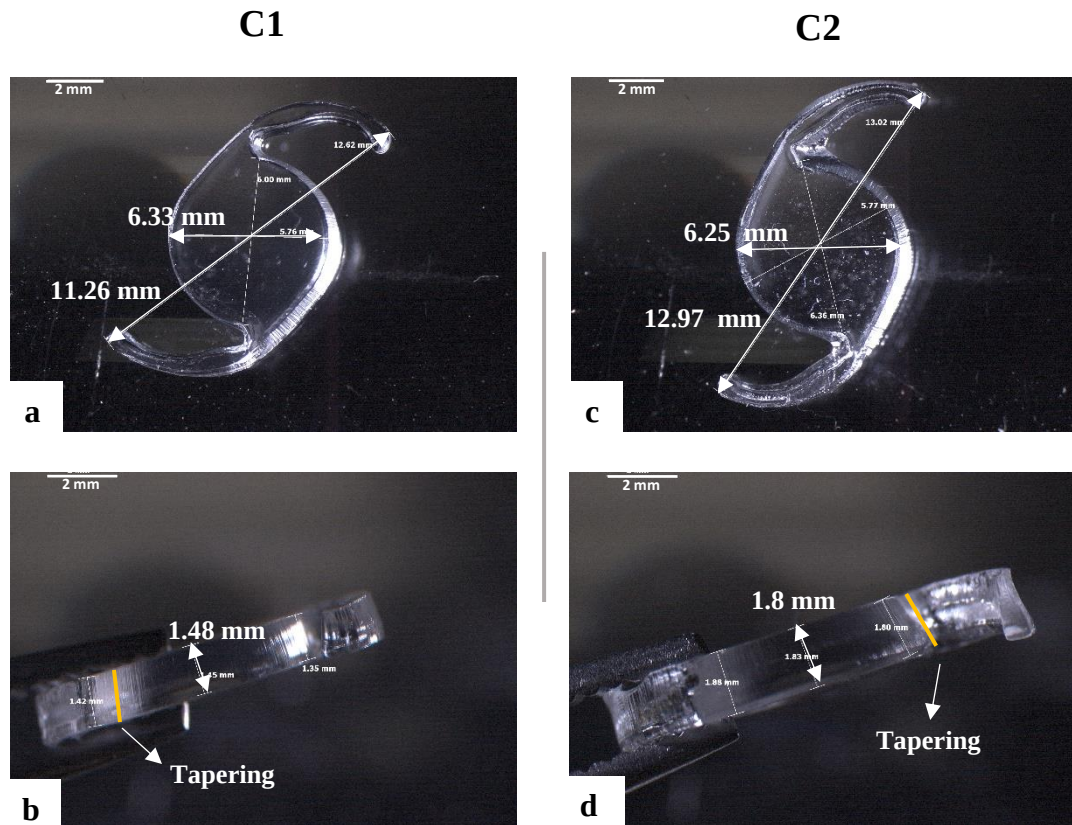


Figure 4.7: Optical microscopy images of IOLs printed with formulation C1 (**a, b**) and C2 (**c, d**) with their dimensions measured using the microscope. (**a**) and (**c**) show the top view of the implants while (**b**) and (**d**) show a side view of the same.

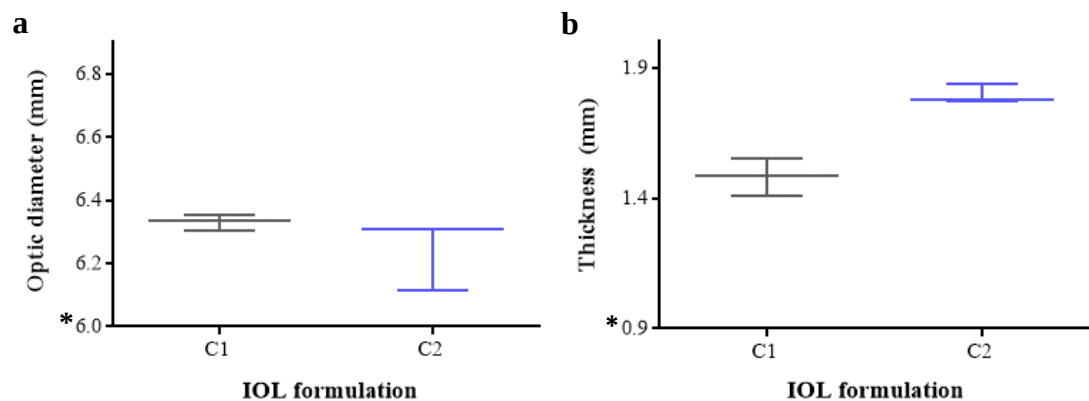


Figure 4.8: Variability in the optic diameter (**a**) and the thickness (**b**) of the IOLs printed with formulations C1 and C2. The dimensions that are denoted on the Y-axis with an asterisk correspond to the diameter and thickness of the CAD model.

The transparency of the printed IOLs is another characteristic which needs to be carefully evaluated due to the ophthalmic purpose of these devices. Whilst both C1 and C2 were translucent when dry, the latter had a greater degree of transparency (Figure 4.9). This was due to the presence of benzyl rings in the crosslinker used in this formulation. It is important to note that these lenses had not been polished, which is an important step in improving the optical quality of these devices. The transparency of the IOLs was maintained when incubated in an aqueous environment at 35 °C (Figure 4.10, a and c). However, they became more opaque as they cooled down back to room temperature (Figure 4.10, (b) and (d)). As no fluid was absorbed by the polymers (see Swelling tests section), it was hypothesised that this could be due to changes in the arrangement of the polymer chains during these transitions between different conditions.

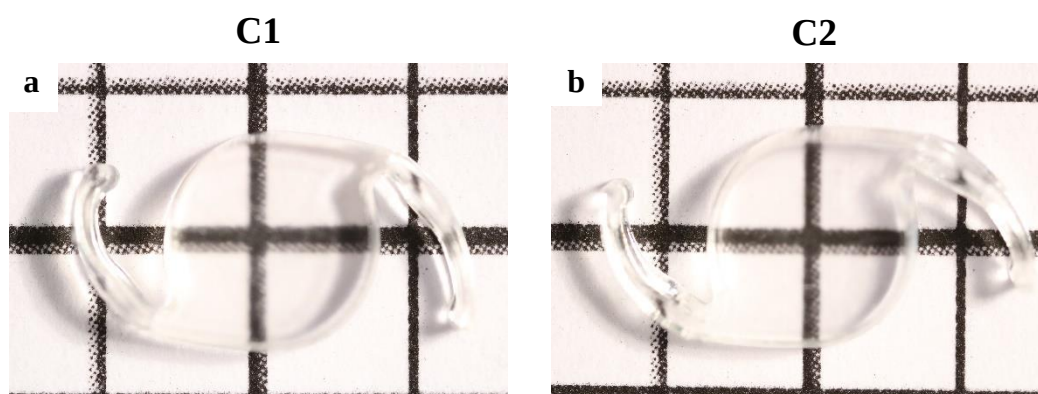


Figure 4.9: Optical images of IOLs printed with formulation C1 (**a**) and C2 (**b**). An increase in the optical quality is perceptible with formulation C2 in comparison with C1.

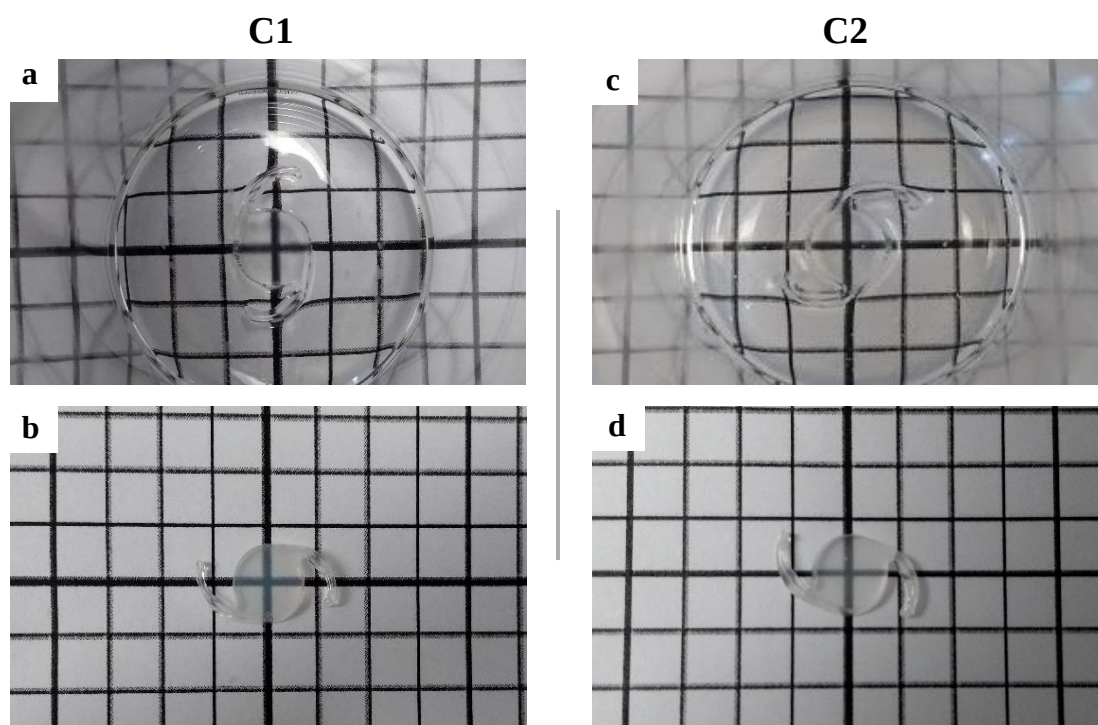


Figure 4.10: Digital images of IOLs printed with formulations C1 **(a, b)** and C2 **(c, d)**. Images **(a)** and **(c)** show the IOLs incubated in PBS at 35 °C, while images **(b)** and **(d)** show the same implants after incubation and cooled down to room temperature. A decrease in transparency is noticeable in the latter.

Finally, it was observed that the printed IOLs demonstrated a good balance between mechanical strength and flexibility. The IOLs were foldable when dry (Figure 4.11) and they maintained their flexibility and mechanical strength after incubation in an aqueous environment. This facilitated their implantation in a human capsular bag model *in vitro* by performing a sham cataract surgery (see Implantability tests section). This indicated that the printed IOLs herein described could potentially be used in a clinical setting after the pertinent improvements in transparency and refinements in the manufacturing process have been made.

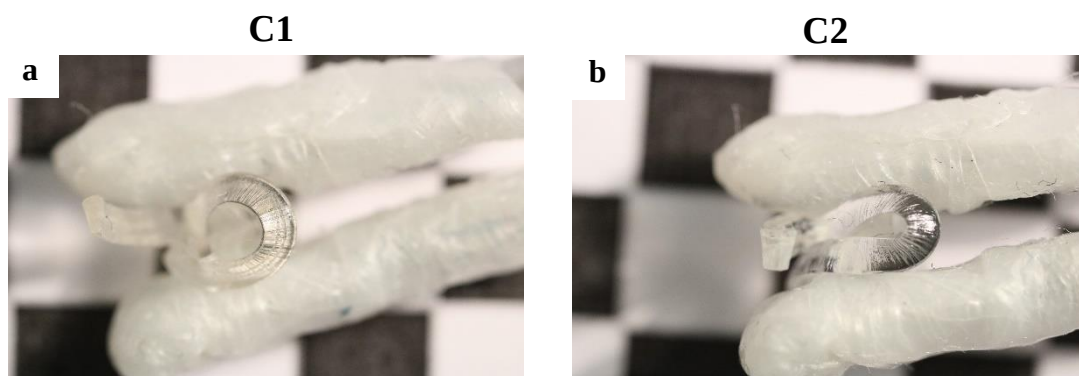


Figure 4.11: Optical images depicting the foldability of an IOL printed with formulation C1 **(a)** and an analogous IOL produced with formulation C2 **(b)**.

4.4.2 Analysis of the efficiency of the post-curing process

Any object produced via stereolithography contains unreacted monomers in its structure. This might compromise the mechanical performance and biocompatibility of the device. Therefore, the printed IOLs were subjected to a post-curing process in order to complete the polymerisation of the residual monomers, thus preventing the cytotoxic effects elicited by these molecules when the implants are introduced in a biological system. The post-curing treatment consisted of two procedures: the irradiation of the IOLs with UV light and a thermal treatment in a vacuum oven at 70 °C. The monomer depletion during these processes was monitored in both C1 and C2 IOLs via proton nuclear magnetic resonance (^1H -NMR, 400 MHz, CDCl_3 , ppm): $\delta = 6.47\text{-}6.42$ (1H, dd, $J = 18.72$ Hz), $\delta = 6.2\text{-}6.13$ (1H, dd, $J = 27.76$ Hz), $\delta = 5.87\text{-}5.84$ (1H, dd, $J = 11.84$ Hz). The results showed that the post-curing protocol was successful in eliminating the unreacted monomers that remained within the polymer structure of the printed IOLs. This experiment also helped to determine the minimum time of UV irradiation and heating required to polymerise all the residual monomers. The number of unreacted molecules decreased considerably after one hour of UV treatment, but it did not decline any further with a longer irradiation time (Figure 4.12). Thermal post-curing was thus needed in order to complete the polymerisation of the radicals that remained within the polymer. As seen in Figure 4.12, no signal was detected for the acrylate groups from monomers after heating the IOLs for four hours in vacuum. This was also the case for the samples that had been heated for either

eight hours or overnight. Therefore, it was concluded that an effective post-curing process should include a UV-irradiation treatment of approximately one hour and a thermal post-curing step that should last for at least four hours.

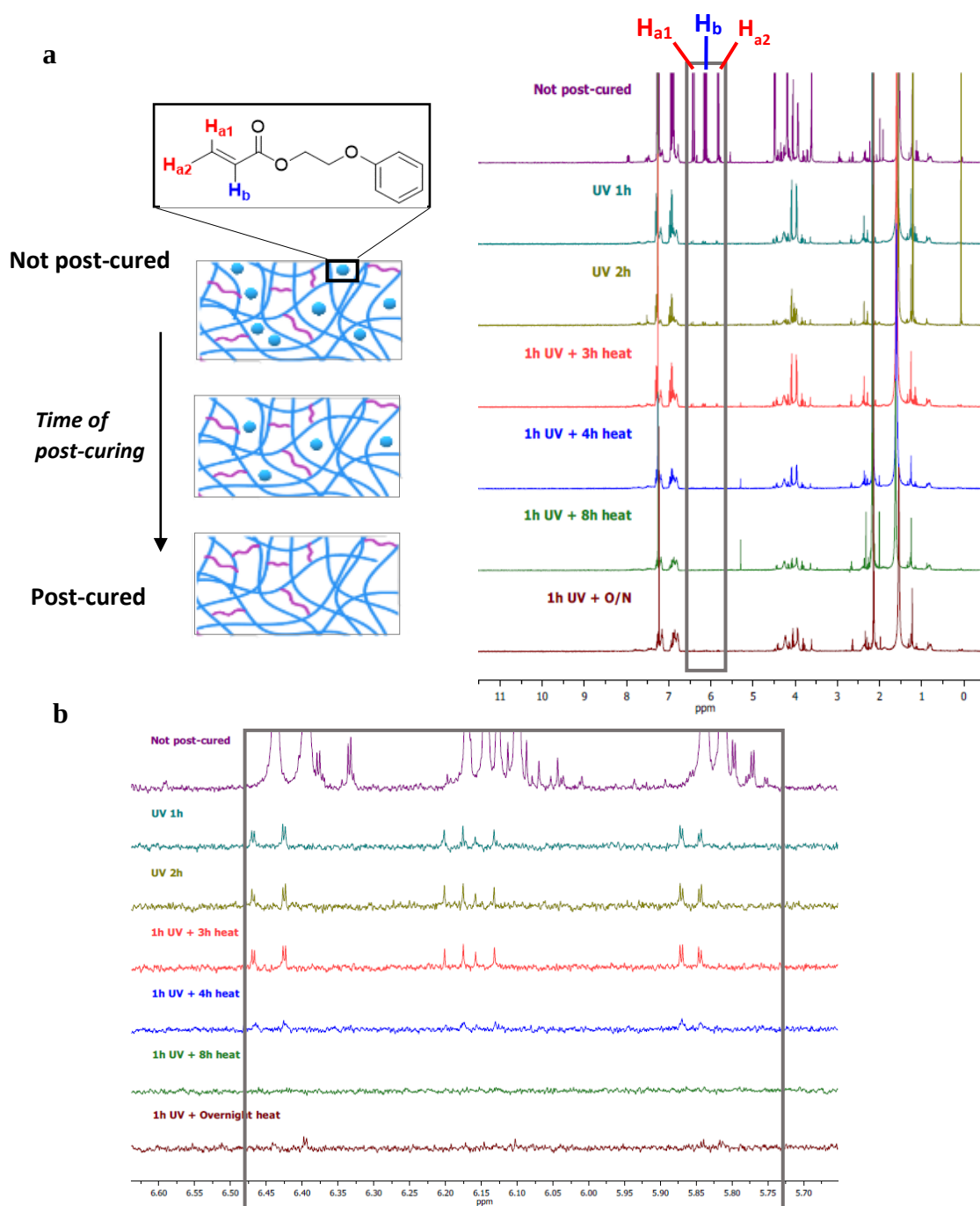


Figure 4.12: ^1H -NMR spectra obtained from the analysis of the efficiency of the post-curing process using IOLs printed with formulation C1. A depletion in the amount of residual monomers was observed with longer post-curing times, hence confirming the effectiveness of this protocol to eliminate the leachable molecules from the polymers **(a)**. An enlarged view of the signal produced by the acrylate groups from the unreacted monomers is shown **(b)**. The same results were obtained when repeating this procedure using IOLs that had been printed with formulation C2 (See Appendix 4).

4.2.3 ATR-FTIR

The effectiveness of the curing and post-curing processes was evaluated via ATR-FTIR by analysing both the resin used for printing the IOLs and samples of printed lenses before and after being post-cured. This analysis was performed for both C1 and C2 formulations and it was based on the detection of the signal produced by the carbon-carbon double bond from the acrylate group of the monomers present in the resin and polymer samples. This signal appeared at a frequency of $1,630\text{ cm}^{-1}$ and, as seen in the resulting spectra (Figure 4.13), its intensity was considerably higher in the spectrum generated from the analysis of the resin than in the printed polymers. Furthermore, the intensity of this signal was lower in the post-cured samples than in the not post-cured counterparts, hence confirming the efficiency of the post-curing process for the elimination of unreacted monomers from the polymer. The intensity of the peak observed at approximately $1,410\text{ cm}^{-1}$ had also decreased after curing and post-curing, since this signal was generated by the $=\text{CH-H}$ bonds. Finally, another confirmation of the efficiency of the curing and post-curing processes was provided by the increase in the intensity of the peak observed at $2,915\text{ cm}^{-1}$, which was generated by the methylene bonds present in the polymer chains.

With regard to the rest of the spectra, the peaks generated by the C-O-C and C=O bonds ($1,160$ and $1,725\text{ cm}^{-1}$, respectively) confirmed the presence of esters in the polymers (Figure 4.14). These functionalities were present in the structure of the acrylate monomers that formed these materials. Moreover, the phenyl groups from the main monomer yielded a series of signals that are characteristic of aromatic functionalities: aromatic C-H stretch ($3,055\text{ cm}^{-1}$), aromatic C-H ring bend ($670\text{--}1,225\text{ cm}^{-1}$) and aromatic ring stretch (a pair of bands at $1,480\text{ cm}^{-1}$) (Table 4.2). The main difference between both formulations was the presence of urethane groups in the crosslinker used for the synthesis of C2. The N-H vibrations from this functional group would generate a band at $1,590\text{--}1,650\text{ cm}^{-1}$, while the N-H stretching would produce a peak at $3,460\text{--}3,510\text{ cm}^{-1}$ ^{495,496}. However, no additional peaks on these regions were detected on the spectra obtained from the analysis of C2 IOLs when compared to C1. This might be a consequence of the overlapping between signals.

Overall, this analysis confirmed the effectiveness of the curing process performed in the 3D printer and the efficiency of the post-curing protocol for the elimination of the unreacted monomers that remained within the polymer after printing. Furthermore, a confirmation of the similarity between the chemical composition of formulations C1 and C2 was provided by this study.

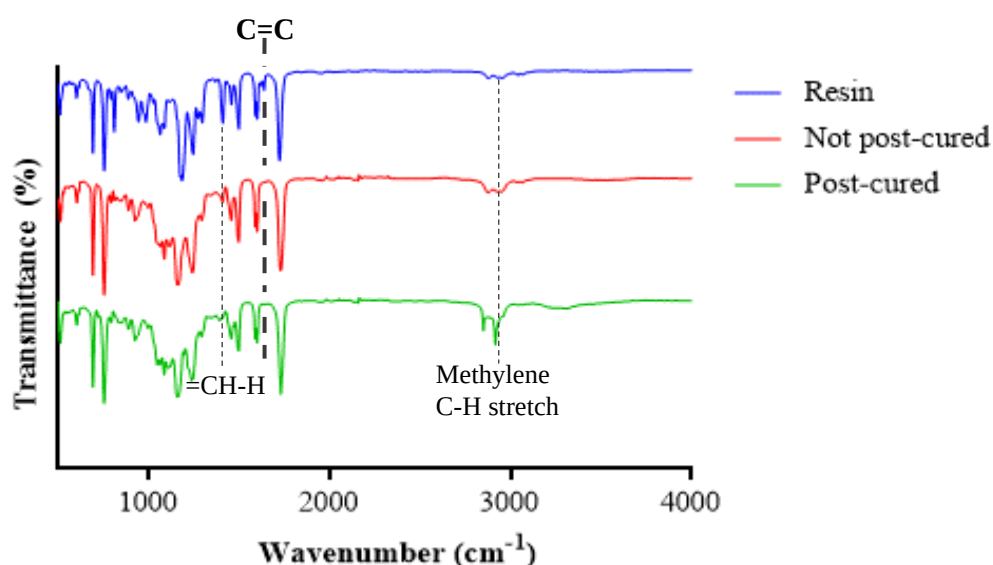


Figure 4.13: ATR-FTIR spectra obtained from the analysis of the resin used for the production of C1 IOLs (blue) and from lenses printed with this formulation, both before (red) and after being post-cured (green). A reduction in the intensity of the signal generated by the C=C functional groups from acrylate monomers was observed after both curing and post-curing. The same results were obtained when repeating this analysis with IOLs that had been printed with formulation C2 (Appendix 5).

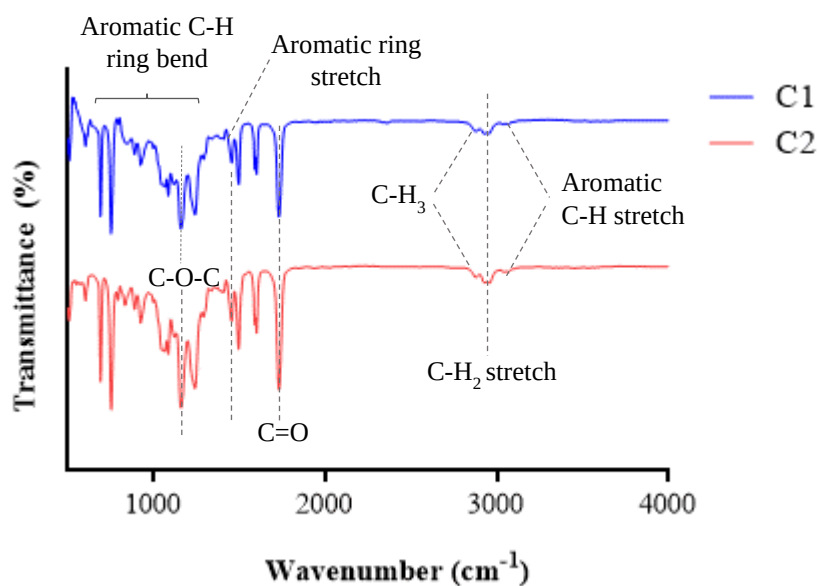


Figure 4.14: Comparison between the ATR-FTIR spectra obtained from the analysis of IOLs printed with either formulation C1 (blue) or C2 (red) and subjected to post-curing.

4.2.4 Water Contact Angle

The hydrophobicity of the printed IOLs was assessed by performing water contact angle measurements. These tests revealed that both C1 and C2 formulations were highly hydrophobic, with a contact angle of $82.3 \pm 3.2^\circ$ and $83.6 \pm 14.5^\circ$, respectively (Figure 4.15). According to the literature, the water contact angle of AcrySof[®] is $84.4 \pm 0.09^\circ$ ⁴⁹⁷. Thus, while the composition of the printed IOLs was different to that of the commercial lens used as a reference, the hydrophobicity of these implants was comparable.

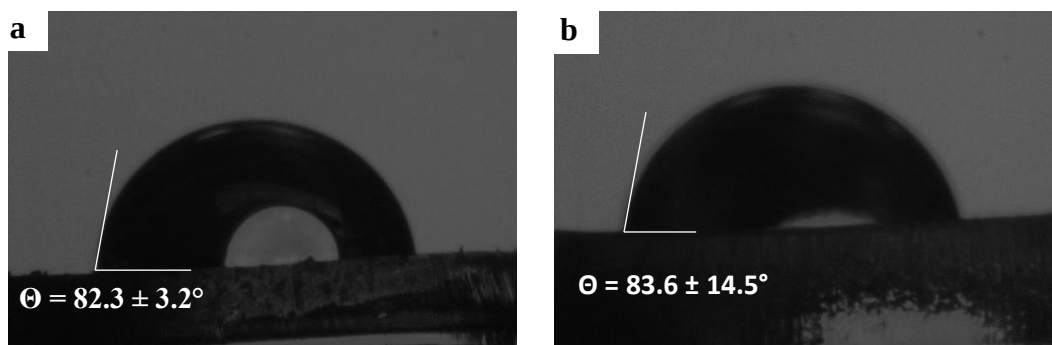


Figure 4.15: Water contact angle of **(a)** C1 and **(b)** C2 IOLs.

4.2.5 Differential Scanning Calorimetry

The T_g of the printed IOLs was evaluated by DSC in order to obtain a quantifiable measurement of the flexibility of the polymers which could be compared to that of the AcrySof[®] IOL. The lower the T_g , the lower the temperature at which the material transitions from a glass-like state to a rubbery form. According to the results obtained from these analyses, the T_g of the IOLs made with formulation C1 ($4.73 \pm 0.32^\circ\text{C}$) was lower than that of the lenses printed with the resin known as C2 ($10.27 \pm 0.59^\circ\text{C}$) (Figures 4.16 and 4.17, respectively). This was in agreement with the hypothesis suggested during the development of the formulations, which stated that the formulation C2 would have a higher mechanical strength as a consequence of the presence of phenyl groups in the crosslinker as well as in the main monomer. The superior mechanical properties of the crosslinker used for the production of formulation C2 is evidenced by the high T_g of the polymer that it forms (55°C).

When compared with AcrySof[®], the values of T_g of the printed lenses were lower in both C1 and C2 IOLs than in the commercial implant, which has an approximate T_g of 15°C . However, the T_g of formulation C2 was within the range of preferable glass transition temperatures recommended by the manufacturer ($10\text{-}20^\circ\text{C}$)¹⁸⁸. Therefore, it can be concluded that formulation C2 would be the most appropriate in terms of flexibility for the fabrication of IOLs.

The T_g of C1 and C2 IOLs that had been incubated in an aqueous environment at 35 °C for one month was also analysed in order to assess the long-term stability of the materials when subjected to physiological conditions (Figures 4.18 and 4.19). The results showed that the T_g of the polymers was not altered when storing the IOLs in an aqueous medium for a relatively long period of time. This conclusion was achieved after performing a Mann-Whitney test in order to compare the T_g of each material before and after incubation. This test revealed that the differences were not statistically significant in any case ($p = 0.7$ for C1 and $p = 0.2$ for C2). However, it would have been beneficial to use larger sample sizes in order to increase the strength of the statistical analyses.

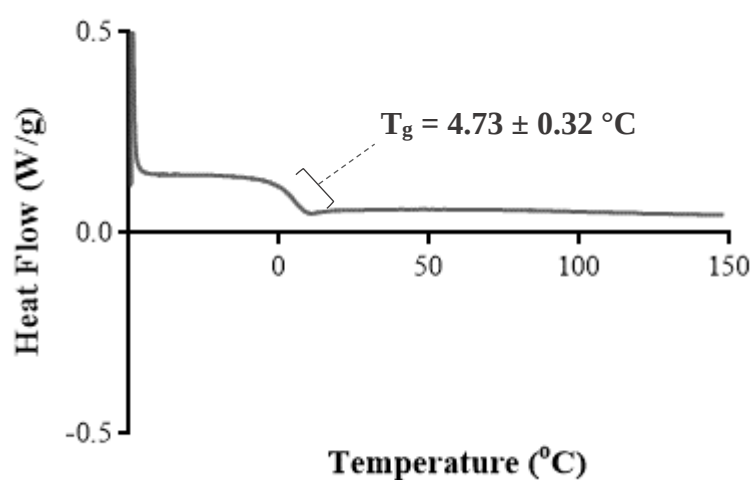


Figure 4.16: Thermogram obtained from the third cycle of the DSC analysis of the polymer that forms the IOLs printed with formulation C1. The value of T_g is $4.73 \pm 0.32 \text{ } ^\circ\text{C}$.

Table 4.2: Values of T_g of formulation C1 obtained from the replicates analysed by DSC.

Sample	$T_g \text{ (} ^\circ\text{C)}$
1	4.42
2	5.06
3	4.72
Average	4.73 ± 0.32

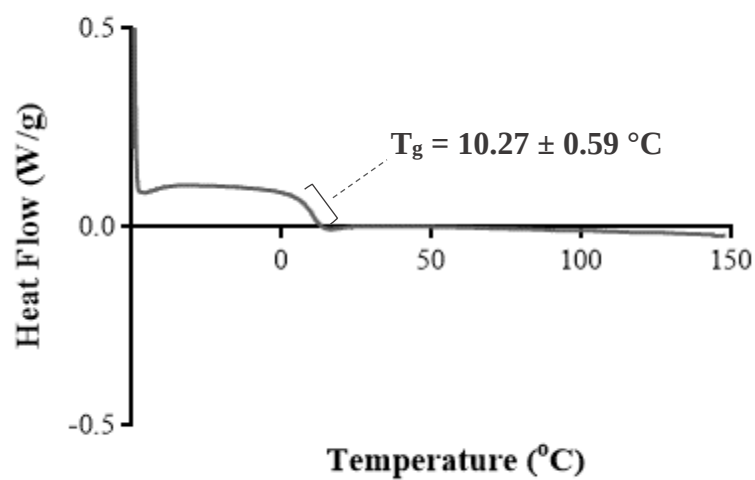


Figure 4.17: Thermogram obtained from the third cycle of the DSC analysis of the polymer that forms the IOLs printed with formulation C2. The value of T_g is 10.27 ± 0.59 °C.

Table 4.3: Values of T_g of formulation C2 obtained from the replicates analysed by DSC.

Sample	T_g (°C)
1	10.04
2	9.84
3	10.94
Average	10.27 ± 0.59

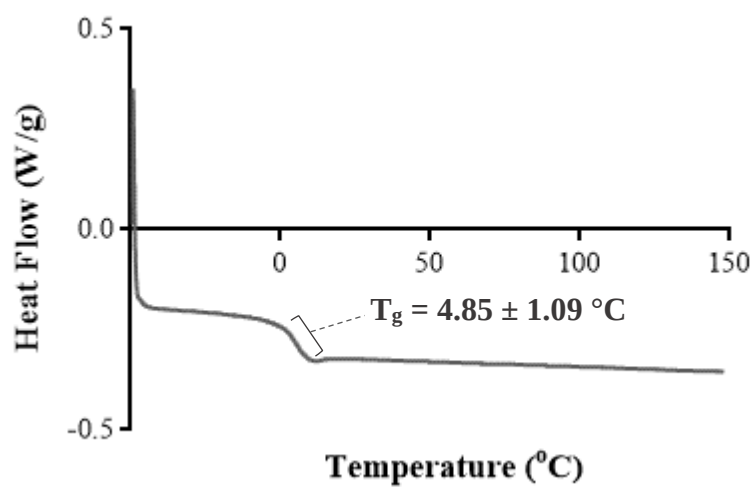


Figure 4.18: Thermogram obtained from the third cycle of the DSC analysis of the polymer that forms the IOLs printed with formulation C1 and incubated in an aqueous environment at 35 °C for one month. The value of T_g is $4.85 \pm 1.09 \text{ } ^\circ\text{C}$.

Table 4.4: Values of T_g of formulation C1 after incubation in an aqueous environment at 35 °C for one month obtained from the replicates analysed by DSC.

Sample	$T_g \text{ (} ^\circ\text{C)}$
1	6.11
2	4.2
3	4.25
Average	4.85 ± 1.09

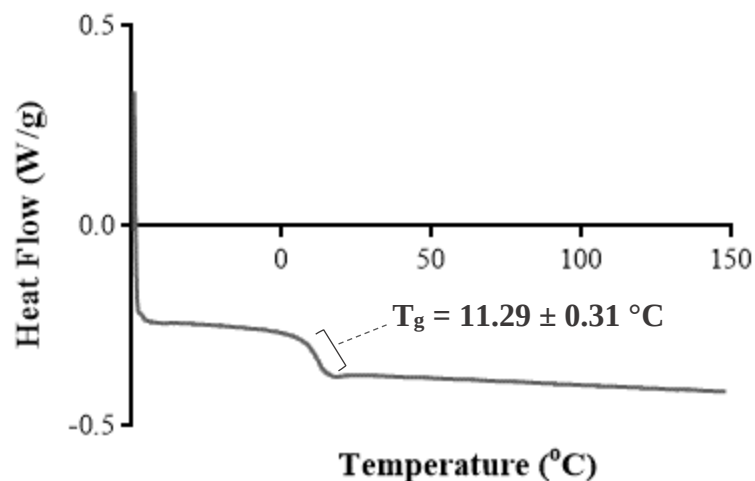


Figure 4.19: Thermogram obtained from the third cycle of the DSC analysis of the polymer that forms the IOLs printed with formulation C2 and incubated in an aqueous environment at 35 °C for one month. The value of T_g is $11.29 \pm 0.31 \text{ } ^\circ\text{C}$.

Table 4.5: Values of T_g of formulation C2 after incubation in an aqueous environment at 35 °C for one month obtained from the replicates analysed by DSC.

Sample	$T_g \text{ (} ^\circ\text{C)}$
1	11.5
2	10.93
3	11.44
Average	11.29 ± 0.31

4.2.6 Thermogravimetric analysis

The thermal stability of the printed IOLs was analysed by TGA. According to the resulting thermograms (Figures 4.20 and 4.21), the polymers started to degrade right before reaching a temperature of 400 °C. The onset of the degradation, also known as initial decomposition temperature (IDT) of formulation C1 was slightly lower ($386.7 \pm 1.72 \text{ } ^\circ\text{C}$) than that of C2 ($389.7 \pm 2.63 \text{ } ^\circ\text{C}$). As the thermal stability of a material is usually proportional to its mechanical strength, these analyses confirmed the superior mechanical properties of formulation C2 in comparison with C1. The printed IOLs

were also analysed after a one-month incubation in an aqueous medium at 35 °C in order to assess the stability of the material when stored in these conditions (Figures 4.22 and 4.23). The results showed that the storage of the lenses in this environment did not affect their thermal stability, as the Mann-Whitney test revealed that the differences in IDT between IOLs which had or had not been incubated under physiological conditions were not statistically significant neither for C1 nor C2 ($p = 0.7$ in both cases). However, the use of larger sample sizes would have increased the strength of the statistical analyses by allowing the use of parametric tests.

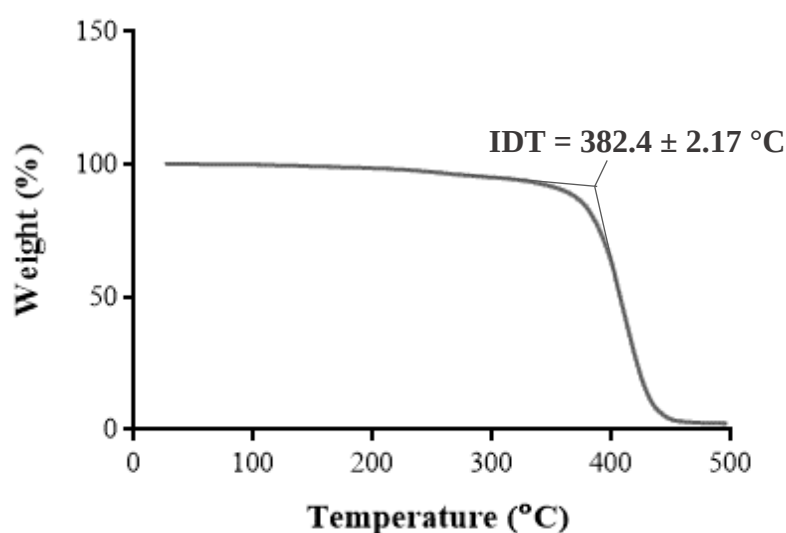


Figure 4.20: Thermogram obtained from the thermogravimetric analysis of C1 IOLs. The IDT is 382.4 ± 2.17 °C.

Table 4.6: Values of IDT of formulation C1 obtained from the replicates analysed by TGA.

Sample	IDT (°C)
1	380.13
2	382.63
3	384.46
Average	382.4 ± 2.17

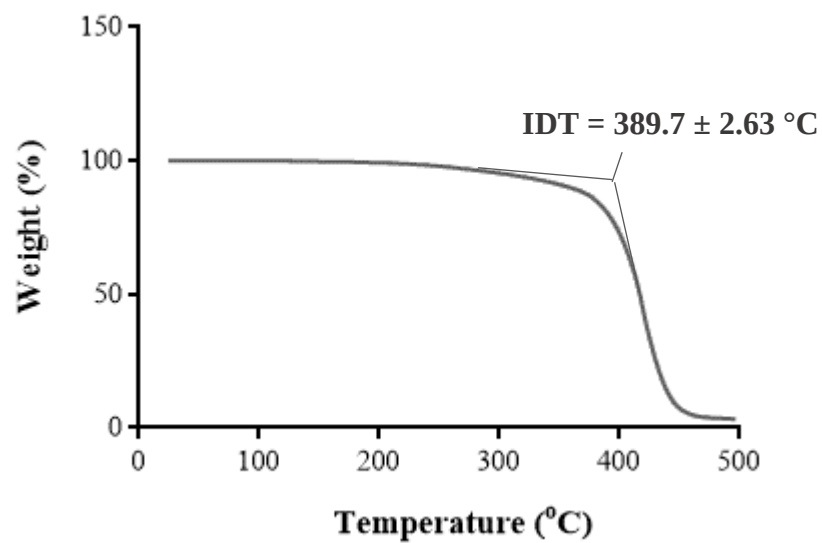


Figure 4.21: Thermogram obtained from the thermogravimetric analysis of C2 IOLs. The IDT is 389.7 ± 2.63 °C.

Table 4.7: Values of IDT of formulation C2 obtained from the replicates analysed by TGA.

Sample	IDT (°C)
1	392.29
2	387.03
3	389.77
Average	389.7 ± 2.63

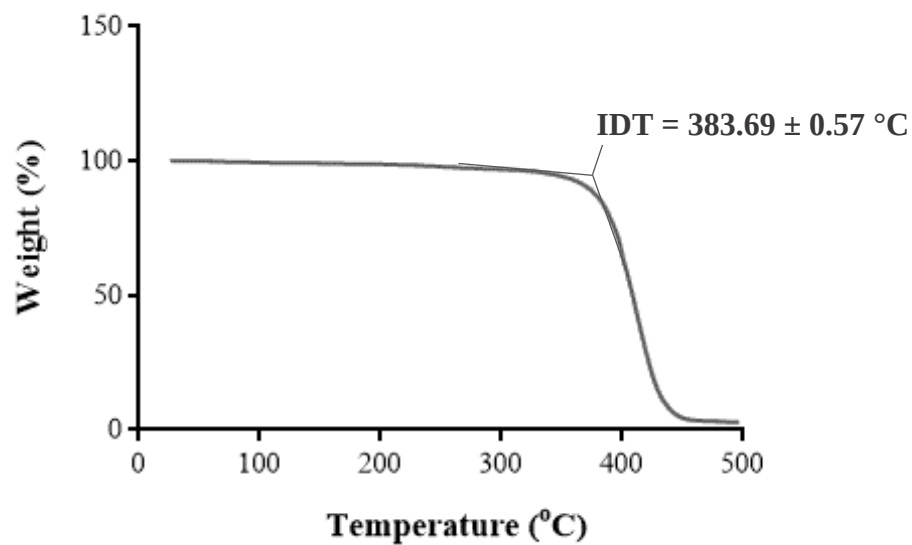


Figure 4.22: Thermogram obtained from the thermogravimetric analysis of C1 IOLs after incubation in an aqueous environment at 35 °C for one month. The IDT is 383.69 ± 0.57 °C.

Table 4.8: Values of IDT of formulation C1 after incubation in an aqueous medium obtained from the replicates analysed by TGA.

Sample	IDT (°C)
1	383.05
2	384.14
3	383.88
Average	383.69 ± 0.57

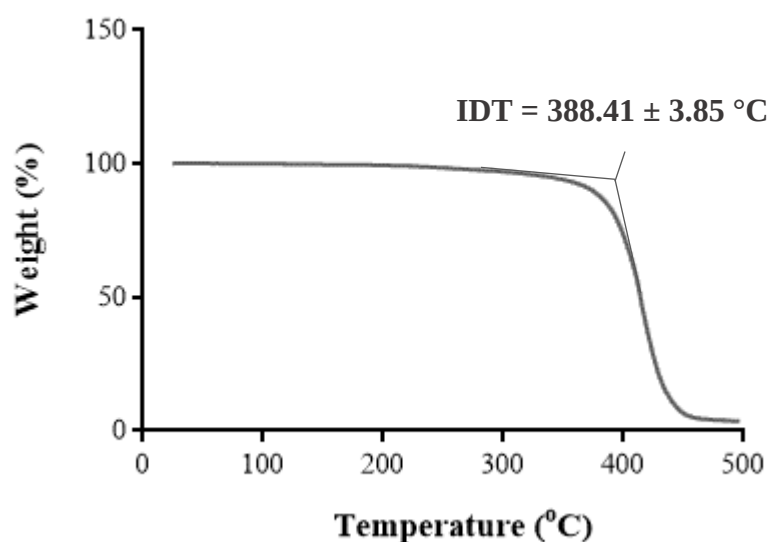


Figure 4.23: Thermogram obtained from the thermogravimetric analysis of C2 IOLs after incubation in an aqueous environment at 35 °C for one month. The IDT is $388.41 \pm 3.85 \text{ }^{\circ}\text{C}$.

Table 4.9: Values of IDT of formulation C2 after incubation in an aqueous medium obtained from the replicates analysed by TGA.

Sample	IDT (°C)
1	384.24
2	389.15
3	391.84
Average	388.41 ± 3.85

4.2.7 Swelling tests

The behaviour of the IOLs in an aqueous environment comparable to that of the ocular posterior chamber was evaluated by performing a swelling test. The implants were incubated in PBS 1X (pH 7.4) at 35 °C for one month. The weight of the IOLs was recorded at different points during this period of time in order to assess the uptake of water. The results showed that the weight of the lenses printed with formulation C1 stayed almost constant over the duration of the analysis, as they did not uptake any water. However, the weight of C2 IOLs decreased by approximately 10% during the

last two weeks of the experiment (Figures 4.24 and 4.25). It was hypothesised that this could be due to the degradation of the crosslinker, since this polymer contains esters that could be hydrolysed in an aqueous environment. Thus, long-term degradation studies should be conducted in order to evaluate this theory.

Overall, this experiment provided further confirmation of the similarity between the chemical properties of the printed IOLs and AcrySof[®], as the latter has an equilibrium water content of approximately 0.1%⁴⁹⁸ and the printed lenses did not absorb any water during the analysis herein described.

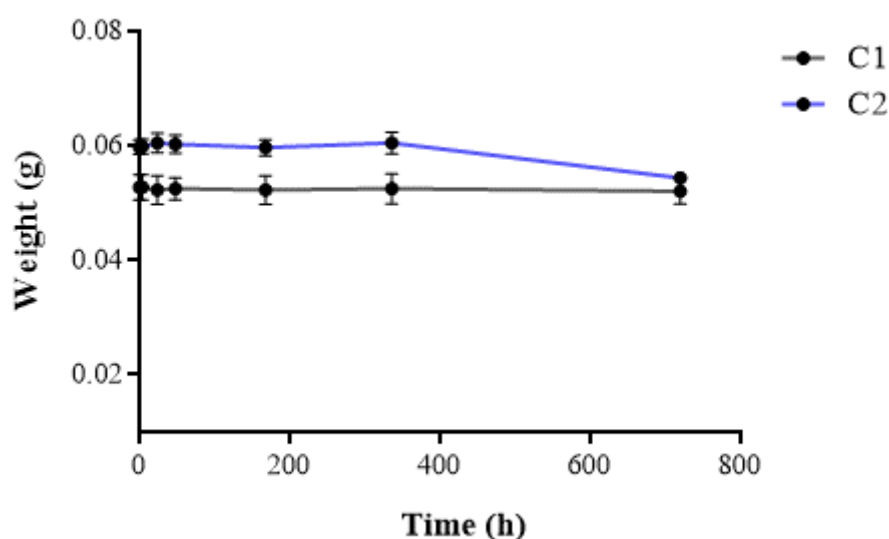


Figure 4.24: Line graph showing the variation in weight of C1 and C2 IOLs incubated in an aqueous environment for one month.

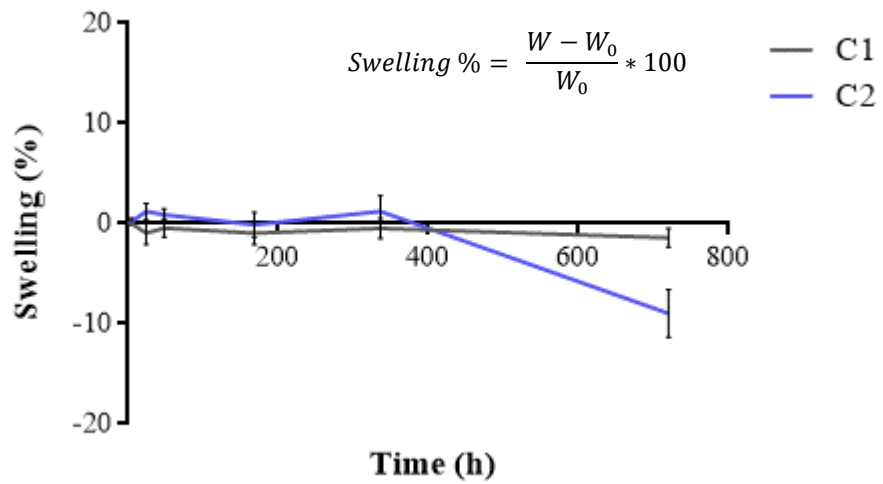


Figure 4.25: Line graph showing the swelling percentage of IOLs made with formulations C1 and C2.

4.2.8 Scanning Electron Microscopy

The morphology of the IOLs was evaluated by taking images of their constituent parts via SEM. A characteristic that an IOL must possess in order to prevent PCO is the presence of a sharp edge on the posterior side of the lens, which prevents the migration of LECs to the posterior side of the capsular bag. As shown in Figure 4.26, the printed IOLs possessed this design feature. Moreover, they also possessed a round anterior edge and a slanted profile which, according to some hypotheses, would provide a higher quality of vision due to a reduction in the incidence of glare effects³²⁶.

When compared to the AcrySof® IOL, the optic-haptic junction of the printed lenses was less defined than that of the commercial implant (Figure 4.27, (a) and (c)). Moreover, the anterior edge of the lens was considerably sharper in AcrySof® (Figure 4.27, (b)). Thus, an increase in the resolution of the stereolithography system would be required in order to increase the resemblance of these features with respect to the AcrySof® lens.

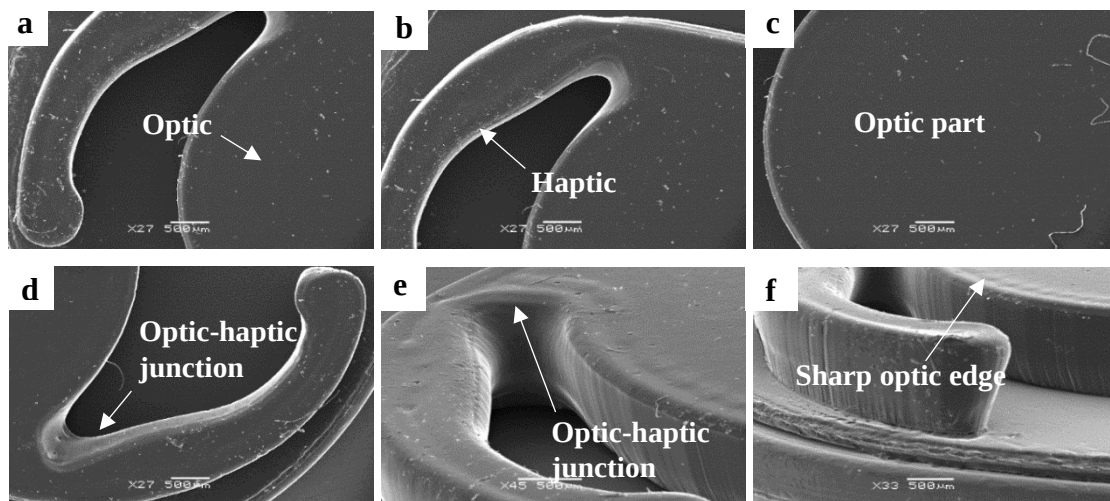


Figure 4.26: SEM micrographs of an IOL printed with formulation C1 showing its different parts: (a) left haptic; (b) left optic-haptic junction; (c) optic part of the IOL; (d) angle formed by the optic-haptic junction; (e) side view of the optic-haptic junction and (f) side view of one of the haptics. The scale bars represent 500 μm.

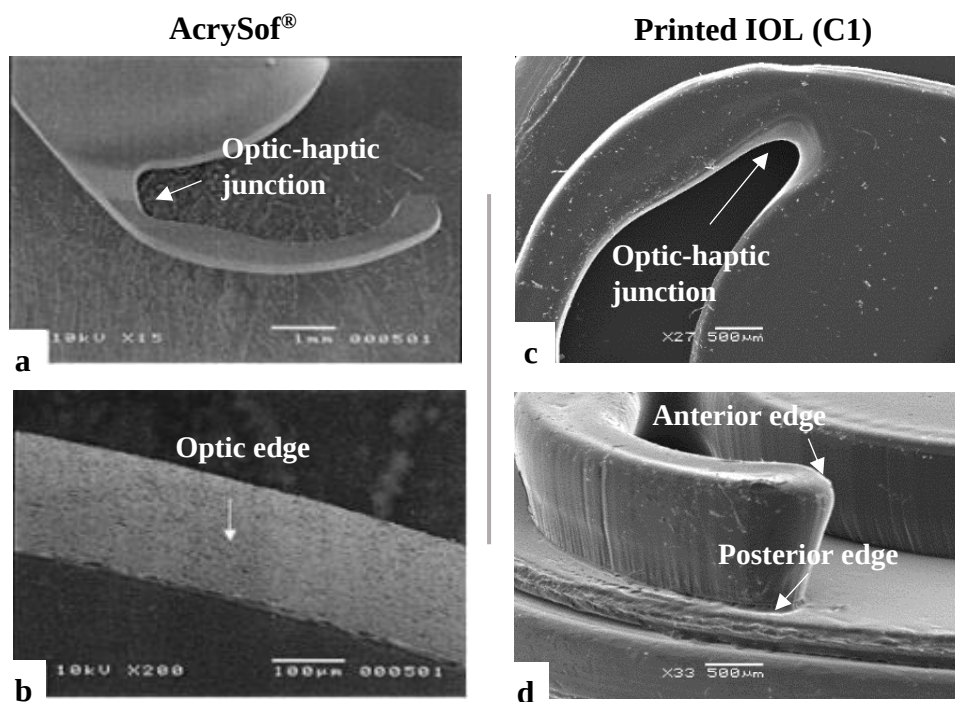


Figure 4.27: SEM micrographs showing the optic-haptic junction and optic edge of an AcrySof® IOL (a, b, respectively) and same for a printed IOL (c, d).

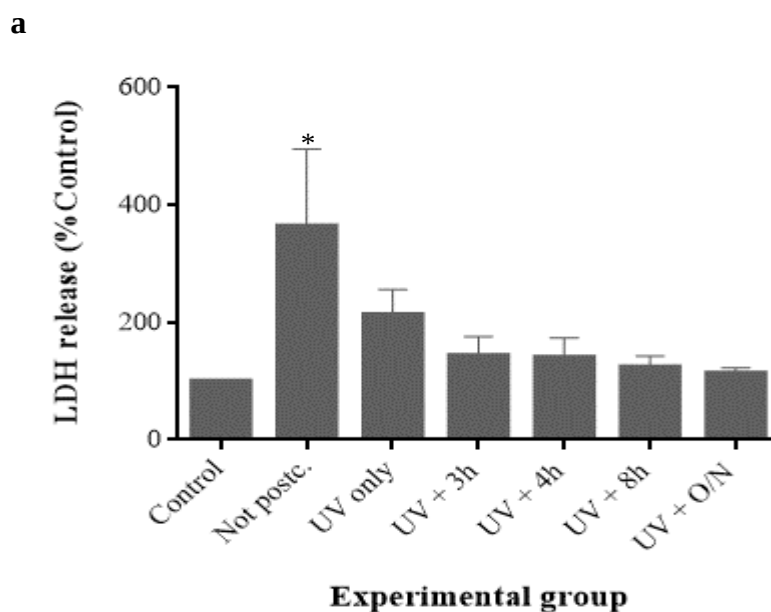
4.2.9 Cytotoxicity assays

The biocompatibility of the materials used in the manufacture of the printed IOLs and the effect of the post-curing process on this attribute were assessed by performing an LDH assay. Samples of polymer that had not been post-cured or which had been subjected to different stages of the post-curing protocol were incubated in contact with FHL-124 lens cells for 48 hours *in vitro*. After this time period, the media was analysed for the presence of LDH enzymes released by any cells that had died due to contact with toxic monomer molecules. The concentration of LDH in the media was significantly higher in the group that contained IOLs which had not been post-cured than in the negative control, which did not contain any IOL (Figure 4.28). The treatment with UV light led to a significant decrease in the mortality of the cells, which was manifested by a decline in the release of LDH. This was further reduced with the application of a thermal treatment to complete the post-curing process. The observations made with an optical microscopy system corroborated these results, as the number of healthy cells observed was substantially higher in the experimental groups that had been subjected to the thermal treatment than in those which had not (Figures 4.29 and 4.30). For both C1 and C2 formulations, longer periods of thermal post-curing resulted in lower levels of LDH release, thus indicating that the biocompatibility of the materials was improved by this treatment. The results from the analysis performed on formulation C1 revealed that the lowest level of cytotoxicity was attained after post-curing the IOLs overnight, while on formulation C2 this was achieved after eight hours of post-curing. However, the difference between the levels of toxicity elicited after either eight or twelve hours of treatment was not significant, as shown by the Tukey's test performed in this analysis.

The results obtained from the ANOVA test indicated that the differences between the means of LDH release measured in different experimental groups were statistically significant ($p = 0.0003$ and $p = 0.0004$ for C1 and C2, respectively). Further information about the differences between each of the experimental groups was provided by the Tukey's multiple comparisons test. This analysis indicated that the differences in LDH release between the IOLs that had not been post-cured and the rest

of experimental groups were statistically significant. It is also important to note that the differences between the levels of cytotoxicity detected in the negative control and in the groups that contained IOLs which had been subjected to a thermal treatment were not statistically significant.

Overall, the results from the LDH test confirmed that a post-curing process was required in order to make the printed IOLs biocompatible. This experiment also indicated that the implants must be subjected to a thermal treatment after UV irradiation in order to reduce the toxicity of the material. The longer the heating time, the lower the levels of cytotoxicity elicited by the implants. Therefore, a post-curing process comprised of a UV irradiation step and a thermal treatment overnight would ensure the biocompatibility of the IOLs prior to implantation in the posterior chamber of the eye.



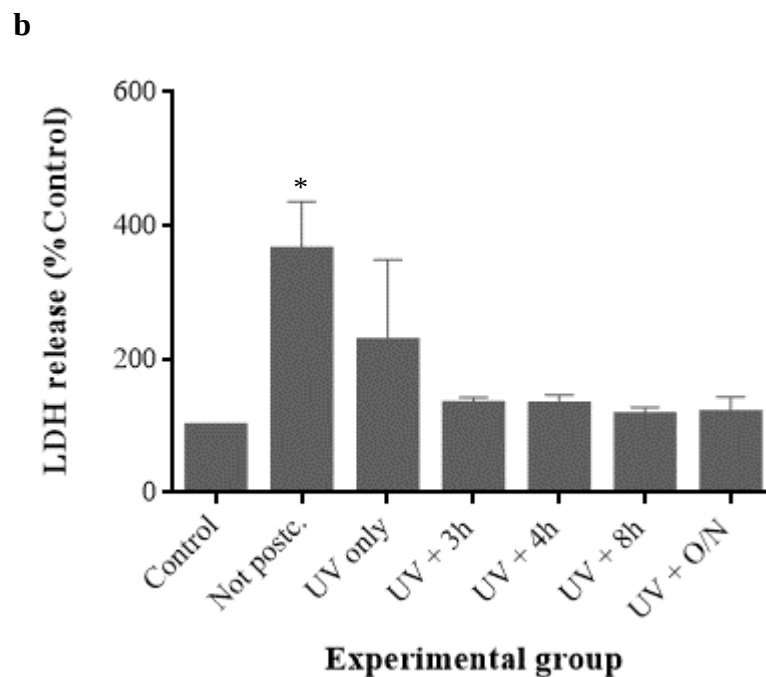


Figure 4.28: Bar graphs showing the LDH release after a 48-hour incubation of FHL-124 cells in the presence of IOLs made with formulation C1 (**a**) or C2 (**b**) and which had been subjected to different stages of the post-curing process: not post-cured, UV-treated for 1 hour, or UV-irradiated and subjected to different heating times in vacuum (3, 4, 8 hours and overnight). One-way analysis of variance (ANOVA) was performed in order to evaluate the statistical significance. Tukey's multiple comparison post-hoc test was used to determine the differences within the experimental groups. The asterisk indicates that the levels of LDH release detected in the not post-cured group were significantly different to those detected in the rest of the experimental groups.

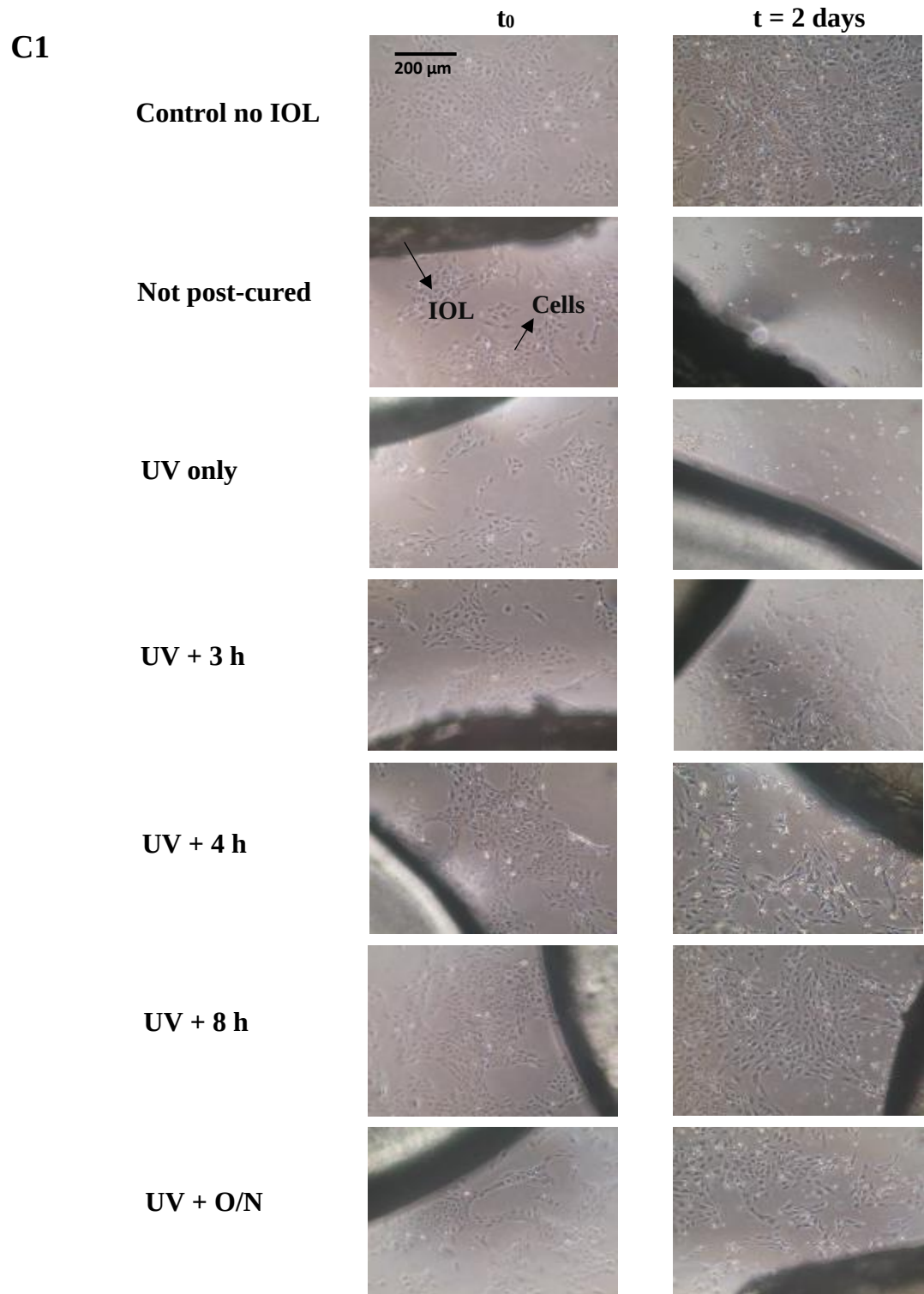


Figure 4.29: Optical microscopy images of lens cells from the control group and cells which had been cultured in the presence of C1 IOLs that had not been post-cured or which had been subjected to different stages of this process. As can be seen, after two days of incubation with IOLs that had not been post-cured or which had been treated only with UV light, the number of healthy cells was lower than on the control group, which did not contain any IOL. However, the thermal treatment performed after the irradiation with UV light led to a considerable increase in the number of cells that survived the incubation with the printed IOLs. The author would like to thank Dr Julie Eldred for obtaining these images.

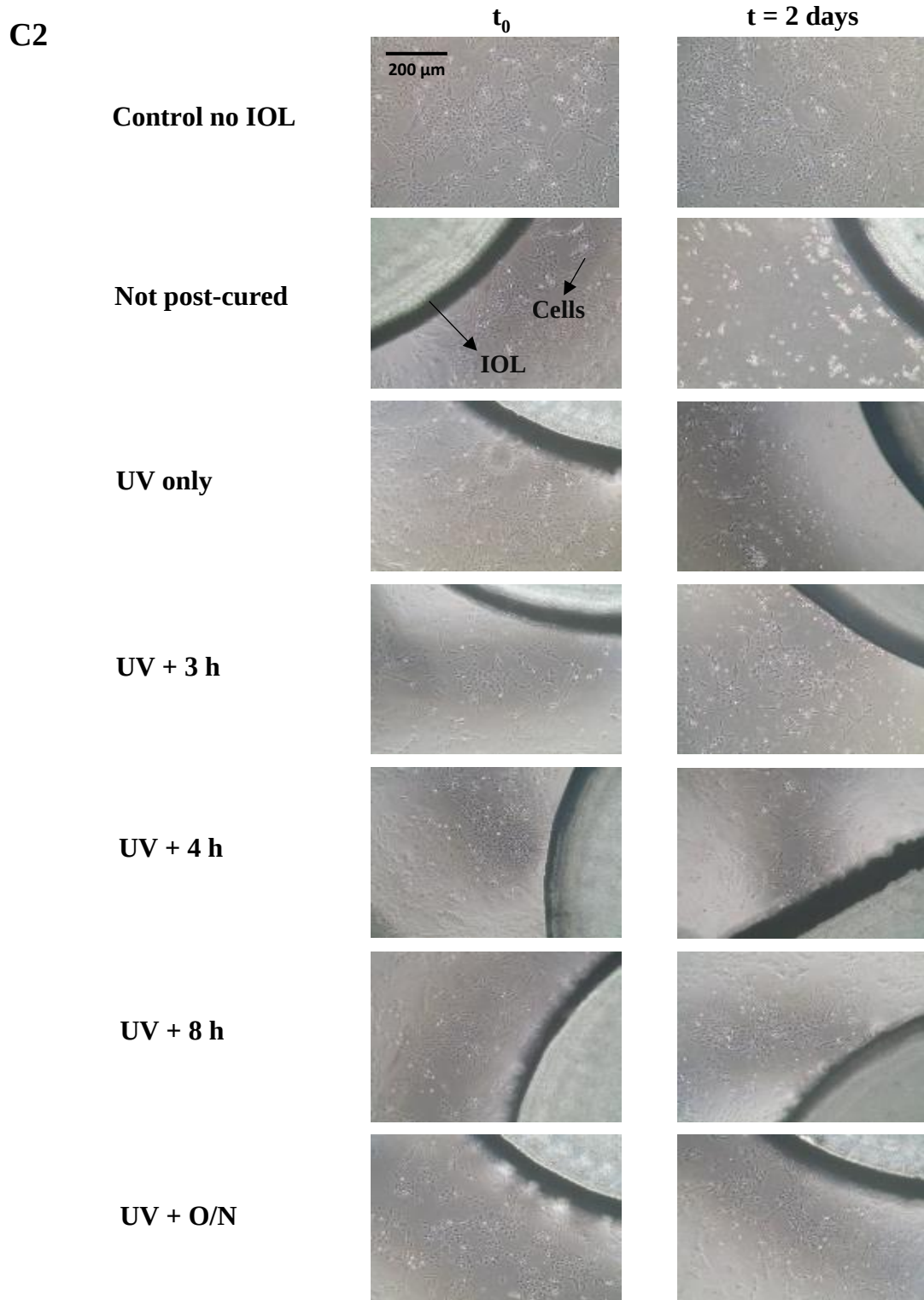


Figure 4.30: Optical microscopy images of lens cells from the control group and cells which had been cultured in the presence of C2 IOLs that had not been post-cured or which had been subjected to different stages of this process. As can be seen, after two days of incubation with IOLs that had not been post-cured or which had been treated only with UV light, the number of healthy cells was lower than on the control group, which did not contain any IOL. However, the thermal treatment performed after the irradiation with UV light led to a considerable increase in the number of cells that survived the incubation with the printed IOLs. The author would like to thank Dr Julie Eldred for obtaining these images.

4.2.10 Implantability tests

The suitability of the printed IOLs for implantation in the posterior chamber of the eye was assessed by injecting them in a human capsular bag *ex vivo*. Prior to this, an injection test was performed in an aqueous medium in order to test the capability of the lenses to withstand the mechanical stress that they are subjected to during this process (Figure 4.32). This experiment confirmed that the printed lenses were flexible enough to facilitate their insertion into the surgical injector used for implantation in the eye. Moreover, their mechanical strength allowed the completion of the process without causing any damage on the implants.

Once the injectability of the IOLs was confirmed, the implantability tests were performed in a human capsular bag *ex vivo*. As shown in Figure 4.33, the lenses fitted in this organ without stretching or deforming it. This is important in order to achieve the restoration of both the anatomical structure and physiological function of the lens after cataract surgery. Therefore, it was concluded that the printed IOLs that resulted from the experimental work described in this chapter were suitable for implantation in the capsular bag, although further improvements in the manufacturing process would be required before translating them to the clinic.



Figure 4.31: Surgical injector DualTec, used in the implantation tests described herein.

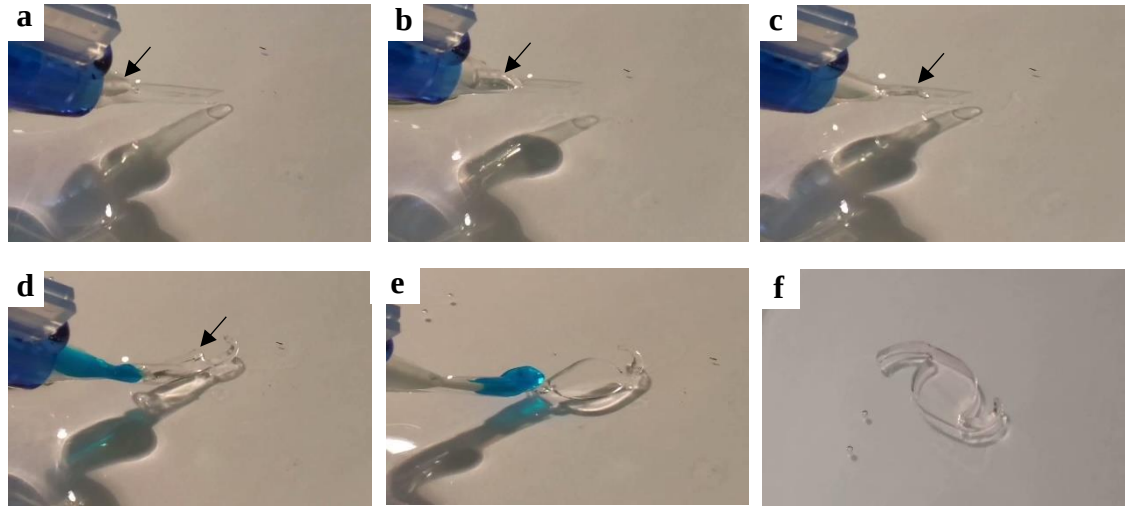


Figure 4.32: Digital images showing the delivery of IOLs from a surgical injector. **(a-c)** IOL, indicated with an arrow, inside the injector and being delivered to the aqueous medium. **(d, e)** IOL unfolding upon release from the injector. **(f)** image showing the IOL completely unfolded in the aqueous medium.

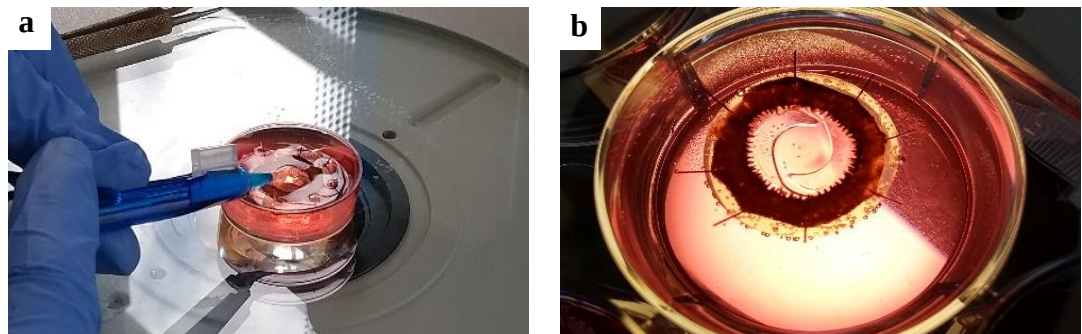


Figure 4.33: Digital images showing the implantation of a printed IOL in a human capsular bag model in vitro. **(a)** Delivery of the IOL into the capsular bag with a surgical injector; **(b)** IOL implanted in the human capsular bag.

4.5 Discussion

Current therapies for PCO cause numerous complications such as retinal detachment, glaucoma, endophthalmitis and cystoid macular edema. Therefore, current research is focusing on the prevention of this syndrome. This can be achieved by creating IOLs with compositional and structural characteristics that contribute to the inhibition of

the cellular responses that lead to the onset of PCO. Additive manufacturing would enable the rapid and inexpensive production of these IOLs for research and clinical purposes. With this motivation, the present study demonstrated the suitability of 3D-printing technologies for the manufacture of functional IOLs. The lenses produced in these experiments were replicas of the hydrophobic acrylic IOL Alcon AcrySof[®]. This choice was based on the superior performance of this IOL in terms of PCO prevention after implantation in the capsular bag. It is widely accepted that the reason for this positive outcome is the presence of a square edge on the posterior side of the lens, which prevents the migration of lens cells onto the posterior side of the capsular bag. Therefore, special attention was given to the replication of this design feature in the experiments herein described.

Another factor that contributes to the low rates of PCO development after the implantation of the AcrySof[®] IOL is its chemical composition. The biomaterial used in the manufacture of these implants is a hydrophobic acrylic polymer, which has a higher capsular biocompatibility than hydrogels. While it was not possible to reproduce the composition of the AcrySof[®] IOL due to the incompatibility of this material with the stereolithography system used in this project, an alternative hydrophobic acrylic formulation was developed which had similar characteristics to that of AcrySof[®]. The monomer of choice for the production of the printable ophthalmic-grade composition was 2-phenoxyethyl acrylate. The selection of this monomer was based on the moderate value of T_g of the polymer that it forms (6 °C), its high refractive index (1.518) and its history of application in the production of ophthalmic devices. Two formulations were then prepared by mixing the main monomer with either PEG-DMA or an aromatic urethane diacrylate crosslinker. It was hypothesised that the addition of either a methacrylate polymer or a macromolecule with phenyl groups to the formulation would improve the mechanical strength of the resulting material. Moreover, the benzyl rings present in the second crosslinker would theoretically improve the transparency of the polymer due to the increase in the refractive index that the presence of conjugated aromatic functionalities would instigate.

After the development of the photopolymerisable resins, functional hydrophobic acrylic IOLs which had the same design as Alcon AcrySof® were successfully produced by stereolithography. While the hydrophilic acrylic lenses described in the previous chapter had been manufactured layer by layer using the build platform during the printing process, this was not possible when employing the Form 2 3D-printer. When using this system, the hydrophobic IOLs that were printed on the platform had larger dimensions on the X-Y plane than the original model, thus resulting in a distortion of the AcrySof® IOL design. Moreover, these implants were so fragile that when they were detached from the build platform they did not maintain their structure and showed a high tendency to roll up (see images in Appendix 6). This issue was still encountered even when trying to increase the mechanical strength of the polymer by adding crosslinkers with higher functionalities (*data not shown*). Therefore, the solution to this problem was to print without the build platform in order to obtain IOLs which had an improved dimensional accuracy on the X-Y plane and a higher mechanical strength. However, the Z resolution was significantly compromised when following this method. This resulted in the fabrication of lenses that were considerably thicker than the commercial model used as a reference. Since the thickness of a lens has a direct effect on its refractive power, it is of paramount importance to achieve a high degree of control over this parameter. Thus, it would be highly desirable to optimise the stereolithography system in order to allow the manufacture of IOLs with a high resolution while using the build platform. In addition, this would open up the possibility of producing convex or concave lenses with a controlled curvature, which is fundamental for the production of functional IOLs.

Once the IOLs were produced, a post-curing process was carried out in order to improve their mechanical properties and biocompatibility. The rationale for this lies in the fact that some of the radicals produced during the curing process may not have polymerised to completion by the time this process finishes. This would have a detrimental effect on the mechanical strength and biocompatibility of the material. Therefore, the printed IOLs were post-cured by irradiating them with UV light for one hour and heating them overnight in a vacuum oven at 70 °C. The FTIR, NMR and cytotoxicity analyses subsequently performed demonstrated the efficacy of this

procedure for the elimination of the unreacted monomers that remained within the polymer after printing. Thus, this protocol increased the biocompatibility of the materials used in the manufacture of the IOLs, as seen on the results obtained from the cytotoxicity assays.

With the aim of evaluating the functionality of the printed IOLs as ophthalmic devices, their characterisation involved the use of various techniques that revealed their physico-chemical properties. The measurement of the water contact angle of the materials confirmed that both C1 and C2 IOLs possessed a similar hydrophobicity to that of AcrySof®. This was further corroborated by the results obtained from the swelling tests, which showed that the printed lenses did not take up any water into their polymer structure. This would have a beneficial effect on the biofunctionality of these implants, as the implantation of IOLs that do not swell in water would prevent any damage in the capsular bag caused by the over-stretching of this tissue. Furthermore, the prevention of water infiltration into the polymer may reduce the incidence of glistenings in these lenses. Finally, it is important to note that a slight decrease in the weight of the IOLs made with formulation C2 was detected after one month of incubation in an aqueous environment. This could be due to the hydrolysis of the ester groups present in the crosslinker used in the production of this polymer. Hence, further studies would be required in order to evaluate the release of degradation products from these lenses, since this could compromise their biocompatibility.

The mechanical properties of the IOLs were assessed by thermal analysis and injection testing. The T_g of the polymers was analysed by DSC, which confirmed that both C1 and C2 IOLs were malleable under both room and physiological temperatures. While the T_g of formulation C1 was lower than that of AcrySof®, C2 possessed a value of T_g that lied within the range recommended for the manufacture of hydrophobic acrylic lenses. Hence, this corroborated that the addition of a crosslinker that contained benzyl rings would increase the mechanical strength of the material. To conclude with the assessment of the mechanical properties of the IOLs, an injection test was

performed. This confirmed that these lenses possessed the adequate balance between flexibility and mechanical strength that allows the implantation of these devices through microincision surgery.

Due to the ophthalmic application of the printed IOLs, the transparency of these lenses was another parameter that required close examination. Both C1 and C2 IOLs were translucent when dry and showed an acceptable level of transparency when incubated in an aqueous medium at physiological temperature. However, the lenses whitened up slightly when they returned to room temperature. As the polymers did not take up any water, and no melting transitions were detected on the thermograms obtained from the DSC analyses, it was hypothesised that the loss of transparency was not due to the occurrence of phase separation phenomena or the presence of crystalline regions. Therefore, this phenomenon could be a consequence of rearrangements in the polymer chains after the changes in temperature that they were subjected to during the swelling testing.

The evaluation of the morphology of the lenses by SEM revealed that the edge of the optic portion was considerably sharper on the posterior side than on the anterior face. According to certain theories, this could have a beneficial effect on the optical properties of the IOLs due to the ability of these design features to prevent dysphotopsias³²⁶. When compared to AcrySof[®], the edges of the printed IOLs were blunter. This may be a consequence of the relatively low resolution achieved with the printer used in these experiments. Therefore, the use of systems with higher resolutions would be advantageous in the manufacture of IOLs. This would enable the production of implants with nanotopographies or other features at the micro or nano-scale that would have the ability to control the behaviour of the lens cells that adhere to these devices upon implantation in the capsular bag. If this approach were successful in reducing cell migration, the incidence of PCO could be substantially reduced by implanting IOLs that present these design features in their structure.

Finally, an implantation test was carried out *ex vivo* in order to assess the implantability of the printed lenses in the capsular bag. Human donor eyes were dissected and the cornea, iris and lens were mounted on a Petri dish. A capsulorhexis

was performed on the lens and the fibres were subsequently extracted through it. The printed IOLs were injected in the capsular bag by using a surgical injector. It was thus demonstrated that the IOLs were implantable in the human capsular bag, as they fitted in this structure and they withstood the mechanical forces exerted on them during surgery.

4.6 Conclusions

The work described in this chapter demonstrated that additive manufacturing can be employed to produce functional IOLs. Moreover, the characterisation techniques performed during the analysis of these devices confirmed that the printed lenses were not cytotoxic and that they had the physico-chemical properties required for a successful implantation in the capsular bag. The design of these lenses was a reproduction of the Alcon AcrySof[®] single-piece IOL, which shows the lowest rates of PCO development in comparison with other commercial IOLs. The materials used in the production of the printed implants were hydrophobic acrylic polymers which, while different to the material that forms the AcrySof[®] IOL, possessed comparable physico-chemical properties. IOLs made with hydrophobic acrylic materials have an excellent capsular biocompatibility when implanted in the capsular bag, thus decreasing the incidence of PCO. Hence, it was assumed that the printed IOLs would have a good capsular biocompatibility when implanted in the eye. However, further experimental work would be required in order to confirm this theory.

Both formulations produced in this work were appropriate for the manufacture of IOLs and possessed the same degree of hydrophobicity as AcrySof[®]. However, some differences were observed between these materials. The IOLs produced with formulation C2 were slightly more transparent and had a higher mechanical strength than those printed with C1. However, the results obtained from the swelling testing suggested that the crosslinker used in the production of the composition C2 may be susceptible to hydrolysis when incubated in an aqueous environment. Since the release of potentially toxic degradation products would compromise the

biocompatibility of the lenses, it was concluded that the use of C1 IOLs would be preferable in future experiments.

With regard to the design of the printed IOLs, both the optic and haptic components of the commercial implant were successfully reproduced. However, the convexity of the lenses could not be replicated using the methodology performed in this work, as it was not based on the layer-by-layer construction of the printed part. Moreover, dimensional inaccuracies were detected on both the optic diameter and thickness of the implants, the latter being more prominent. The relatively low resolution of the system also resulted in the production of lenses with a lower degree of edge sharpness when compared with AcrySof[®], which could increase the incidence of PCO upon implantation of these IOLs. Consequently, it would be highly desirable to optimise the manufacturing process in order to obtain higher resolutions, or to employ systems that allow the production of devices with significantly higher dimensional accuracies, such as microstereolithography.



CHAPTER 5

Optimisation of the 3D-Printing Resolution



5. Optimisation of the 3D-Printing Resolution

5.1 Introduction

One of the main advantages of stereolithography is the high resolution that can be achieved when building structures with this technique. However, dimensional inaccuracies still occur as a consequence of the limitations of the system and the physico-chemical properties of the resins. A decrease in the resolution would have a detrimental effect on the biofunctionality of any medical device that is manufactured with this technology. Therefore, it is fundamental to adopt strategies that lead to an increase in the dimensional accuracy of the 3D-printed parts³⁶⁰.

The resolution achievable in stereolithography systems is determined by the properties of the material and the parameters of the process³⁶⁰. The light absorption and curing kinetics of the photopolymerisable resin are influenced by the monomers, photoinitiator and additives that it is composed of. On the other hand, the process parameters that determine the resolution on the X-Y plane include the laser spot size and the laser scanning pattern, while the accuracy along the Z-axis is dependent on the layer thickness, the cure depth, the precision of the engine that operates the build platform and the efficiency of the recoating process^{360,365,499}. The cure depth, or thickness of the cured gel, is directly dependent on the penetration depth of the laser beam and the exposure dose, which is influenced by the laser intensity and the illumination time³⁶⁰. In order to ensure the successful adherence between the printed layers, the conversion at the boundary between these structures should be slightly higher than the gel point. This would result in an “over-cure” effect that extends into the previous layer and results in the formation of chemical bonds that maintain the constitutive layers bonded to each other. However, if the cure depth is too high, the resolution of the printed part will be compromised by the formation of polymer in areas that should be left empty³⁵⁵.

The cure depth can be controlled by adding light absorbers to the photopolymerisable resin. These chemical agents reduce the penetration depth of the laser, which results

in the production of thinner layers and a subsequent increase in the dimensional accuracy along the Z-axis³⁶⁰. The mechanism of action of these compounds is based on the competitive absorption of the light emitted by the laser before it reaches the photoinitiator molecules. The absorber then converts this luminous radiation into vibrational and rotational energy, which cannot initiate the photopolymerisation reaction. For any light blocker to be effective, its absorption spectrum should overlap with the emission range of the light source. Furthermore, this compound should absorb the light emitted by the laser more readily than the photoinitiator⁵⁰⁰⁻⁵⁰².

As mentioned in chapter four, the low resolution achieved when producing IOLs with the system employed in the present project posed a challenge in the manufacture of lenses with the appropriate dimensions and design features. While the dimensional accuracy on the X-Y plane was improved by removing the build platform while printing, this method resulted in a significant decrease in the Z-resolution of the printed objects and the impossibility to produce concave or convex lenses. Given that both the thickness and curvature of a lens have a direct effect on its refractive power, it is essential to control these characteristics when printing IOLs. This can only be achieved by building the objects layer by layer, for which the use of the build platform is essential. However, the high cure depth of the resins developed in the present project resulted in an excessive overlap between the IOL and the support structures generated by the printer software, which made it impossible to separate them once the print was complete. While this issue was overcome by carrying out the print without any supports, the dimensional inaccuracy of the printed lenses was still a problem. Furthermore, an additional disadvantage of removing the supporting structures was that the optic component was damaged when detaching the IOLs from the build platform. As this would have a deleterious effect on the functionality of the printed lenses, it was concluded that the use of supports was strictly necessary when employing the build stage to produce the implants layer by layer. In addition, the use of support structures enables the production of the printed parts with a tilted orientation, which has proven to increase the tensile strength of the objects^{503,504}.

The challenges described above made it desirable to optimise the printing process in order to increase the resolution achieved with the stereolithography system used in this project while making use of the platform and support structures. Since the most prominent over-curing effects occurred along the Z-axis, it was hypothesised that the main cause of the dimensional inaccuracies observed in the IOLs was the excessive cure depth of the resin. Thus, the addition of a light absorber would be the most effective approach to increase the resolution of the system. An additional advantage of using UV/visible absorbers in the manufacture of IOLs is their proven capability to protect the retina from the photooxidative stress elicited by the exposure to short-wavelength radiation^{7,83}. Some of the absorbers employed in the manufacture of IOLs include benzotriazole, benzophenone, triazine and azo compounds. These absorbers are preferably covalently bond to the polymeric network of the lens material instead of physically entrapped within the polymer, as the leaching of the absorber may cause toxic responses and compromise the light blocking effect of the IOL⁵⁰⁵.

Given the potential benefits provided by the addition of a light absorber into the photopolymerisable resin, this was tested in the experiments described in this chapter. The absorber employed in this work was Eusorb UV-1995 (Eutec Chemical Co., Ltd. Taipei, Taiwan). This choice was based on the fact that its maximum absorption occurs at 424 nm, which is very close to the wavelength of the light emitted by the printer laser (see Appendix 7). The effect of the use of this compound on the resolution of the system was evaluated by printing the AcrySof® IOL for comparison with the results obtained previously when using resins that did not contain any light blocker in their formulation. Furthermore, the ability of the system to print two novel IOL prototypes which possessed elements of design that increased their bioactivity was also tested after the addition of Eusorb to the formulation. The first model was a prototype of an IOL designed to reduce the incidence of PCO by allowing a higher flow of aqueous humour within the capsular bag. This would result in a decrease in the concentration of EMT-inducing cytokines in the environment that surrounds the LECs, hence reducing the incidence of PCO. The presence of perforations in the haptics of this IOL would contribute to the attainment of this objective. The second prototype was a disk-design IOL which had a linear surface topography on the haptic.

The rationale for this design was based on previous experimental results that showed the ability of these topographies to control the adhesion and migration of lens cells^{506,507}. Therefore, it was hypothesised that the creation of novel IOLs with design features which can control the responses of the cells that adhere to their surface could be a novel avenue to explore for PCO prevention. Only those topographies that are built at the nanoscale would be effective in establishing interactions with cells, as the dimensions of the proteins that adhere to the substrate are on the nanometer level. However, the printer used in this project did not allow the creation of structural elements at the nanoscale. Therefore, the prototype herein described had a topography built at the microscale which acted as a model of what could have been built at the nanoscale if systems such as two-photon polymerisation had been used in this project.

5.2 Aim and Objectives

The aim of the work described in this chapter was to increase the resolution of the printed IOLs by adding a light absorber to the photopolymerisable resin used in the manufacture of these lenses. The formulation of choice for the completion of these experiments was the resin named C1, which was described in chapter four. This selection was based on the biofunctionality and implantability of the IOLs made with this composition, as seen on the characterisation experiments described in the previous chapter.

The first objectives were to evaluate the effect of the light absorber on the cure depth of the resin and to elucidate the range of concentrations of this additive that could be used without compromising the printability of the material. Furthermore, the chemical structure of the absorber was analysed in order to elucidate the mechanism of action of this compound.

The second objective was to print the AcrySof[®] IOL in order to compare the dimensional accuracy of the resulting lenses with that of the implants which had been previously produced without adding any absorber to their composition. Moreover, two

novel IOL prototypes were produced in order to assess the printability of various design features which can contribute to the prevention of PCO.

Finally, the last objective of these experiments was to characterise the resulting IOLs in order to assess the effect of the light absorber on both the physico-chemical properties of the material and the morphology of the 3D-printed lenses.

5.3 Materials and Methods

5.3.1 Cure depth measurements

The effect of the addition of Eusorb UV-1995 on the cure depth of the photopolymerisable resin used in these experiments was evaluated by using the printer laser as a light source. Five different printable resins were prepared, all of which were composed of 93% (w/w) 2-phenoxyethyl acrylate (>93% purity, TCI Chemicals, Tokyo, Japan), 7% (w/w) PEG-DMA (M_n 750 Da, Sigma-Aldrich, UK) and additional 1% (w/w) TPO (>98% purity, TCI Chemicals, Tokyo, Japan) in proportion to the total mass of the pre-polymer mix. While the first resin did not contain light absorber, increasing concentrations of Eusorb UV-1995 (kindly provided by Eutec Chemical Co., Ltd. Taipei, Taiwan) were added to the other formulations. After that, the resins were stirred under an atmosphere of nitrogen until complete dissolution was observed. The concentrations of absorber tested in this experiment included 0.006%, 0.012%, 0.024% and 0.048% (w/w).

The cure depth measurements were carried out by using three Eppendorf tubes which had been cut at one of their extremes (Figure 5.1). These were used to confine the resin within the print area, hence allowing the production of multiple structures while using a relatively low amount of resin. Three cylinders were then created in Blender with a lower diameter than the Eppendorf tubes and a thickness of 0.9 mm. These were then printed without the build platform in order to not limit the cure depth of the resin. Before printing, the open tubes were placed on the print area and the same volume of resin was pipetted in each of them, making sure that the height of the liquid

was at least three times higher than the predicted thickness of the printed structures. This was done in order to ensure that the cure depth was not limited by the volume of resin available in each tube. Once the print started, it was allowed to continue for seven layers in order to obtain polymers with enough mechanical strength to facilitate the measurement of the thickness, which is equivalent to the cure depth. The resulting polymeric cylinders were post-cured for one hour in a UV-curing chamber (Meccatronicore, Trento, Italy) and their thickness was measured using a calliper. This operation was repeated for each of the five formulations tested in this experiment.

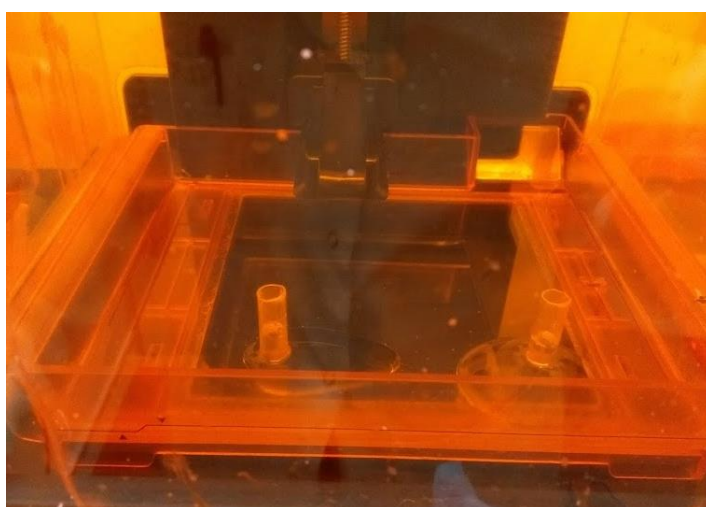


Figure 5.1: Digital image showing the method followed for the production of the cylinders used for the evaluation of the effect of Eusorb UV-1995 on the cure depth of the resin.

5.3.2 ^1H -NMR

The chemical structure of Eusorb UV-1995 was characterised by ^1H -NMR. First, a 10-mg sample of Eusorb was dissolved in 800 μL chloroform-d (99.96 atom% D, Sigma Aldrich). Then, the spectrum was acquired at 20 $^{\circ}\text{C}$ on a Bruker 400 spectrometer at 400 MHz (Bruker Corporation, Massachusetts, USA). All chemical shifts (δ) reported are in parts per million (ppm) and were referenced to the residual protons (^1H) of the deuterated solvent. The data was processed using Topspin 3.5 pl6 software (Bruker Corporation) and Mnova v6.0.2-5475 (Mestrelab Research, Santiago de Compostela, Spain).

5.3.3 ATR-FTIR

The polymers used in the manufacture of the 3D-printed IOLs were evaluated by ATR-FTIR in order to determine the chemical functionalities present in these materials. The spectra were recorded using a Bruker Vertex 70 FTIR spectrophotometer (Bruker Corporation, Massachusetts, USA) by running 32 scans between 4,000 and 500 cm^{-1} at a resolution of 4 cm^{-1} .

5.3.4 Manufacture of IOLs

The IOLs were manufactured using the bottom-up stereolithography system Form 2 (Formlabs, Massachusetts, USA). Firstly, the CAD models of the IOLs were generated using Blender. The designs used in this work included Alcon AcrySof® and two prototypes of novel IOL designs. The CAD model of the AcrySof® IOL was the same as described in previous chapters. The diameter of the convex optic portion was 6 mm, while the haptic-to-haptic length was 13 mm. The optic part of the novel prototypes was also built with a diameter of 6 mm and the thickness was set at 0.9 mm. In the disc-design prototype the optic part was convex. As performed for the AcrySof® model, the convexity was created by adding a hemisphere to the 0.9-mm-thick optic portion of the implant (Figure 5.2). The dimensions of the rest of the features are shown in Figure 5.2. Once the CAD models were created, the printable resins were prepared. The formulation used for the manufacture of the lenses was the same as for the cure depth measurements (see section 5.3.1 for details). The light absorber Eusorb UV-1995 was then added at either 0.006% (w/w) or 0.012% (w/w), and the resin was stirred in a nitrogen atmosphere until complete dissolution. The print was performed with the build platform and using the supports generated by the PreForm software (Figure 5.2). In addition, a ring-shaped support structure was created for the IOL prototype with cylindrical haptics in order to protect these structures from the forces created during the printer's peel process (Figure 5.2). The supports that the software attached in the middle of the optic part of the IOL were removed in order to obtain a smooth surface. The layer thickness was set at 0.025 mm/layer and the laser selected was "Black". All the 3D-printed IOLs were

subsequently post-cured by irradiating them with UV light for one hour and heating them overnight in a vacuum oven at 70 °C.

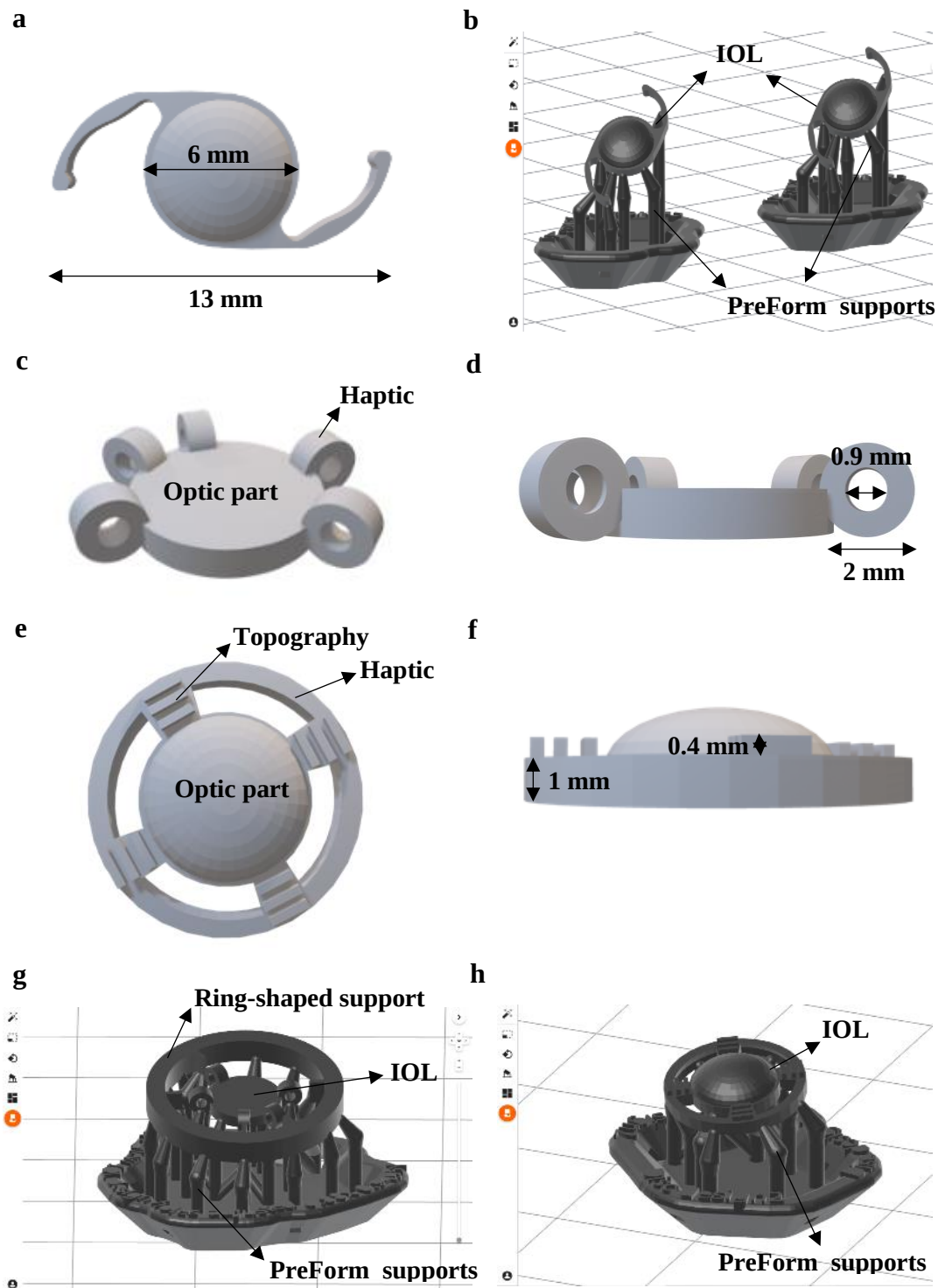


Figure 5.2: (a) CAD model of the AcrySof® IOL; (b) AcrySof® IOLs with the supports generated by PreForm; (c, d) CAD model of the prototype with cylindrical haptics; (e, f) CAD model of the disc-design prototype with topography on its surface; (g) IOL with cylindrical haptics mounted on the supports; (h) disk-design IOL with the supports generated by PreForm. These designs were chosen due to their potential ability to reduce the incidence of PCO after cataract surgery.

5.3.5 Optical microscopy

Optical microscopy images of the 3D-printed IOLs were obtained using a Leica MZ7.5 stereomicroscope. When imaging the replicas of the AcrySof® IOL, the dimensional accuracy with respect to the CAD model was assessed by measuring the thickness, optic diameter and haptic-to-haptic length of three IOLs printed with each of the formulations used in the manufacture of these lenses.

5.3.6 Differential Scanning Calorimetry

The T_g of the polymers used in the manufacture of the 3D-printed IOLs was evaluated by DSC using a DSC Q2500 system (TA Instruments, New Castle, USA). Samples with a mass of 1-3 mg were pressed into aluminium DSC pans that were introduced into the equipment used to perform the analyses. The samples were subjected to a heating cycle from -50 to 150 °C, followed by a cooling step from 150 to -50 °C and a second heating ramp back to 150 °C. The experiment was performed in triplicate. The scan rate for all cycles was 10 °C/min and the nitrogen flow was maintained constant at 50 mL/min. The resulting data was analysed using TRIOS software v4. 3. 1. 39215. The T_g value was obtained from the thermogram generated during the second heating cycle as the temperature at the midpoint of the decline in the heat capacity that takes place during this thermal event.

5.3.7 Thermogravimetric analysis

The thermal stability of the polymers used for the production of IOLs was evaluated by TGA. The analysis was carried out using a TGA 5500 system (TA Instruments, New Castle, USA). Samples with a mass of 1-3 mg were placed in platinum pans and were heated from 25 to 500 °C at a heating rate of 10 °C/min, under a nitrogen atmosphere. The experiment was performed in triplicate. The resulting data was analysed using TRIOS software v4. 3. 1. 39215.

5.3.8 Scanning Electron Microscopy

SEM images of the 3D-printed IOLs were taken in order to evaluate their morphology. The samples were mounted on aluminium stubs and coated in an argon-filled sputter gold coater (Polaron SC7640, Quorum Technologies, UK) for 30 seconds before acquiring the images in a JEOL JSM 5900 LV microscope (Jeol Ltd., Tokyo, Japan) with a 20-kV accelerating voltage.

5.3.9 Statistical analysis

The statistical significance of the differences in thickness between the cylinders printed for the cure depth measurements was assessed by performing the non-parametric Kruskal-Wallis test.

The significance of the differences between the dimensions of the IOLs printed with different concentrations of Eusorb UV-1995 was evaluated by carrying out a Mann-Whitney test.

Finally, the differences in T_g and IDT caused by the addition of different amounts of light absorber were evaluated for statistical significance by performing a Dunn's test after the Kruskal-Wallis test.

The statistical package GraphPad Prism v.6.01 was used for all statistical analysis. The level of significance was set at $p < 0.05$.

5.4 Results

5.4.1 Cure depth measurements

The effect of the addition of different concentrations of Eusorb UV-1995 on the cure depth of formulation C1 was evaluated by measuring the thickness of cylinders which had been printed after adding different amounts of this compound to the photopolymerisable resin. The build platform was not used in these experiments, so

the presence of the light absorber was the only factor that limited the cure depth. Firstly, the depth of cure of a resin that did not contain Eusorb was assessed. The average thickness of the cylinders printed with this formulation was 3 ± 0.26 mm. The addition of 0.006% (w/w) Eusorb resulted in a 60% reduction in the cure depth of the resin. When the concentration of the light absorber was doubled, the thickness of the cylinders decreased by a further 40%. The subsequent addition of 0.024% (w/w) and 0.048% (w/w) of this additive resulted in increasingly higher reductions in the cure depth, with a decline of more than 100% in each case with respect to the preceding study group. The results from the non-parametric Kruskal-Wallis test indicated that the differences in cure depth between the experimental groups evaluated in this study were statistically significant ($p < 0.0001$). However, the Dunn's multiple comparisons test revealed that the only difference deemed significant was the one obtained from the comparison between the resin that did not contain Eusorb and the formulation which contained 0.048% of this compound. Given the substantial differences in thickness that had been observed between the rest of study groups, it was hypothesised that larger sample sizes would have been needed in order to increase the reliability of the data obtained in this experiment.

It is important to note that the cylinders obtained when printing with the resin that contained 0.048% (w/w) Eusorb had a very poor mechanical strength and they showed a high tendency to break. This indicated that the inhibitory effect of the absorber at this concentration was high enough to affect the mechanical performance of the printed parts.

The cure depth of a resin should be slightly higher than the layer thickness in order to obtain an optimal resolution and to allow for an appropriate adhesion between the constitutive layers. If a 20% of hatch over-cure respect to the layer thickness is considered necessary to comply with this requirement, the cure depth that would lead to the attainment of an optimum resolution would be 0.03 mm. According to the data obtained from the cure depth measurements, more than 0.05% (w/w) Eusorb would be needed in order to obtain this depth of cure (see the graph shown in Figure 5.3).

Since it had been observed that the mechanical strength of the polymer was drastically compromised when adding 0.048% (w/w) Eusorb to the formulation, an optimisation of the composition of the resin would be required in order to compensate for this effect when adding relatively high amounts of absorber. This could be achieved by adding crosslinkers with increased functionalities or which have a high value of T_g . Overall, further experiments would be required in order to optimise the concentration of absorber in the formulation in such a way that the resolution is improved but the mechanical performance of the resulting material is not significantly compromised.

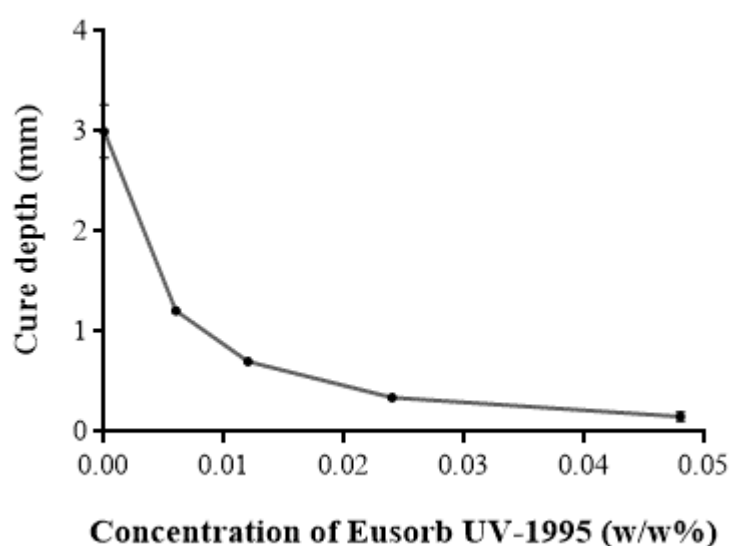


Figure 5.3: Line graph showing the reduction in the cure depth of formulation C1 with increasing concentrations of Eusorb UV-1995. The results from the non-parametric Kruskal-Wallis test indicated that the differences in cure depth between the experimental groups evaluated in this study were statistically significant ($p < 0.0001$).

5.4.2 ^1H -NMR

The chemical structure of the light absorber was evaluated by ^1H -NMR in order to elucidate its mechanism of action (Figure 5.4). The spectrum indicated the presence of a monosubstituted benzyl ring in its structure, as indicated by the following signals:

(^1H -NMR, 400 MHz, CDCl_3): $\delta = 8.06$ (1H, s), 7.94 – 7.91 (2H, d, $J = 12$ Hz), 6.69 - 6.67 (2H, d, $J = 8$ Hz)

The results also indicated the presence an ethyl group near an electron-withdrawing group: $\delta = 4.33$ (2H, q, $J = 7.21$ Hz), 1.37 (3H, t, $J = 7.12$ Hz) and two equivalent methyl groups: $\delta = 3.09$ (6H, s).

The absence of any signals at 6.47 – 5.84 ppm indicated that the compound does not possess any acrylate functionalities.

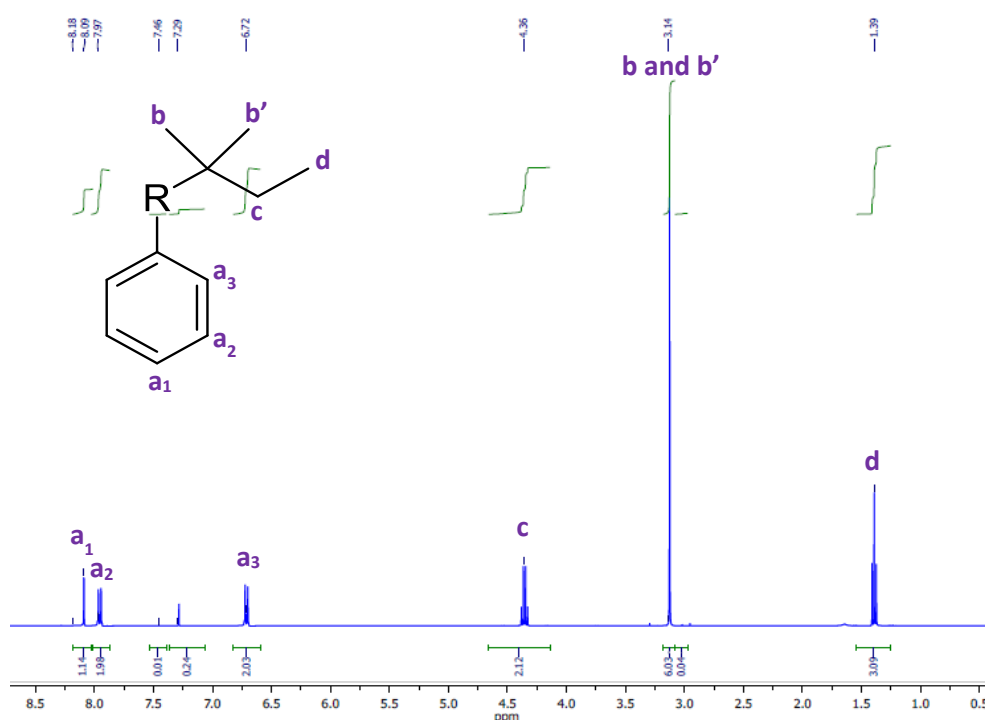


Figure 5.4: ^1H -NMR spectrum of Eusorb UV-1995 and possible chemical structure of this compound. The signals detected indicate the presence of a monosubstituted benzyl ring ($\delta = 8.06$, 7.94 - 7.91 and 6.69 - 6.67), an ethyl group located near an electron-withdrawing group ($\delta = 4.33$ and 1.37) and two equivalent methyl groups ($\delta = 3.09$).

5.4.3 ATR-FTIR

ATR-FTIR analysis was carried out in order elucidate the chemical structure of Eusorb UV-1995 (Figure 5.5). The spectrum generated by this analysis suggested the presence of a monosubstituted aromatic group, which was indicated by the following

signals: aromatic C-H stretch ($3,080\text{ cm}^{-1}$), aromatic C-C bend ($1,690\text{ cm}^{-1}$), aromatic C-H ring bend ($670 - 1,225\text{ cm}^{-1}$) and monosubstituted aromatic C-H bend ($710 - 770\text{ cm}^{-1}$). The presence of a signal at $2,215\text{ cm}^{-1}$ was an indicator of the presence of either a carbon-carbon triple bond ($2,190 - 2,260\text{ cm}^{-1}$), a nitrile group ($2,200 - 2,280\text{ cm}^{-1}$) or a cyanate ($1,190 - 2,260\text{ cm}^{-1}$). The absence of the rest of the characteristic peaks from an alkyne in this spectrum (C-H stretch at $3,310 - 3,320\text{ cm}^{-1}$ and C-H bend at $610 - 680\text{ cm}^{-1}$) suggested that this band was possibly produced by either a nitrile or a cyanate. Finally, the methyl groups in the molecule generated a band at $2,980\text{ cm}^{-1}$, while the methylene produced a signal at $2,927\text{ cm}^{-1}$.

As well as the characterisation of the absorber, a comparative analysis was performed between a polymer printed with formulation C1 and the same material with either 0.006% or 0.012% (w/w) Eusorb added to it (Figure 5.6 and Appendix 8). The spectra showed that all formulations produced the same signals, indicating that the peaks from the absorber may not be detectable due to the low concentration of this compound or the overlapping with the signals generated by the polymer.

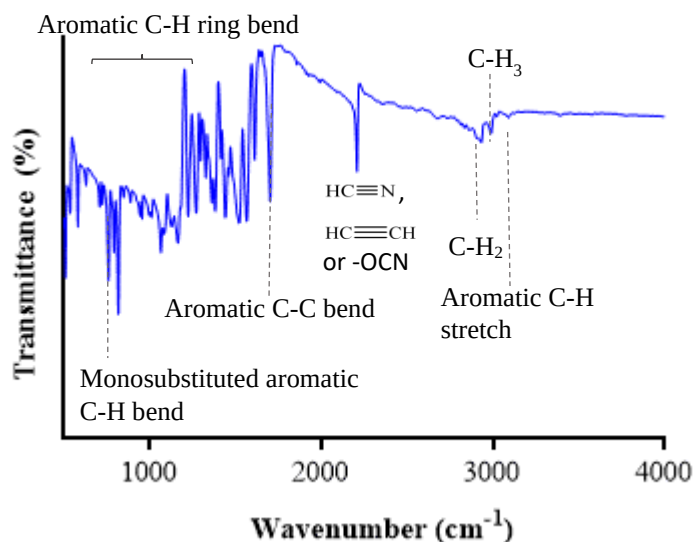


Figure 5.5: ATR-FTIR spectrum of Eusorb UV-1995.

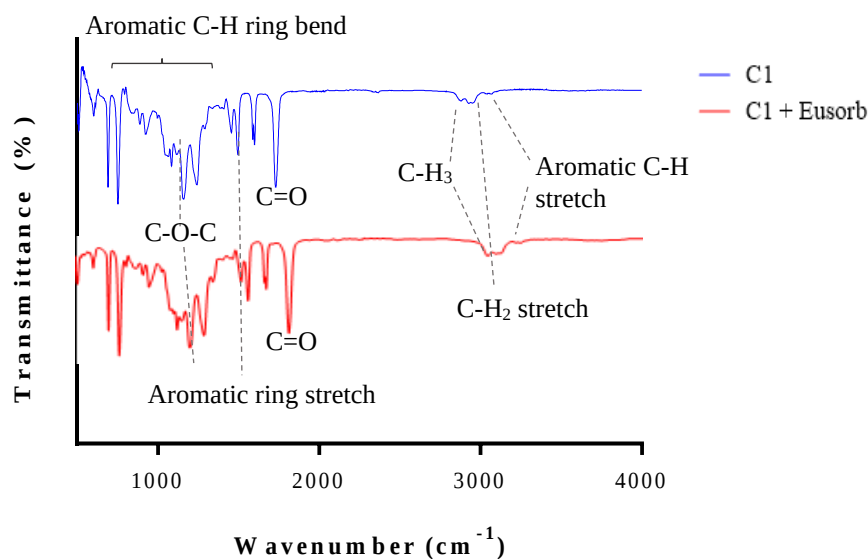


Figure 5.6: ATR-FTIR spectra of formulation C1 (blue) and C1+0.006% Eusorb UV-1995 added to it (red). The same spectrum was obtained when analysing the polymer that contained 0.012% Eusorb (Appendix 8).

5.4.4 Manufacture of IOLs

The IOLs obtained by printing with formulations that contained Eusorb UV-1995 had a significantly higher resolution than those which had been produced without this compound. The use of a light absorber made it possible to print the IOLs using the build platform and the support structures generated by the printer software. When this had been attempted by using a formulation that did not contain this additive, the high degree of over-curing in the polymer led to the formation of IOLs with a low resolution and larger dimensions than the CAD model. The optic and haptics were wider and thicker than those of the AcrySof® IOL, while the overall shape of the implant was irregular and had a low fidelity with respect to the commercial model (Figure 5.7, (a)). Moreover, the IOL and support structures were overlapped as a consequence of the low resolution and over-curing effects obtained with the system employed in this project. Thus, the IOLs could not be completely separated from the supports after printing. A novel IOL prototype with cylindrical haptics had also been printed with this formulation, resulting in similar effects to those observed in the AcrySof® IOL. The optic was significantly thicker than in the original model, while the haptics were completely overlapped with the support structures (Figure 5.11, (a)).

Furthermore, the perforations that this IOL design had in the haptics could not be replicated as a consequence of the low resolution obtained with this formulation (Figure 5.11 (b)).

The addition of Eusorb UV-1995 to the photopolymerisable resin allowed the production of IOLs that contained structural elements at microscale dimensions. This was a consequence of the high resolution achievable when adding this compound to the formulation. Based on the results obtained from the cure depth measurements previously described, the addition of both 0.006% (w/w) and 0.012% (w/w) Eusorb were tested when printing the lenses. These concentrations were chosen due to the significant reduction that they elicited in the cure depth of the resin and the fact that the mechanical properties of the resulting polymers had not been significantly compromised upon addition of these amounts of absorber. The addition of 0.006% (w/w) Eusorb UV-1995 allowed the production of AcrySof[®] IOLs that could be easily detached from the supports and which reproduced the design of the original implant with a high fidelity. However, when printing the novel design which had perforations in the cylindrical haptics, these structural elements were not formed due to the curing of the resin in spaces that should have been left empty (Figure 5.12 (a)). This indicated that, while this concentration was high enough to allow the production of IOLs where most of the design features were successfully formed, a higher amount of absorber may be required in order to reproduce the smallest structural elements of the implants. Therefore, a formulation with 0.012% (w/w) Eusorb UV-1995 was subsequently tested. The successful generation of the perforations in the haptics indicated that this concentration was adequate for the production of 3D-printed IOLs with design features that could contribute to the prevention of PCO (Figure 5.12 (b)). Furthermore, it was demonstrated that the manufacture of another IOL prototype which had a microscale topography on its surface was possible with this formulation (Figure 5.13).

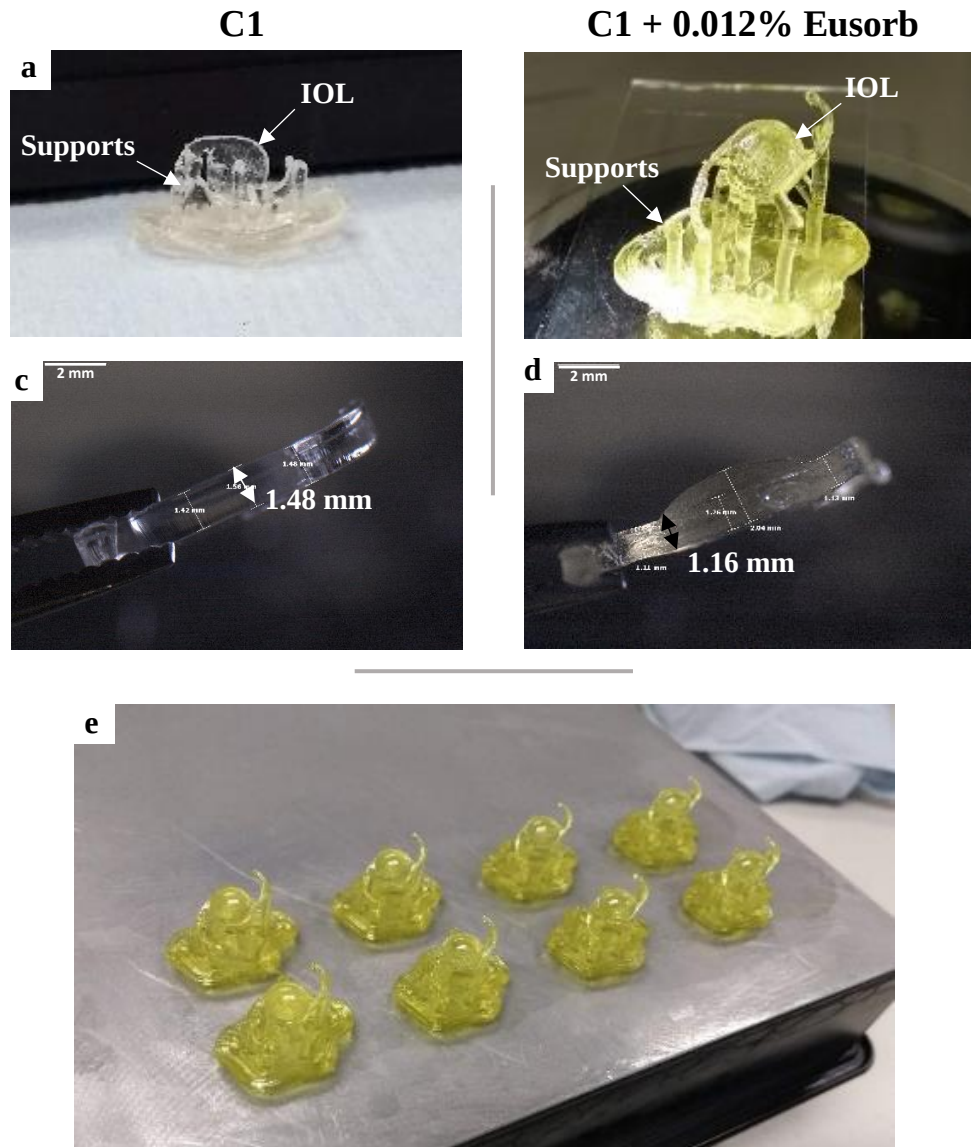


Figure 5.7: Digital images showing a comparison between an AcrySof® IOL printed with formulation C1 (a) and the same model printed with C1 + 0.012% Eusorb UV-1995 added to the formulation (b). Image (c) shows the side view of an IOL printed with formulation C1, while image (d) shows the same for another IOL printed with C1 + 0.012% Eusorb. Image (e) shows the IOLs composed of formulation C1 + 0.012% Eusorb printed on the build platform.

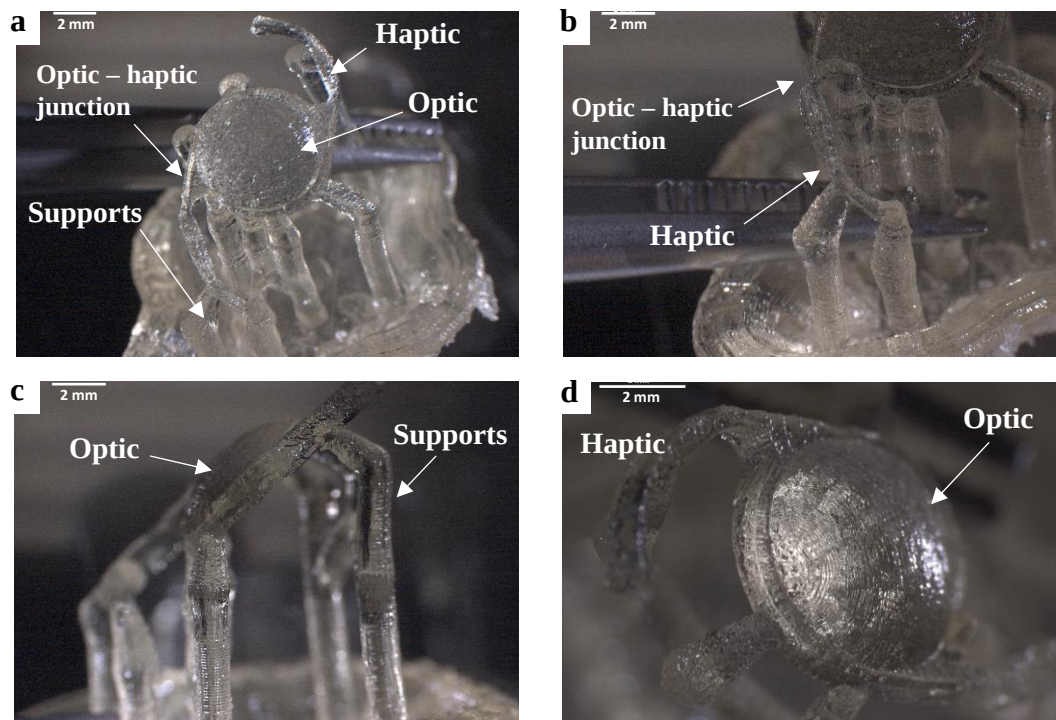


Figure 5.8: Optical microscopy IOLs of the replicas of AcrySof[®] printed with formulation C1 + 0.012% Eusorb and with the supports attached to it. **(a)** General view of the implant; **(b)** detail of one of the haptics; **(c)** side view of the IOL and supports; **(d)** detail of the optic part of the implant.

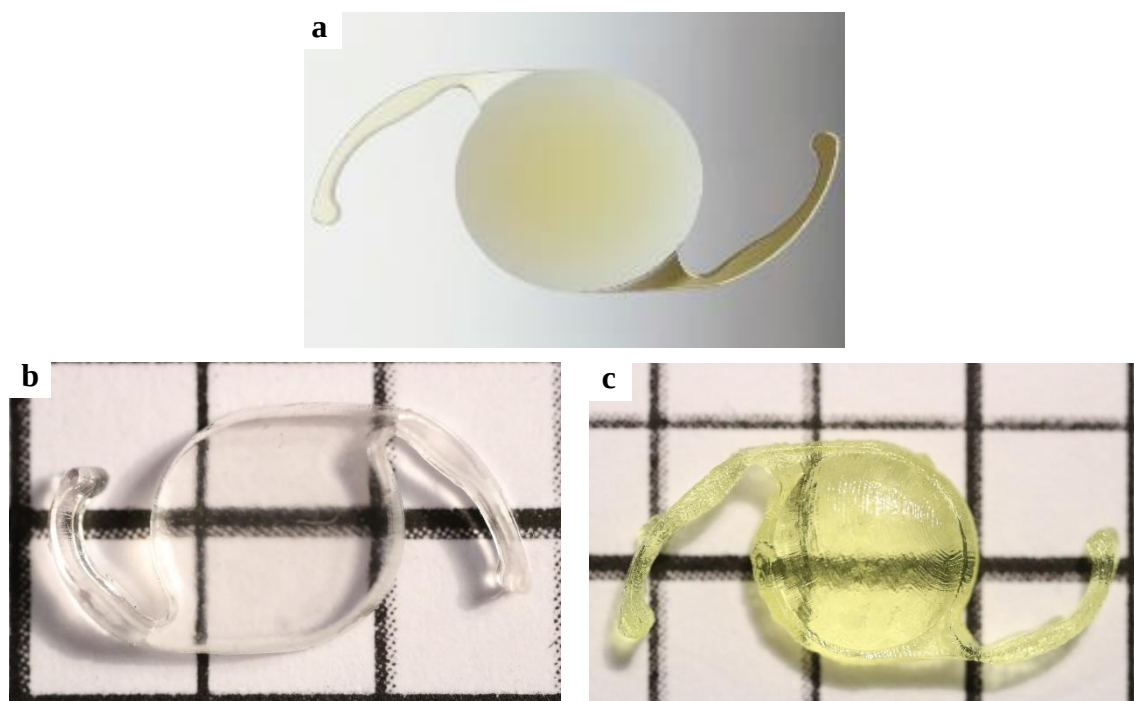


Figure 5.9: Comparison between the original AcrySof[®] IOL and the printed analogues. **(a)** one-piece AcrySof[®] IOL; **(b)** IOL printed with formulation C1; **(c)** IOL printed with formulation C1 + 0.012% Eusorb. A higher fidelity respect to the original model can be observed in the latter, as the haptic-optic junction is as defined as in the AcrySof[®] implant and the shape of the optic is not distorted.

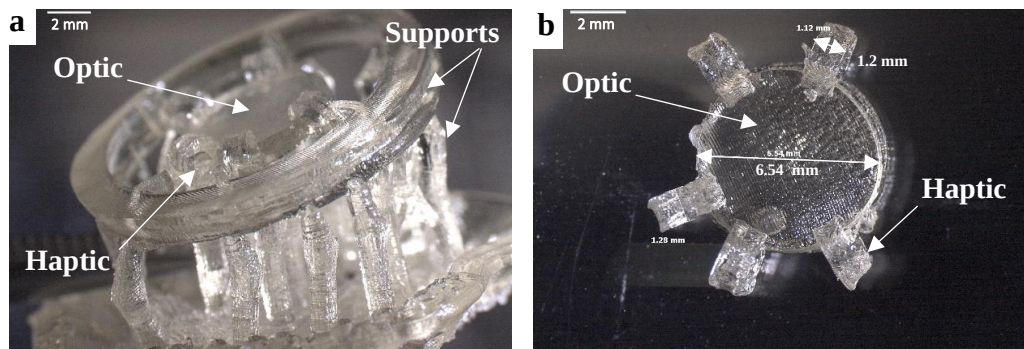


Figure 5.10: Optical microscopy images of the IOL prototype with cylindrical haptics, printed with formulation C1 + 0.012% Eusorb. Image (a) shows the IOL with the support structures attached to it, while (b) shows the implant after removing the supports.

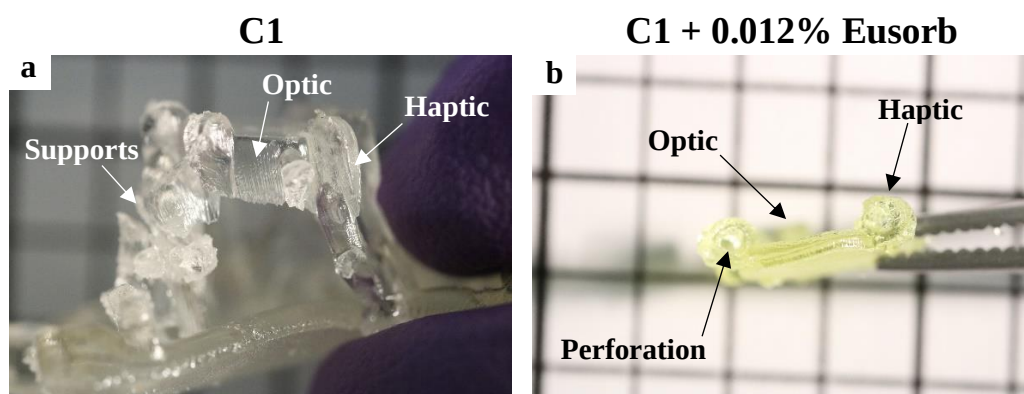


Figure 5.11: Optical images showing the IOL prototype with cylindrical haptics, printed either with formulation C1 (a) or C1 + 0.012% Eusorb (b). A decrease in the thickness of the optic portion can be observed upon addition of Eusorb to the formulation. Furthermore, the use of the absorber enabled the formation of the perforations present in the haptics, which was not possible when using the resin that did not contain this compound.

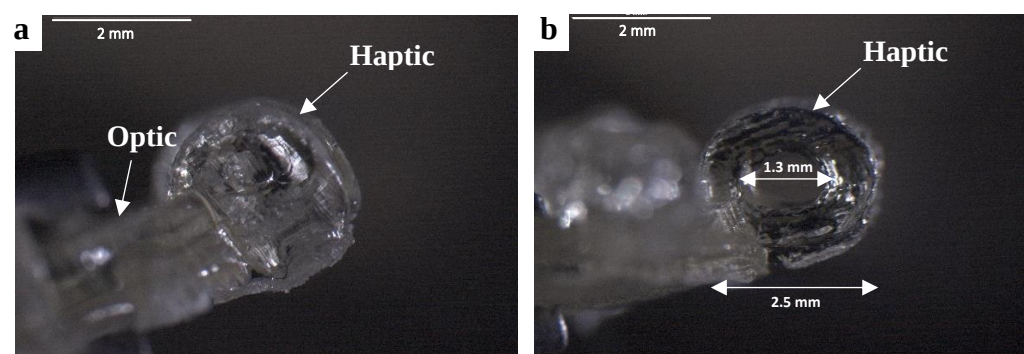


Figure 5.12: Optical microscopy images of a cylindrical haptic of an IOL printed with formulation C1 + 0.006% Eusorb (a) and the haptic of another implant which had been printed with formulation C1 + 0.012% Eusorb (b). The perforation could only be formed when adding the highest concentration of absorber to the photopolymerisable resin.

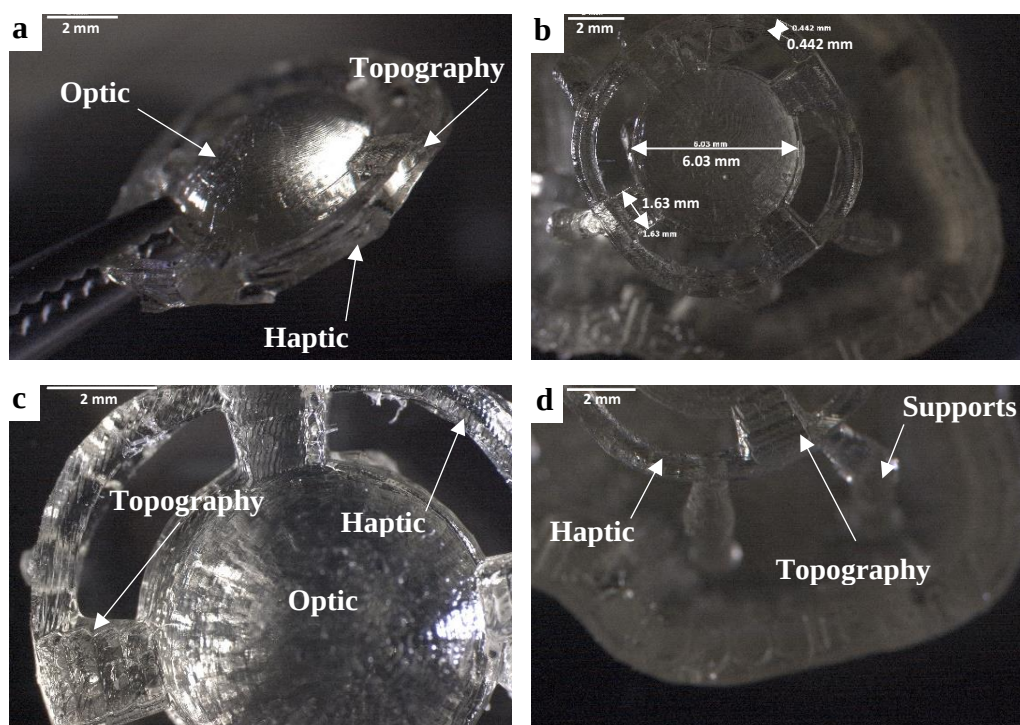


Figure 5.13: Optical microscopy images of the IOL prototype with a disk-design and a linear topography on the anterior surface. **(a)** Side view of the IOL showing its convexity and the topography; **(b)** top view of the IOL attached to the supports, with the dimensions annotated; **(c)** detail of the optic part and haptics with topography; **(d)** detail of the topography created on one of the linkers between the optic and haptic.

Measurements of the 3D-printed AcrySof[®]-like IOLs were taken using a stereomicroscope with the aim of quantifying the dimensional accuracy achieved when adding a light absorber to the formulations. The thickness of the lenses was measured with the purpose of comparing the cure depth obtained with each concentration, as this is the parameter that the light absorber controls. The average thickness of the three IOLs printed with 0.006% (w/w) Eusorb UV-1995 was 1.29 ± 0.04 mm, while the lenses printed with double the concentration of this compound had a thickness of 1.16 ± 0.01 mm (see dimensions in Appendix 9). Considering that the thickness of the CAD model was 0.9 mm, the errors along the Z-axis were $+43.8 \pm 4.5\%$ and $+29.3 \pm 0.6\%$ for the IOLs printed with 0.006% (w/w) and 0.012% (w/w) Eusorb, respectively. The results from the Mann-Whitney test revealed that the difference between these dimensional errors was not statistically significant ($p = 0.1$). However, the analysis of larger sample sizes would have increased the reliability of this data. The comparison with the results obtained when printing IOLs without

adding any light absorber to the resin revealed that the differences in dimensional error between these samples were not statistically significant either. The error along the Z-axis obtained when printing in the absence of absorber was $+64.6 \pm 7.98\%$, and the results from the Mann-Whitney tests showed that the p value was 0.1 for both comparisons performed between formulations where the absorber had or had not been added (C1 vs. C1 + 0.006% Eusorb and C1 vs. C1 + 0.012% Eusorb). Therefore, it was concluded that the addition of a light absorber to the printable formulations resulted in an increase in the dimensional accuracy along the Z-axis which, while deemed insignificant by the statistical tests performed after these experiments, was definitely perceptible. This was in agreement with the results obtained from the cure depth measurements previously described, which revealed that the reduction in cure depth upon addition of Eusorb was not significant below a concentration of 0.048% of this compound. However, further experiments with larger sample sizes would be required in order to increase the reliability of this data.

The dimensional accuracy on the XY-plane was calculated by measuring the diameter of the optic part of the 3D-printed implants. The errors calculated on this dimension with respect to the CAD model were $+1.39 \pm 4.27\%$ and $+2.44 \pm 0.29\%$ upon addition of either 0.006% or 0.012% Eusorb, respectively. As discussed in chapter four, the error obtained when printing without any absorber was $+5.5 \pm 0.42\%$. Therefore, an increase in the dimensional accuracy was observed when adding Eusorb at the concentrations tested. The results from the Mann-Whitney tests indicated that the difference between the errors obtained with 0.006% and 0.012% Eusorb were not statistically significant ($p = 0.7$). Furthermore, this was also the case for the differences detected between the errors measured with either of the absorber-containing formulations and those of the IOLs printed without this compound ($p = 0.1$ in both cases). The increase in the concentration of light absorber led to an increased error in the haptic-to-haptic length, rising from $+10.46 \pm 1.36\%$ with 0.006% Eusorb to $+11.97 \pm 3.2\%$ with 0.012% of this compound. However, the Mann-Whitney tests indicated that this difference was not significant ($p = 0.9$). Moreover, this dimensional error was not used for comparison of the printing accuracies obtained with different

formulations, as the variations in the haptic-to-haptic length might be slightly biased by the different haptic-optic angles that each IOL may adopt.

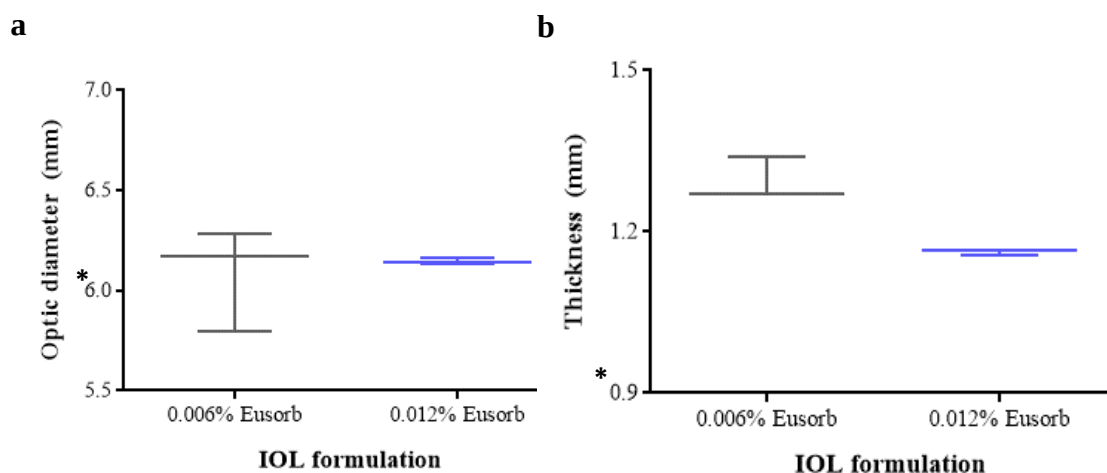


Figure 5.14: Variability in the optic diameter **(a)** and the thickness **(b)** of the IOLs printed with formulations C1 + 0.006% Eusorb and C1 + 0.012% Eusorb. The dimensions that are denoted on the Y-axis with an asterisk correspond to the diameter and thickness of the CAD model.

Overall, it was concluded that the addition of 0.012% Eusorb provided the best results in terms of dimensional accuracy along the Z-axis and reproducibility of results due to the higher degree of light absorption obtained at this concentration. However, the statistical analyses indicated that the differences with respect to the results obtained with formulations C1 and C1 + 0.006% Eusorb were not significant. Further measurements would be required in order to confirm this, as the sample sizes used in these experiments were too small to use parametric tests, which have a higher statistical power than their non-parametric counterparts.

The main disadvantage of using the light absorber was the decrease in the transparency of the lenses. When compared with IOLs composed of formulation C1 and printed without adding Eusorb, the lenses that were produced after the addition of this light absorber to the resin had a lower optical quality (Figure 5.9, (b) and (c)). As well as the effects exerted by this compound on the transparency of the material, the defects created by the layer-by-layer manufacture of the lenses printed with Eusorb may be another factor that contributed to this effect. Thus, a polishing step would be

required in order to improve the optical properties of the IOLs before evaluating their transparency.

5.4.5 Differential Scanning Calorimetry

The T_g of 3D-printed polymers which contained different concentrations of Eusorb UV-1995 was evaluated by DSC. The material obtained by printing with a resin that contained 0.006% (w/w) Eusorb had an average T_g of 8.4 ± 0.31 °C, while the T_g of the polymer obtained after the addition of 0.012% (w/w) Eusorb was 6.46 ± 0.34 °C (Figures 5.13 and 5.14). When compared with the T_g of the polymers obtained by printing with the same formulation before adding the light absorber (4.73 ± 0.32 °C), it was observed that the addition of 0.006% (w/w) Eusorb almost doubled the value of T_g . However, when the concentration of this compound was increased to 0.012% (w/w), the T_g was only 1.73 °C higher than that of the polymer which had been printed in the absence of the absorber. The results of the Dunn's multiple comparisons test revealed that the difference between the T_g of formulations C1 and C1 + 0.006% Eusorb was statistically significant. However, the variations in this parameter between the rest of the formulations were not statistically significant, according to the same test.

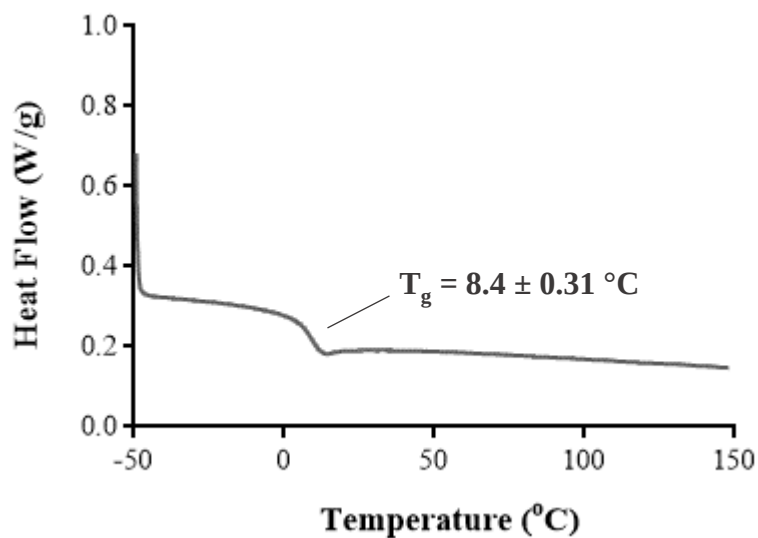


Figure 5.15: Thermogram obtained from the third cycle of the DSC analysis of the polymer that forms the IOLs printed with formulation C1+0.006% Eusorb. The value of T_g is $8.4 \pm 0.31 \text{ } ^\circ\text{C}$.

Table 5.1: Values of T_g of formulation C1+0.006% Eusorb obtained from the replicates analysed by DSC.

Sample	$T_g \text{ (} ^\circ\text{C)}$
1	8.61
2	8.55
3	8.04
Average	8.4 ± 0.31

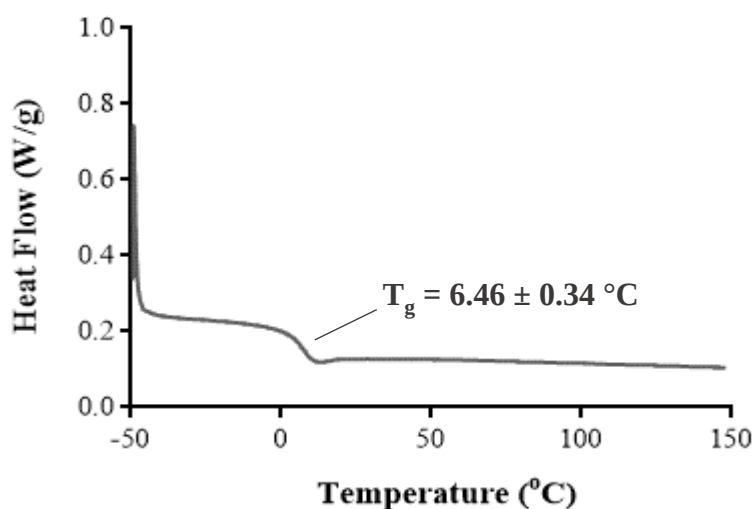


Figure 5.16: Thermogram obtained from the third cycle of the DSC analysis of the polymer that forms the IOLs printed with formulation C1+0.012% Eusorb. The value of T_g is $6.46 \pm 0.34 \text{ } ^\circ\text{C}$.

Table 5.2: Values of T_g of formulation C1+0.012% Eusorb obtained from the replicates analysed by DSC.

Sample	T_g (°C)
1	6.79
2	6.48
3	6.11
Average	6.46 ± 0.34

5.4.6 Thermogravimetric analysis

The thermal stability of the polymers which had been printed after the addition of either 0.006% (w/w) or 0.012% (w/w) Eusorb UV-1995 to formulation C1 was evaluated by TGA. Both materials had similar thermal profiles and had completely degraded at 500 °C (Figures 5.17 and 5.18). The material obtained after the addition of 0.006% (w/w) Eusorb had an IDT of 386.24 ± 1.41 °C, while the IDT of the polymer that contained double the concentration of this compound was 384.24 ± 2.2 °C. According to the results obtained from the Dunn's test, this difference was not statistically significant. When compared to the IDT of formulation C1 (382.41 ± 2.17 °C), an increase of 3.83 °C was observed upon addition of 0.006% (w/w) Eusorb, while the difference in this parameter between formulations C1 and C1 + 0.012% was 1.83 °C. The statistical analyses revealed that these difference were not statistically significant.

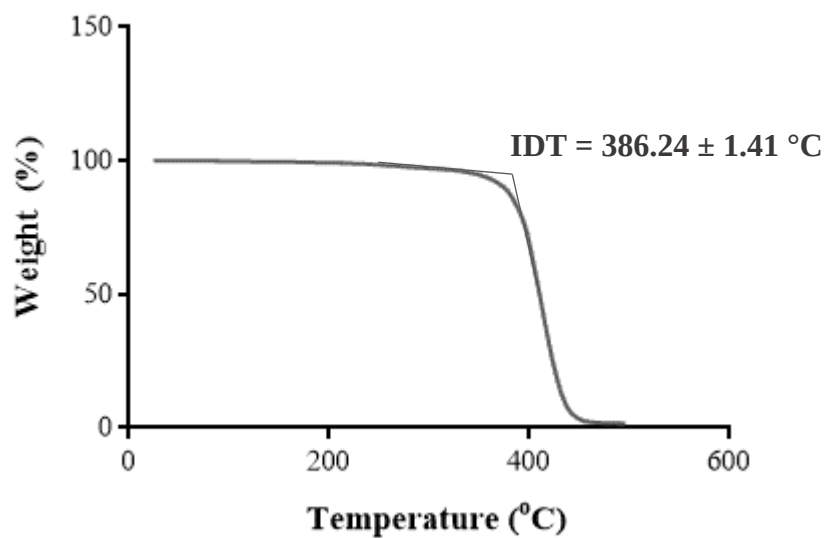


Figure 5.17: Thermogram obtained from the thermogravimetric analysis of IOLs printed with formulation C1+0.006% Eusorb. The IDT is 386.24 ± 1.41 °C.

Table 5.3: Values of IDT of formulation C1+0.006% Eusorb obtained from the replicates analysed by TGA.

Sample	IDT (°C)
1	385.24
2	385.62
3	387.85
Average	386.24 ± 1.41

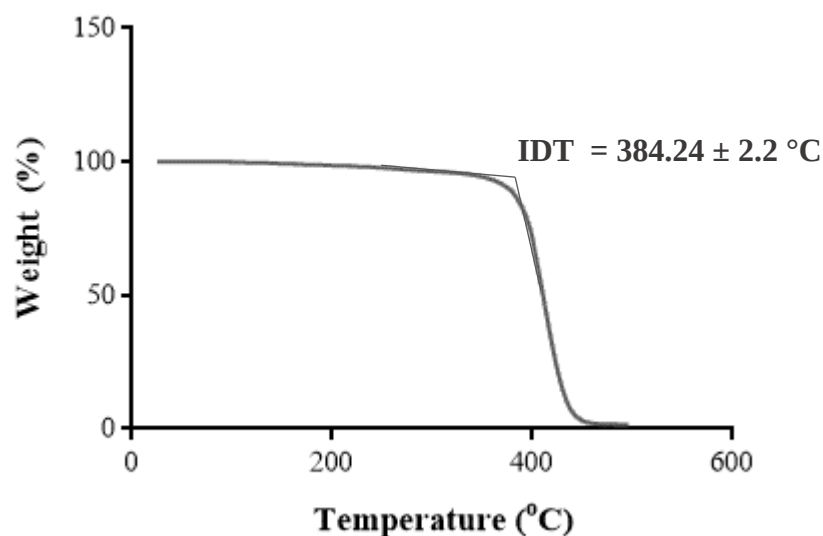


Figure 5.18: Thermogram obtained from the thermogravimetric analysis of IOLs printed with formulation C1+0.012% Eusorb. The IDT is 384.24 ± 2.2 °C.

Table 5.4: Values of IDT of formulation C1+0.012% Eusorb obtained from the replicates analysed by TGA.

Sample	IDT (°C)
1	382.61
2	383.35
3	386.75
Average	384.24 ± 2.2

5.4.7 Scanning Electron Microscopy

SEM images were obtained in order to assess the structural characteristics of IOLs which had been printed with either 0.006% (w/w) or 0.012% (w/w) Eusorb added to their formulation (Figure 5.19). The morphology of the haptics, optic part and optic-haptic junctions were similar in both cases. However, the IOLs which had been produced with the resin that contained the highest concentration of absorber showed a high surface roughness. Since this effect had not been observed in IOLs that contained lower amounts of this compound or which had been printed in the absence

of any light blocker, it was hypothesised that it was a consequence of the inhibitory effect that Eusorb exerted on the photopolymerisation reaction.

With regard to the sharpness of the edge of the implants, this was higher in the IOL printed with 0.006% (w/w) Eusorb than in the analogue which contained double the amount of this compound. The defects formed in the polymer structure as a result of the higher degree of inhibition over the polymerisation reaction may account for this difference. A comparison was also made between the original AcrySof® IOL and the implants that had been printed either in the absence of absorber or with the two concentrations of this compound which had been tested in the manufacturing experiments. It was observed that the implant which had been produced with the resin that contained 0.006% Eusorb had the sharpest edges, and that these were comparable to those of the commercial IOL (Figure 5.20, (b) and (f)). A significant difference in the sharpness of the edges was detected when comparing the IOL that had been printed without the absorber and the implant that contained 0.006% of this compound, the former being noticeably rounder than the latter. This confirmed that an increase in the resolution was required in order to replicate the edge profile of the original model. Despite the improvements made in the edge sharpness upon addition of 0.006% Eusorb to the printable formulation, it was observed that the side of this lens was slightly slanted, in a similar way to the IOLs that had been produced without any light blocker (Figure 5.20, (f)). As this effect was visibly reduced in the IOL that had been printed with 0.012% Eusorb in its formulation (Figure 5.19, (k) and (l)), it was hypothesised that the reduction in the cure depth and the subsequent decrease in the thickness of the lenses had mitigated the tapered effect that can be observed when relatively thick objects are built by stereolithography.

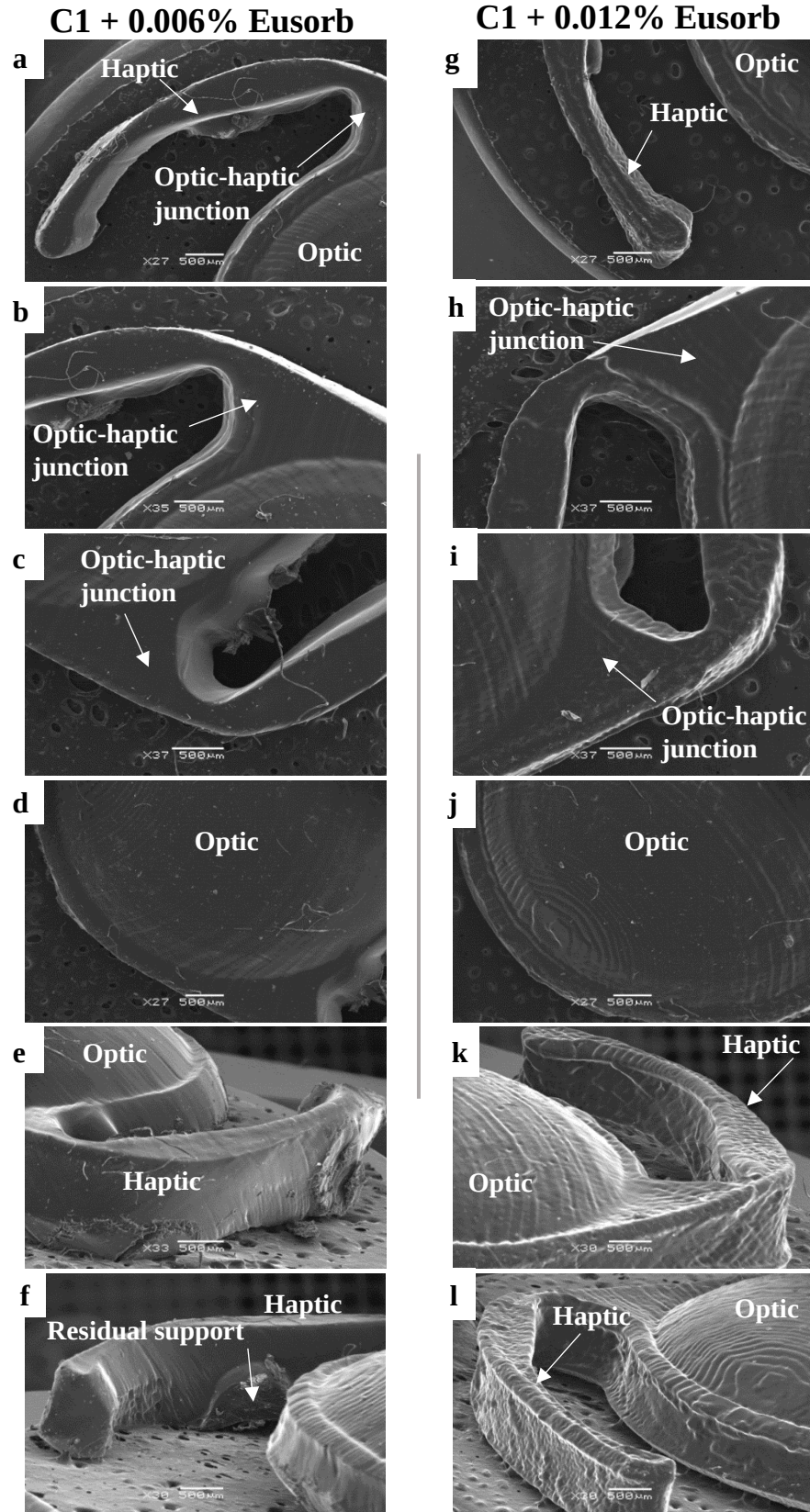
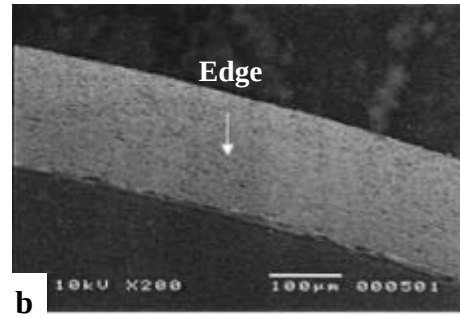
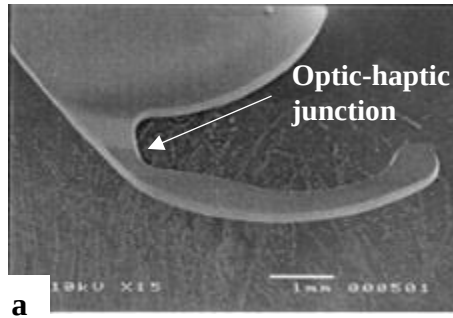
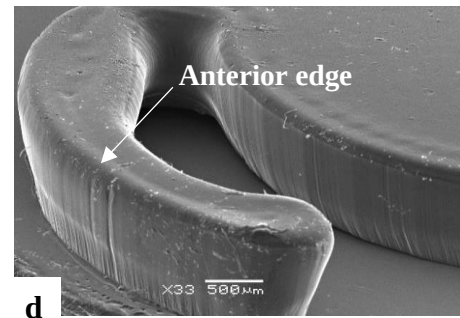
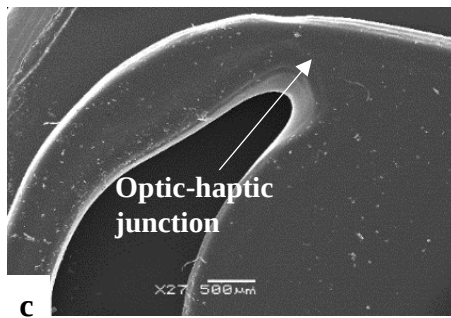


Figure 5.19: SEM micrographs showing the optic-haptic junctions (a-c, h, i), optic part (d, j), and haptics (e-g, k, l) of IOLs that had been printed with C1 + 0.006% Eusorb (a-f) or C1 + 0.012% Eusorb (g-l).

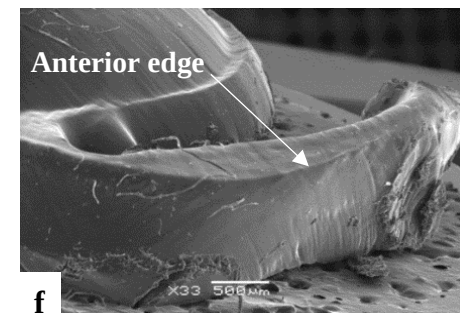
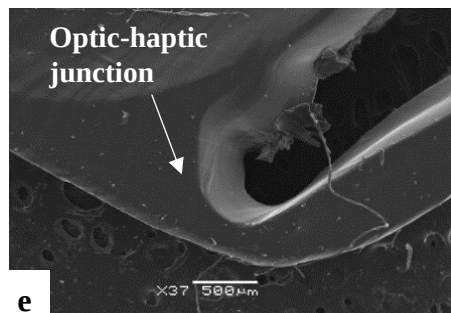
AcrySof®



C1



C1+0.006% Eusorb



C1+0.012% Eusorb

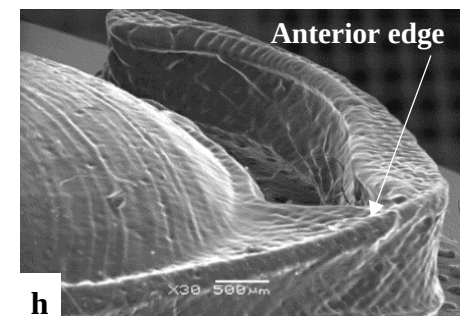
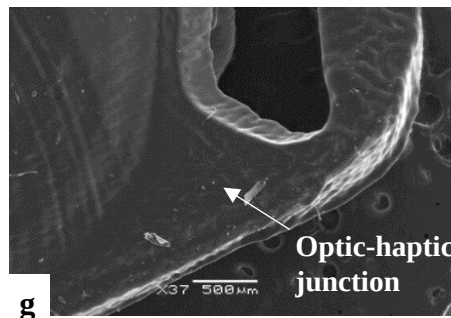


Figure 5.20: SEM micrographs of the original AcrySof® IOL (a, b) and IOLs that had been printed with formulation C1 (c, d), C1 + 0.006% Eusorb (e, f) and C + 0.012% Eusorb (g, h). An increase in the definition of the optic-haptic junction can be observed when adding Eusorb to the formulation (c, e, g), making it comparable to that of AcrySof®. The sharpness of the edge increased upon addition of 0.006% Eusorb, while it decreased slightly when increasing the concentration to 0.012%.

5.5 Discussion

As discussed in previous chapters, the IOLs that were printed with formulations which did not contain any light absorber had a very low resolution as a consequence of the excessive cure depth of these resins. The experiments described in this chapter proved that the addition of a blue-light absorber to the printable formulations resulted in a significant improvement in the resolution, hence reducing the dimensional inaccuracies of the 3D-printed IOLs. The mechanism of action of organic light-absorbing compounds like the one used in these experiments is based on the conversion of the incident light into heat or radiation of a higher wavelength. This causes a reduction in the intensity of the light beam and a subsequent decrease in the cure depth. On the other hand, inorganic absorbers exert their effect by scattering the radiation emitted by the light source⁵⁰². Two fundamental requirements for any absorber to be effective are the possession of a higher tendency to absorb the incident radiation than the photoinitiator present in the formulation and the absorption of light which is in the same spectral region as the radiation emitted by the light source⁵⁰⁸.

Light absorbers have been widely used in the fabrication of ophthalmic materials. These additives have the capability to protect the retina from the photooxidative stress caused by the exposure to short-wavelength radiation^{7,83}. Furthermore, they can act as stabilisers which protect the polymeric materials from the detrimental effect that UV light has on their durability. Thus, this would contribute to the preservation of the optical and mechanical properties of the ophthalmic devices for longer periods of time⁵⁰⁹. A desirable characteristic for any absorber that is to be added to an ophthalmic-grade material is its ability to establish covalent bonds with the polymer chains. This would prevent the molecules from leaching out of the material once the device is implanted in the body, hence avoiding the cytotoxic responses that this would cause in the surrounding tissues^{510,511}.

The absorber used in the present project was Eusorb UV-1995 (Eutec Chemical Co.). This choice was based on the ability of this molecule to absorb light of approximately 400 nm, since the light emitted by the stereolithography system employed for the production of IOLs had a wavelength of 405 nm. As the chemical structure of this compound was not provided by the supplier, an analysis was performed in order to determine its composition and thus its mechanism of action. The results from the analytical techniques employed in these experiments indicated the presence of a benzyl ring in the structure of this compound and the absence of any acrylate groups. This suggested that the mechanism of action of this absorber was based in the absorption of photons by the aromatic rings and the subsequent conversion of this energy into heat or radiation with a higher wavelength. Moreover, the absence of acrylate functionalities indicated that this compound could not bind covalently to the polymer that formed the IOLs, unless it contained any other photoreactive groups such as thiols. The incorporation of the absorber into the polymer via physical entrapment could compromise the biocompatibility of the material, as the molecules could leach out from the device into the intraocular environment if the IOL was implanted in the capsular bag. Therefore, it would be essential to assess the potential toxicity of this material before testing the functionality of the IOLs *in vivo*.

Once the mechanism of action of the light blocker had been elucidated, its effect on the cure depth was assessed. The measurements of the resin cure depth that were carried out using formulations which contained different concentrations of this compound confirmed that the addition of higher amounts of light absorber led to an exponential reduction in the depth of cure. Therefore, it was expected that a significant improvement in the Z resolution would be observed when adding this absorber to the resin employed in the production of IOLs. However, special attention should be given to the reduction in the mechanical strength of the polymer upon addition of Eusorb. Thus, the determination of the minimum concentration of this compound that would lead to a significant alteration in the mechanical performance of the material would have been valuable in these experiments.

Having assessed the influence of the light absorber on the cure depth of the resin, the effect of this compound on the resolution of the 3D-printed IOLs was subsequently evaluated. The addition of 0.006% Eusorb to the photopolymerisable resin resulted in a considerable increase in the dimensional accuracy of the lenses printed on the build platform. This enabled the use of support structures while printing, as no over-curing effects were observed that could impede the separation of the IOLs from these structures once the process was finished. Furthermore, the printed AcrySof®-like IOLs had sharp edges and possessed all the design features that the original model has. However, when other prototypes were printed, some of the structural features of these designs were not properly formed in the resulting lenses. An example of this was the failure to replicate the haptic perforations of one of the IOL designs produced in these experiments. Thus, it was concluded that the amount of absorber used in these tests could not increase the resolution to an extent which allowed the replication of the CAD models with a high fidelity. Therefore, the next formulation tested in these experiments contained double the concentration of Eusorb. The AcrySof® IOLs produced with this formulation were similar to those that had been printed with 0.006% Eusorb. However, a substantial improvement on the Z-resolution was observed, which was attributed to a greater reduction in the cure depth with an increased amount of absorber. This allowed the successful replication of all the design features present in the novel IOL prototypes, including the perforations and topographies that they possessed. Hence, the addition of a higher amount of light absorber could have a beneficial effect on the bioactivity of the IOLs, since it resulted in the successful replication of structural elements which could control the physiological processes that lead to the development of PCO.

While the addition of Eusorb had a positive effect on the resolution of the 3D-printed IOLs, the effect that this compound could have on the physico-chemical properties of the material should also be considered. The SEM images taken of the AcrySof® replicas showed that the IOL which contained 0.012% Eusorb had a high surface roughness. This effect was not observed in lenses that had been printed with resins which contained half the amount of light absorber. Thus, it was hypothesised that the

decrease in the smoothness of the IOLs might have been a consequence of the formation of defects within the polymer network as a result of the inhibitory effect that the light blocker exerted on the photopolymerisation reaction. Since these artefacts could have a detrimental effect on the optical and mechanical performance of the IOLs, further experiments would be required in order to find the optimum concentration of absorber that would increase the resolution of the lenses while preserving the smoothness of their surface⁵¹².

The influence of the light absorber on the mechanical strength of the polymer was assessed by thermal analysis. The addition of this compound caused variations in both the T_g and the thermal stability of the material. The analysis of IOLs which contained 0.006% Eusorb revealed that the T_g of the polymer had doubled its value with respect to the formulation which did not contain any absorber. This could be due to the intercalation of the benzyl rings from this compound between the polymer chains. These bulky functional groups increase the steric hindrance in the system, hence reducing the mobility of the constituent polymer chains and increasing the T_g of the material. However, an opposite trend was observed when increasing the concentration of absorber to 0.012%. While the difference between the T_g of the resulting material and that of the polymer which contained 0.006% Eusorb was not statistically significant, it was hypothesised that the inhibitory effect that the absorber has on the polymerisation reaction could be deleterious for the mechanical strength of the polymer when this compound is added at relatively high concentrations. However, further experiments would have to be conducted in order to confirm this theory.

While the addition of light absorbers to IOL formulations is common practice, the use of Eusorb UV-1995 in the production of ophthalmic implantable devices has not been previously reported. Therefore, the optical quality, mechanical properties and biocompatibility of IOLs that contain this compound should be carefully evaluated. When compared with other 3D-printed IOLs that did not contain any light absorber in their formulation, a decrease in the transparency was observed in the replicas that had been produced after the addition of this compound to the photopolymerisable resin.

However, conclusive results can only be achieved after polishing the lenses in order to remove any artefacts or surface defects that might be compromising the optical quality of the polymer. An additional consideration to take into account when using light absorbers is the long-term stability of the resulting materials under physiological conditions, as IOLs are permanent implants. Therefore, thermal stability studies should be conducted after a prolonged incubation of the lenses in a medium that mimics the intraocular environment.

5.6 Conclusions

The addition of a blue-light absorber to the photopolymerisable resins had a positive effect on the manufacture of IOLs, as it resulted in a considerable increase in both the resolution and the reproducibility of the results. This enabled the production of the lenses layer by layer by using the build platform of the stereolithography system, which made it possible to control the thickness of the implants and to create design features which could not be generated otherwise. This is of particular importance for the manufacture of IOLs, as the thickness and curvature of a lens have a direct effect on its focal length and therefore on its optical performance. Furthermore, some of the structural elements of an IOL have a significant impact on its bioactivity. As previously mentioned, the sharpness of the edge of the implant can contribute to the prevention of PCO by creating a capsular bend that hinders the migration of lens cells to the posterior capsule, while the creation of certain surface topographies can modulate cellular responses such as adhesion, differentiation and migration.

Despite the positive results obtained when adding the light absorber to the resins used in the manufacture of IOLs, the results from the characterisation tests suggested that the addition of relatively high concentrations of this compound could have a detrimental effect on the mechanical strength of the material. Hence, the formulation should be optimised in order to elucidate the concentration of absorber that allows the attainment of the highest resolution possible with the system employed while preserving the mechanical strength of the material. This could involve the adjustment

in the amount of crosslinker and photoinitiator present in the formulation in order to compensate for the inhibitory effects exerted by the light blocker. Once the composition has been optimised, further characterisation experiments would be required in order to assess the suitability of the resulting material for the manufacture of IOLs. Thus, the optical quality, mechanical properties and biocompatibility of the lenses should be carefully evaluated.

Overall, the introduction of a light absorber in the printable formulation allowed the manufacture of different prototypes with elements of design at the microscale that could not be produced when using resins that did not contain this compound. This is of particular importance in the development of bioactive IOLs that have the ability to prevent the development of PCO or other ophthalmic pathologies. However, the production of nanoscale features is critical if the cellular responses are to be controlled. Since this cannot be achieved in conventional stereolithography systems, the use of other photopolymerisation-based technologies such as micro-SLA or two-photon polymerisation would be essential for the production of IOLs with enhanced bioactivity.



CHAPTER 6

Surface Functionalisation of Intraocular Lenses



6. Surface Functionalisation of Intraocular Lenses

6.1 Introduction

The use of an IOL as a drug delivery vehicle offers numerous advantages, including the reduction in the invasiveness of the post-surgical treatments and the local delivery of the therapeutic agent within the capsular bag²⁷⁶. As mentioned in chapter two, numerous approaches exist for loading a drug into the IOL, which include the attachment of loaded devices to the haptics, the immersion of the implant in a drug solution, supercritical fluid impregnation, the chemical grafting of the drug and the deposition of loaded coatings on the lens surface²⁸³. The latter has the added advantage of enabling the modification of the surface properties of the IOL in order to modulate the adhesion of lens cells to the implant. Numerous studies have reported a reduction in the incidence of PCO after the functionalisation of the surface of the lens with highly hydrophilic molecules which possess antifouling properties²⁰⁶. Therefore, the combination of this approach with the entrapment of drugs that target the signalling pathways involved in the development of secondary cataract could be a highly effective method for the prevention of this syndrome. However, special care must be taken in order to preserve the transparency of the lens if the delivery system is bound to the optic part of the IOL. Thus, transparent polymers should be used in the production of the coatings and only therapeutic agents that do not interfere with the quality of vision of the patient are allowed for administration through this method.

Numerous methodologies can be employed to functionalise the surface of an IOL in order to facilitate the attachment of a drug delivery system. Plasma treatment is probably the most common procedure for the modification of the surface of medical implants. A plasma is a partly ionised gas that contains electrons, photons, ions, free radicals and neutral particles^{513,514}. Some of these particles are in an excited state and they eventually return to their ground state, emitting UV radiation that contributes to the modification of the surface. Plasmas are generated when gases are excited to higher energy states by radiofrequency or microwave radiation or by electrons emitted

from a hot filament discharge⁵¹⁴. Depending on the plasma conditions and the abundance of each chemical species in the gas, this treatment can have different effects on the substrate. These include the etching, or degradation of the material by radicals or ions, and the grafting of chemical functionalities onto the surface. The chemical groups generated on the substrate would impart the desired characteristics on the material or provide active sites for further functionalisation⁵¹⁵.

Despite the advantages offered by the utilisation of plasma, there are also some issues associated with the application of this technique. These are mainly related with the stability of the resulting plasma films, as they can be damaged or lost when subjected to abrasion and are prone to accumulate oxygen when exposed to air¹⁹⁸. It has also been shown that the hydrophilicity of plasma-treated IOLs decreases after relatively long times of storage, as the polar groups can rotate away from the surface⁵¹⁶. Consequently, the biological functionality provided by plasma is not permanent. This poses a challenge in the clinical application of implants whose surfaces have been functionalised by following this technique⁵¹⁷.

In view of the potential issues that may be encountered when using plasma to functionalise the surface of IOLs, it is important to consider the alternative techniques that are available for this purpose. These include physical adsorption, irradiation, oxidation with ozone, chemical grafting and the deposition of polyelectrolyte multilayers. Any technique that results in the formation of stable covalent bonds between the substrate and the coating is preferable over physical modifications. Therefore, chemical grafting is becoming a popular choice in the production of functionalised IOLs⁵¹⁸.

The functionalisation of a substrate via chemical grafting can be performed through two different techniques: a coupling reaction between pre-polymerised chains and functional groups from the surface (*grafting-to* approach) or the graft polymerisation of monomers from an initiator that has been previously attached to the substrate

(*grafting-from* method). Chemical grafting requires the presence of reactive functional groups on the substrate. Therefore, inert surfaces must be activated in order to create reactive sites that can undergo chemical conjugation with the molecules that are to be attached to the substrate. This can be achieved by irradiating the surface with UV or gamma radiation or by exposing it to high-energy electrons, ozone or, as explained above, to plasma⁵¹⁹. Furthermore, numerous studies have reported the activation of the surface of IOLs through the immobilisation of the initiator or chain transfer agent used in polymerisation reactions such as (ATRP), (RAFT) or photo-grafting^{203,520,521}.

As discussed at the beginning of this introduction, one of the approaches that can be taken to load drugs into IOLs is the deposition of drug-loaded polymeric coatings. The films deposited on the surface of the IOLs must have a high uniformity, since the even distribution of the material over the surface of the lens is essential for the attainment of an optimum optical performance. This can be achieved by making use of a number of deposition techniques, which include sputtering, dip-coating, chemical vapour deposition and spin coating, among others^{522,523}. Hydrogels are commonly used in this endeavour, as their soft consistency reduces the incidence of irritation in the surrounding tissues once implanted in the body, while their hydrophilicity confers them with an excellent biocompatibility. Furthermore, as these materials swell when incubated in water, a solvent-activated controlled-release of the therapeutic agent can be achieved when using these systems. Different methodologies have been developed for the incorporation of drugs within hydrogels. The direct encapsulation of the therapeutic agent within the polymer structure is perhaps the most straightforward method^{524,525}. This is achieved by mixing the drug with the polymer precursors, which leads to the entrapment of the agent within the gel that results from the polymerisation reaction. An alternative approach for the incorporation of the drug within the hydrogel is the incubation of the gel in a drug aqueous solution, which allows for diffusion of the agent into the pores of the polymer. In cases where a higher degree of control over the release of the drug is required, alternative methods such as the incorporation of drug-loaded micro- or nanoparticles and the chemical conjugation of drug molecules

to the polymer chains lead to a decrease in the release rate and allow the creation of stimuli-responsive systems⁵²⁵.

As one of the main objectives of this thesis was to fabricate bioactive IOLs with the ability to prevent PCO, it was hypothesised that the conjugation of a drug delivery system to the printed implants described in previous chapters would improve their ability to prevent this disorder. The therapeutic agent of choice was Axitinib[®], a small-molecule drug whose capacity to prevent the development of PCO has been recently proven *in vitro*¹. As the IOLs were highly hydrophobic and they did not possess any functional group that could be easily conjugated to Axitinib[®], it was assumed that the most suitable approach for the attachment of the delivery system to the IOLs would be the deposition of coatings on their surface. Various studies have described the formation of polymeric films on the surface of ophthalmic lenses and the entrapment of drugs within these structures in order to achieve a controlled release of the agent^{298,302,526,527}. Moreover, as previously mentioned, a potential benefit of this technique is the increase in the uveal biocompatibility of the IOLs by depositing hydrophilic polymers on their surface. However, special consideration should be given to the effect that this method might have on the development of PCO, since the implantation of hydrophilic IOLs has been related with a higher incidence of this disease.

The polymer used for the synthesis of the coatings produced in the experiments herein described was pHEMA. This choice was based in the hydrophilicity of this polymer, which would allow a swelling-controlled release of the drug, and its historical application for the production of ophthalmic devices³⁰². The molecular weight of the polymer that forms a drug delivery system has a significant effect on the release of the therapeutic agent. Given the ability of RAFT polymerisation to provide a high degree of control over the molecular weight of the resulting material, this was the method of choice for the synthesis of pHEMA⁴³³. Once the polymer was synthesised, coatings made of this material were deposited on the surface of the IOLs via spin coating. The working principle of this technique is based on the deposition of a

polymer solution onto a solid substrate and the subsequent spreading of this material as a consequence of the centrifugal force exerted during high-speed rotation (Figure 6.1)^{523,528,529}. This method has been widely used in the formation of uniform polymer films on the surface of intraocular or contact lenses^{530–532}. While not tested in these experiments, the therapeutic agent could be entrapped within the resulting coatings by dissolving the drug in the polymer solution used for spin coating.

The results of the experimental work reported in this chapter showed that the deposition of a hydrophilic polymer on the surface of the printed IOLs resulted in the formation of unstable coatings that would not resist the mechanical stress exerted on them during the implantation in the capsular bag. Thus, an alternative method based on the chemical conjugation of the drug delivery system to a film created on the surface of the IOLs was envisaged. To achieve this, the IOL was functionalised with polymer brushes made of acrylate-PEG-thiol (Ac-PEG-SH). It was hypothesised that these molecules could be covalently conjugated to a drug delivery system that contains electron-deficient alkene groups which can act as Michael addition acceptors. The subsequent formation of a thioether bond between the coating and the drug delivery system would increase the durability of the resulting assembly, hence preventing the detachment of the delivery system from the surface of the IOL upon implantation in the capsular bag.

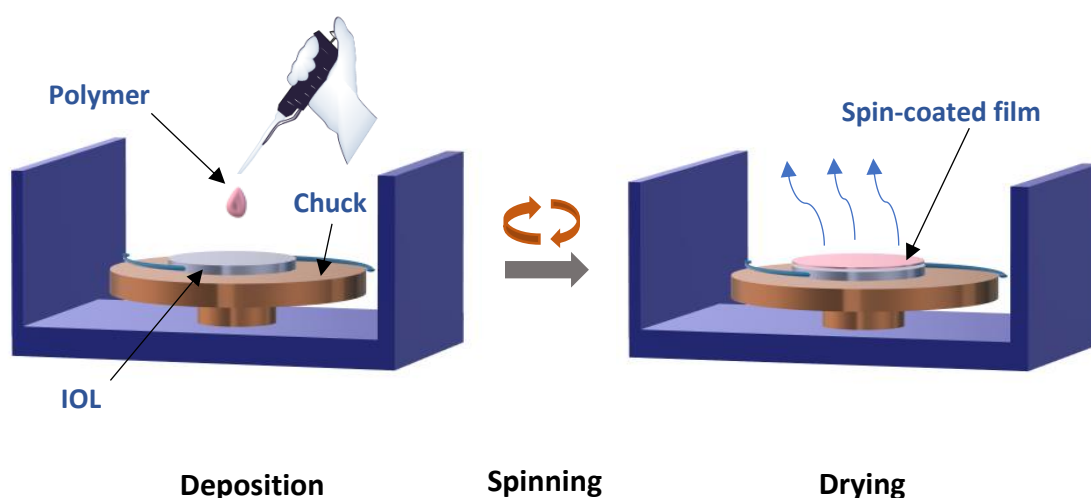


Figure 6.1: Diagram depicting the deposition of a polymeric film on an IOL via spin coating.

6.2 Aim and Objectives

The aim of the experiments reported in this chapter was to attach a drug delivery system to the surface of the printed IOLs which resulted from the work described in previous chapters. The therapeutic agent delivered by this device would be Axitinib[®], since this drug has been reported to prevent the cellular responses that lead to the development of PCO when tested *in vitro*¹.

The first objective was to test the feasibility of using spin-coating for the deposition of polymeric layers on the surface of the printed lenses. The resulting film could be used to encapsulate the drug and control its release once the IOL is implanted in the capsular bag. The first step in this process was the synthesis of pHEMA via RAFT polymerisation. This would be followed by the pertinent characterisation techniques required to elucidate the molecular weight of the polymer. Finally, a solution of this polymer would be prepared in order to facilitate its deposition on the surface of the IOLs via spin coating.

As the results of the spin coating experiments revealed that this method would not be suitable for the attachment of a controlled-release drug delivery system to the surface of the IOLs, an alternative approach was devised. It was hypothesised that an enzyme-responsive delivery system could be synthesised via Michael addition reaction between 4-arm PEG-maleimide and MMP2-cleavable peptides. Thus, the work described in the second part of this chapter focused on the development of a methodology for the functionalisation of the surface of the printed IOLs in order to facilitate the conjugation of this delivery system. A series of characterisation techniques, including SEM, EDS, FTIR, water contact angle measurements and Ellman's assay, were subsequently performed in order to evaluate the success of this technique.

6.3 Materials and Methods

6.3.1 Synthesis of pHEMA for spin coating

Poly(hydroxyethyl methacrylate) with a molecular weight of 5,000 Da was synthesised via RAFT polymerisation. All materials were purchased from Sigma Aldrich and were used without any further purification, unless otherwise stated. The synthesis was carried out by mixing 2-hydroxyethyl methacrylate (98% purity), 2,2'-azobis (2-methylpropionitrile) (98% purity) and 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPTPA) at a molar ratio [HEMA]: [CPCTPA]: [AIBN] = 265: 5: 1 (Figure 6.2). AIBN had been previously recrystallised in methanol and stored at -20 °C before use.

As five grams of polymer were produced, 0.038 moles of HEMA were mixed with 1.46×10^{-4} moles of AIBN and 7.3×10^{-4} moles of RAFT agent. These reagents were poured in a round-bottom flask and mixed with 20 mL DMF. The resulting mix was stirred in a nitrogen atmosphere at 70 °C until complete dissolution. The polymerisation reaction was then carried out by incubating the solution at the same temperature for 24 hours in an oxygen-free atmosphere.

The resulting polymer was purified by performing a precipitation in diethyl ether. The solution of pHEMA in DMF was mixed with twice its volume of methanol. The resulting solution was then slowly poured in five times its volume of diethyl ether. The mix was centrifuged at 4,000 rpm for five minutes and the supernatant was subsequently discarded. The precipitate was re-suspended in double the volume of methanol used for the first precipitation and poured in five times its volume of diethyl ether. The resulting mixture was centrifuged at 4,000 rpm for five minutes. All the pHEMA that had precipitated after carrying out this procedure was collected and dried in a desiccator for 48 hours.

6.3.2 ^1H -NMR

The molecular weight of the pHEMA synthesised by RAFT polymerisation was assessed by ^1H -NMR. First, a 20-mg sample of dry pHEMA was dissolved in 750 μL DMSO- d_6 (99.5 atom% D, Thermo Fisher Scientific, UK). The spectrum was then acquired at 20 $^\circ\text{C}$ on a Bruker 400 spectrometer at 400 MHz (Bruker Corporation, Massachusetts, USA). All chemical shifts (δ) reported are in parts per million (ppm) and were referenced to the residual protons (^1H) of the deuterated solvent. The data was processed using TopSpin 3.5 pl6 software (Bruker Corporation) and Mnova v6.0.2-5475 (Mestrelab Research, Santiago de Compostela, Spain).

6.3.3 Gel Permeation Chromatography

The molecular weight and molecular weight distribution was further characterised by GPC. This analysis was performed using a G7810A Agilent PL-GPC50 system connected with a refractive index detector. A PLgel 5 μm MiniMIX-C (50 x 4.6 mm) guard column was connected to two PLgel 5 μm Mixed-D (300 x 7.5 mm) columns in series and was subjected to a temperature of 30 $^\circ\text{C}$. A mixture of HPLC-grade chloroform/triethylamine (95 : 5, v/v) was degassed and used as the eluent. The machine was calibrated using Agilent EasiVial GPC calibration polystyrene standards with a molecular weight that ranged from 180 Da to 300 Da. The flow rate was set at 1 mL/min. Data were collected and analysed with CIRRUSTM data stream software. A 6-mg pHEMA sample was dissolved in THF to a final concentration of 3 mg/mL and filtered with a 0.45 μm PTFE membrane before manual injection into the machine through the injection valve. The number average molecular weight (M_n), weight average molecular weight (M_w) and the polydispersity (Đ) were obtained from this analysis.

6.3.4 Spin coating IOLs

IOLs which had been printed using formulation C1 and that had been subjected to the post-curing protocol described in chapter four were coated with pHEMA via spin coating. The polymer that had been synthesised via RAFT polymerisation was subsequently dissolved in methanol in order to produce a 10% (w/v) solution of pHEMA. Then, the IOLs were coated with this solution using an Ossila spin coater (Ossila Ltd., Sheffield, UK). The process started by covering the lenses with a layer of methanol by pipetting 6 μ L of this solvent on the surface of the implants and spinning at 800 rpm. This step eased the deposition of the subsequent layers. Two variants of the spin coating procedure were tested in these experiments: static and dynamic coating. The static coating was performed by pipetting 6 μ L of pHEMA solution on the surface of the lenses and spinning at the desired speed for a specific time (see Table 6.2). The resulting layer was dried by flushing with nitrogen before the next one was deposited by following the same procedure. On the other hand, dynamic spin coating was carried out by pipetting 70 μ L of pHEMA solution on the lenses while these were spinning at the desired speed. The resulting coating was dried by flushing with nitrogen.

6.3.5 Optical microscopy

Optical microscopy images of the spin-coated IOLs were obtained using a Leica MZ7.5 stereomicroscope. Images were also taken of non-coated analogues for comparison of the optical quality.

6.3.6 Production of polymer brushes

Polymer brushes composed of Ac-PEG-SH were tethered on the surface of IOLs which had been printed with formulation C1 and that had been subsequently subjected to the post-curing protocol described in chapter four. The “grafting-to” approach described in the Introduction of this chapter was carried out for the production of these polymer brushes. Firstly, a 0.75% (w/v) suspension of Ac-PEG-SH ($\geq 95\%$ purity, M_n 2,000 Da, Biochempeg, Massachusetts, USA) in water was prepared by using

ultrapure water from a Milli-Q[®] water purification system (Millipore Corporation, Massachusetts, USA). The resulting mix was stirred overnight at 50 °C in an oxygen-free atmosphere in order to prevent the oxidation of the thiol groups. After that, TPO (>98% purity, TCI Chemicals, Tokyo, Japan) was adsorbed onto the surface of the implants. To achieve this, the photoinitiator was dissolved in ethanol in order to prepare solutions which contained the concentration of TPO that was required for each experiment. Two different approaches were tested for the adsorption of TPO onto the surface of the lenses. The first one consisted in pipetting 10 µL of TPO solution on the optic part of each IOL and incubating the implants at room temperature for 15-20 minutes before drying them at 110-120 °C for five minutes. The second approach was based on the immersion of the implants in 2 mL of TPO solution for 15-20 minutes at room temperature. After that, the IOLs were dried at 110-120 °C for five minutes.

Once the photoinitiator molecules were adsorbed on the surface of the lenses, the conjugation to Ac-PEG-SH was carried out. First, 20 µL of Ac-PEG-SH suspension were pipetted on top of each IOL. After that, the lenses were incubated in a UV-curing chamber (Meccatronicore, Trento, Italy) at 55 °C for two hours, adding 20 µL of the Ac-PEG-SH suspension every 15 minutes in order to prevent the coating from drying out and to incorporate as many molecules of Ac-PEG-SH as possible into the resulting film. Once this step was finished, the excess of polymer was washed away by incubating the IOLs in approximately 50 mL of Milli-Q[®] water for three hours under continuous agitation at 100 rpm. The vessels used for washing had been covered with Parafilm[®] and purged with nitrogen in order to minimise the oxidation of the thiol groups present in the polymer brushes.

6.3.7 Scanning Electron Microscopy

SEM images of IOLs that been coated either with pHEMA via spin coating or with Ac-PEG-SH by synthesising polymer brushes were generated in order to assess the efficiency of the methods that had been followed to fabricate these structures. The samples were mounted on aluminium stubs and coated in an argon-filled sputter gold coater (Polaron SC7640, Quorum Technologies, UK) for 30 seconds before acquiring

the images in a JEOL JSM 5900 LV microscope (Jeol Ltd., Tokyo, Japan) with a 20-kV accelerating voltage.

6.3.8 Ellman's reaction

The amount of thiol groups present on the IOLs that had been coated with Ac-PEG-SH was assessed by performing an Ellman's assay. All materials used in this reaction were purchased from Sigma Aldrich and were used without any further purification, unless otherwise stated. Sodium phosphate ($\geq 99\%$ purity, Thermo Fisher Scientific, UK), ethylenediaminetetraacetic acid ($\geq 99\%$ purity) and triethanolamine ($\geq 99\%$ purity) were used in the preparation of the buffers. The reaction was carried out as per the manufacturer's protocol. Ellman's reagent solution was prepared by dissolving 4 mg 5,5-dithio-bis-(2-nitrobenzoic acid) ($\geq 98\%$ purity) in 1 mL of reaction buffer (0.1 M Na_2HPO_4 , pH 8.0, containing 1 mM EDTA). Each IOL was then introduced in a 20-mL glass vial and 2.5 mL of reaction buffer were added. Then, 50 μL of Ellman's reagent solution were added to each vial and the samples were incubated for 30 minutes at room temperature. The absorbance was read at 412 nm using a CLARIOstar[®] plate reader (BMG Labtech, Aylesbury, UK) and the concentration of thiol groups was calculated using the Beer-Lambert law:

$$A = \varepsilon c l \quad (5)$$

where "A" is the absorbance of the solution, " ε " is the molar extinction coefficient of the analyte in the reaction buffer used in this protocol ($14,150 \text{ M}^{-1}\text{cm}^{-1}$, as per the manufacturer's protocol), "c" is the concentration of analyte and "l" denotes the length of the light path, which is equal to the width of the cuvette (1 cm).

6.3.9 Water Contact Angle

The efficiency of the synthesis of polymer brushes was assessed by performing water contact angle measurements using a goniometer built in-house. This device was

comprised of a red LED light source, a Hamilton needle and an imaging source video camera (1,080 x 768 pixel) connected to image capture software. The water contact angle was measured using ImageJ (NIH, Maryland, USA).

6.3.10 ATR-FTIR

The coating efficiency was evaluated by ATR-FTIR spectroscopy. The spectra were recorded using a Bruker Vertex 70 FTIR spectrophotometer (Bruker Corporation, Massachusetts, USA) by running 32 scans between 4,000 and 500 cm^{-1} at a resolution of 4 cm^{-1} .

6.3.11 Statistical analysis

The statistical significance of the differences in the concentration of thiol groups between IOLs that had been coated by following different methodologies, as measured by Ellman's assay, was evaluated by performing the non-parametric Kruskal-Wallis test. Furthermore, the differences between each of the experimental groups were analysed by carrying out the Dunn's multiple comparisons test. The statistical package GraphPad Prism v.6.01 was used for all statistical analysis. The level of significance was set at $p < 0.05$.

6.4 Results

6.4.1 Coatings produced by spin coating

6.4.1.1 Synthesis of pHEMA via RAFT polymerisation

The polymers obtained from the RAFT polymerisation of HEMA were analysed by ^1H -NMR in order to elucidate the average molecular weight of the constituent polymer chains. This was done by calculating the ratio between the number of protons in the polymer macromolecules and those that were present in the benzyl ring of the RAFT agent. Since each polymer chain has, in theory, one benzyl ring from the RAFT agent,

and it is known that this functional group has five protons, the number of protons from a specific functional group present in each of the monomer units that constitute a polymer chain could be estimated by using the integrals of each signal. In this case, the functional group from the monomer that was used for this calculation was the methylene named “c” (Figure 6.4). Since it is known that this functional group has two protons, and that each monomer unit has one methylene “c”, the number of monomer units per chain could be estimated from this data. Once the approximate number of monomers per chain was known, an estimation could be made for the molecular weight of the polymer chains by considering that the molecular mass of each monomer is 130.14 Da and the molecular weight of the RAFT agent bound to each chain is 278.38 Da. The approximate value of M_n obtained from these calculations was 6,803.95 Da, which was relatively close to the target value (5,000 Da).

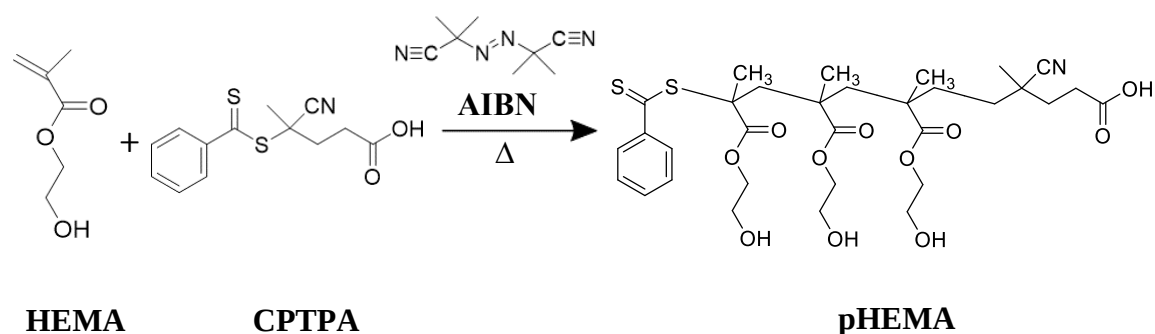


Figure 6.2: Reaction scheme of the synthesis of pHEMA via RAFT polymerisation. The RAFT agent used in this reaction was 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPTPA).



Figure 6.3: Digital image of the pHEMA obtained via RAFT polymerisation, after drying.

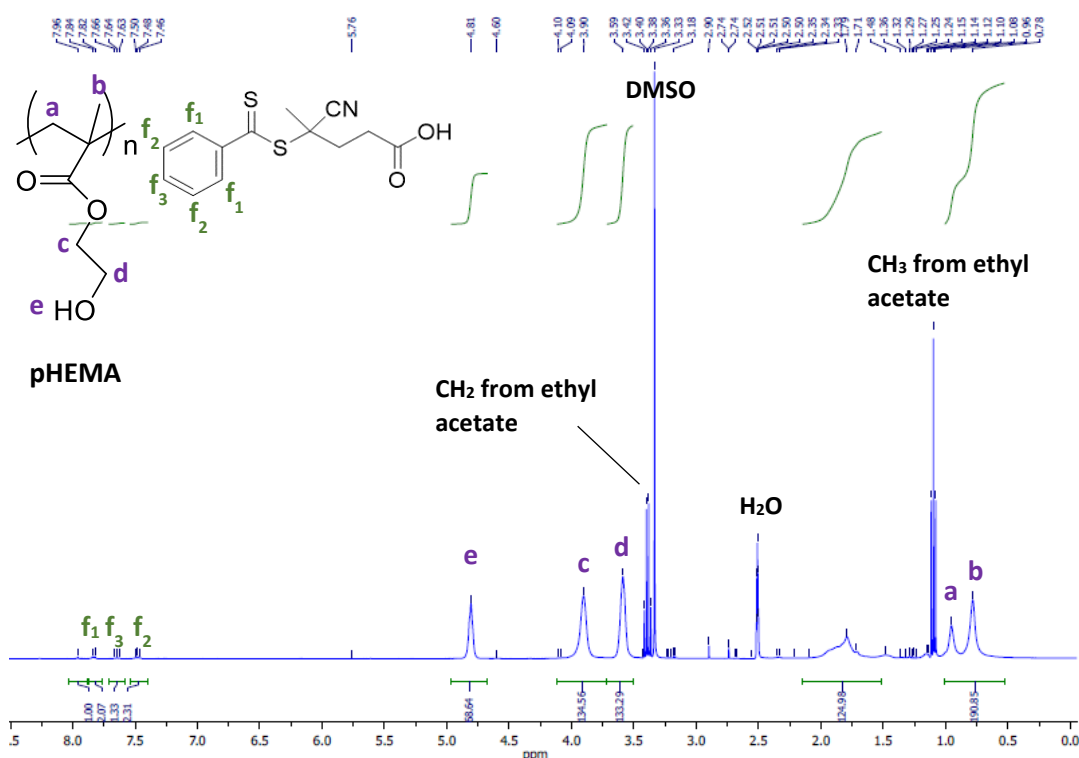


Figure 6.4: ^1H -NMR spectrum of pHEMA synthesised via RAFT polymerisation. The integral values for the signals produced by the benzyl ring are 1, 2.07, 1.33 and 2.31, while the integral of the signal produced by the methylene named “c” was 134.56. These values were used in the calculation of the approximate molecular weight of the polymer chains.

The calculations performed to calculate the average molecular weight of the polymer are described as follows:

$$\Sigma(\text{integrals from benzyl ring}) = 1 + 2.07 + 1.33 + 2.31 = 6.71$$

$$6.71/5 = 1.342 \text{ per proton}$$

$$\text{Number of protons from (c)methylene group} = \frac{134.56}{1.342} = 100.27 \text{ protons}$$

$$\begin{aligned} \text{Number of (c) methylene groups per polymer chain} &= \frac{100.27 \text{ protons}}{2 \text{ protons/group}} \\ &= 50.135 \text{ (c)groups} = 50.135 \text{ monomer units per chain} \end{aligned}$$

Estimated molecular weight for each chain

$$= 130.14 \frac{\text{Da}}{\text{monomer}} * 50.135 \text{ monomers} + 278.38 \text{Da} = \mathbf{6,803.95 \text{ Da}}$$

6.4.1.2 Gel Permeation Chromatography

GPC was employed to confirm the molecular weight of pHEMA and to evaluate the polydispersity of the polymer. The number average molecular weight (M_n), weight average molecular weight (M_w), conversion and polydispersity ($\mathcal{D}$) are shown in Table 6.1.

Table 6.1: Molecular weight and polydispersity of pHEMA synthesised via RAFT polymerisation.

Sample	Conversion	M_n (Da)	M_w (Da)	$\mathcal{D}$
pHEMA	98%	3,579	4,046	1.13

According to the results obtained from this analysis, the molecular weight of the polymer was 4,046 Da, which was relatively close to the target value (5,000 Da). The polydispersity index was 1.13, which indicated that the polymer did not have a high polydispersity. However, this value was not within the range that is typical from highly controlled synthetic polymers, which usually possess a polydispersity index that ranges from 1.02 to 1.1⁵³³.

6.4.1.3 Spin coating IOLs

Various IOLs were coated with pHEMA via spin coating. When following the static coating approach, the minimum spinning speed and time required to obtain relatively uniform coatings were 800 rpm and 45 seconds. The deposition of both single and multiple layers was tested, resulting in the formation of coatings with different thicknesses. However, the production of multiple layers resulted in a reduction in the uniformity of the coatings. In addition, the resulting films had a high tendency to form cracks when drying. This had a detrimental effect on the transparency of the lenses, as the presence of fractures resulted in the occurrence of light scattering phenomena.

It is also important to note that the optical quality of the lenses was compromised by the slightly pink coloration that pHEMA presented as a consequence of the use of CPTPA in its production (Figure 6.5). This effect was intensified when creating multiple layers of pHEMA on the surface of the lenses. Finally, as a consequence of the hydrophobicity of the IOLs, the distribution of pHEMA over the surface of the lenses proved to be be a challenge at speeds lower than 800 rpm.

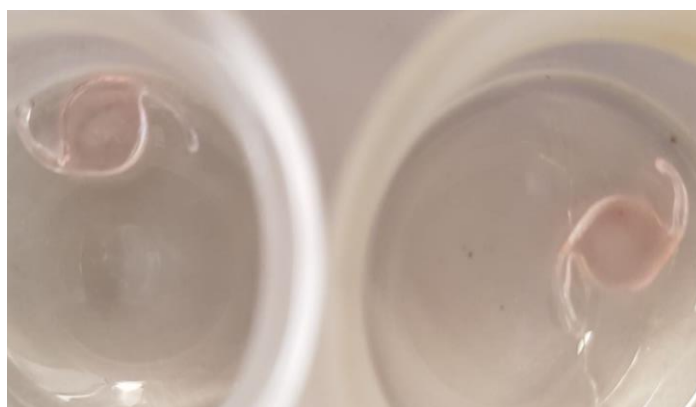


Figure 6.5: Digital image of two IOLs coated with two layers of pHEMA via spin coating. The slightly pink coloration of the coatings can be observed.

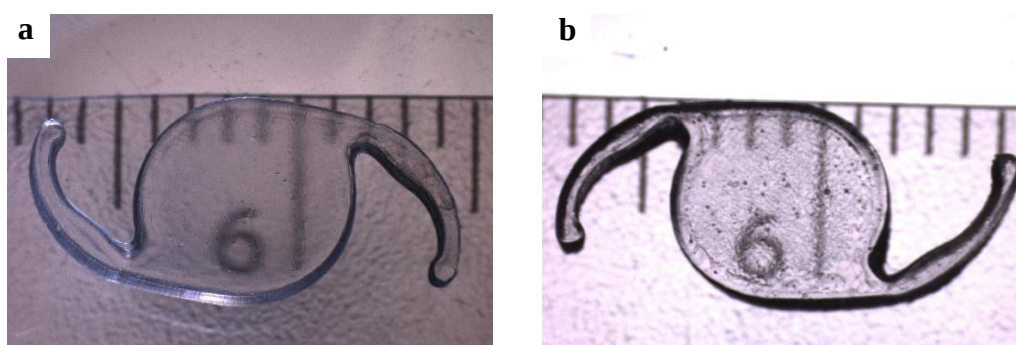


Figure 6.6: Optical microscopy image of a non-coated IOL (a) and an IOL that had been coated with one layer of pHEMA via spin coating (b).

6.4.1.4 Analysis of the coating efficiency via SEM

The stability of the pHEMA-based coatings in an aqueous environment was assessed by obtaining SEM micrographs of the coated IOLs after a four-hour incubation in 1mL of PBS at 35 °C in order to mimic the conditions found in the posterior chamber of the eye. The IOLs had been coated with either two or eight layers of polymer in order to compare the stability of coatings that had different thicknesses. Before incubation in the aqueous medium, it was observed that the coatings were not uniformly distributed over the surface of the lenses (Figures 6.7 and 6.8 (a)). Most of the polymer had accumulated at the centre of the optic portion of the implants, while the periphery was barely covered by the coating. This could be a consequence of the differences in hydrophilicity between the coating and the IOL, which would cause a tendency to reduce the contact surface between them. Furthermore, as mentioned in the previous section, another problem encountered was the formation of multiple cracks over the surface of the polymeric coatings. This had a detrimental effect on the optical quality of the lenses and should therefore be prevented.

After the incubation in an aqueous medium, most of the coating had washed away from the surface of both IOLs. When compared to the dry lenses, the coating densities of the IOLs that had been incubated in PBS were much lower (Figures 6.7 and 6.8 (b)). The SEM images showed that a thin layer of polymer was still attached to the IOLs, as the cracks were visible on the surface of the lenses and some proportion of the coating could still be observed. Therefore, it was concluded that the stability of the polymeric films in an aqueous environment was minimal, as most of this structure had washed away from the surface of the lenses after only four hours of incubation. The results also indicated that the creation of thicker coatings did not result in an increase of the residence time of these structures when immersed in an aqueous medium. This indicated that the deposition of coatings made of pHEMA on the surface of the lenses was not an appropriate strategy for the development of a drug delivery system, as the short residence time of the polymer on the surface of the IOLs would make it impossible to control the release of the drug entrapped within these coatings.

The rapid solubilisation of pHEMA would lead to a rapid burst-release of the drug. This would reduce the control over the release kinetics of the therapeutic agent, hence increasing the incidence of side effects in the surrounding tissues if the IOL was implanted in the eye. While this effect could be delayed by increasing the molecular weight of the polymer used in the production of the coatings, this would not guarantee a higher degree of control over the release of the drug in the long term. Therefore, the use of crosslinked polymers which cannot dissolve in water would be required in order to achieve an appropriate regulation of the drug release kinetics. Furthermore, the adherence of the coatings to the surface of the lenses should be enhanced by creating physical or chemical interactions that would prevent the detachment of the polymer chains from the surface of the implants.

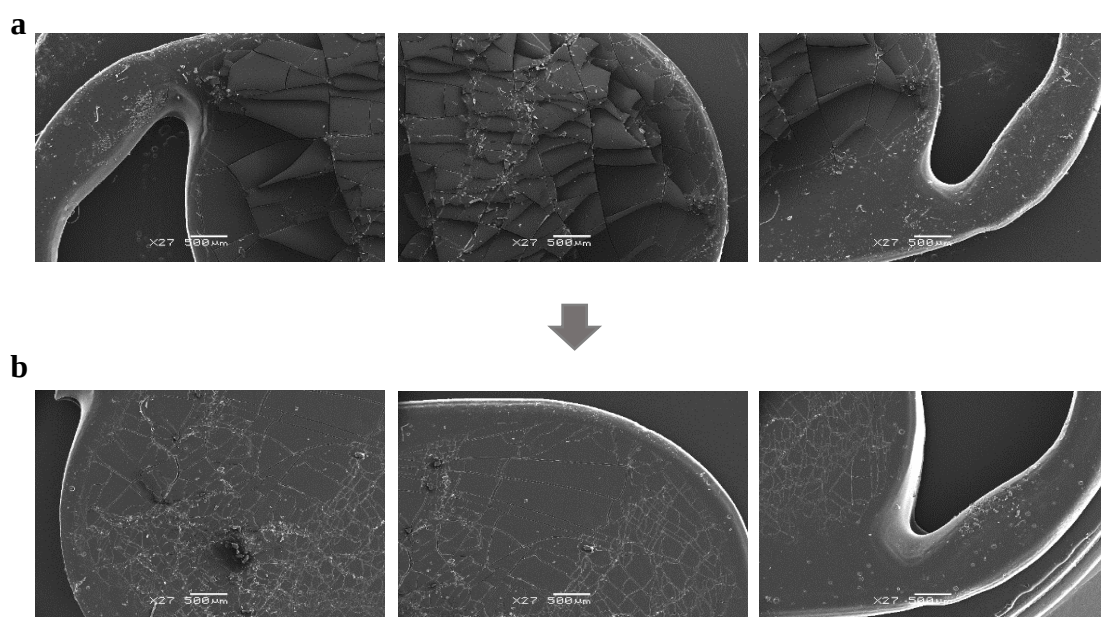


Figure 6.7: SEM images of an IOL spin-coated with two layers of pHEMA before incubating in water **(a)** and after incubation **(b)**. It can be seen how most of the coating had detached from the surface. All scale bars represent 500 µm and the magnification was 27X.

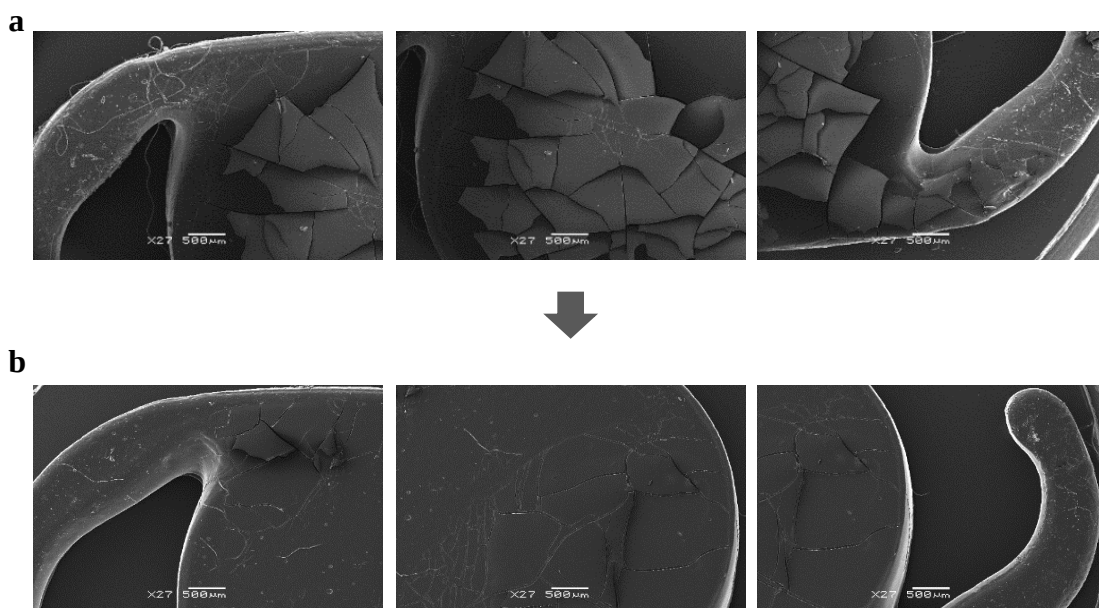


Figure 6.8: SEM images of an IOL spin-coated with eight layers of pHEMA before incubating in water **(a)** and after incubation **(b)**. It can be seen how most of the coating had detached from the surface. All scale bars represent 500 μm and the magnification was 27X.

6.4.1.5 Optimisation of the spin coating process

As well as the low stability of the coatings when immersed in an aqueous medium, other issues that needed to be addressed were the formation of cracks in these structures and the relatively low uniformity of the resulting coatings. It was hypothesised that an increase in the spinning speed would result in a higher uniformity in the distribution of the polymer over the surface of the lenses, while the formation of cracks could be prevented by adding water to the pHEMA solution in order for it to act as a plasticiser and to slow down the evaporation of the solvent.

Firstly, the effect of higher spinning speeds on the distribution of the polymeric coatings was evaluated. The methodology followed in these experiments was static spin coating. It was observed that higher spinning speeds generally resulted in a slightly higher uniformity of the coatings (Table 6.2). However, the efficiency of the process was compromised by the low reproducibility of the results and the fact that some IOLs moved away from the chuck when spinning at such high speeds.

Furthermore, a complete coverage of the lenses was not achieved in these experiments, which could be due to the relatively low volumes of polymer solution used (6 μL). Therefore, further optimisation was required in order to improve the results obtained with this technique in terms of coating coverage and reproducibility.

Having observed that an increase in the spinning speed resulted in an improvement in the uniformity of the coatings, the effect of using higher volumes of polymer solution was subsequently evaluated. The results of these tests indicated that the use of higher volumes of solution, up to 70 μL , resulted in higher coating coverages. However, the excess of liquid showed a tendency to flow from the top of the lens onto the chuck. It was hypothesised that this could be solved by following the dynamic coating method, as the solution would be slowly dispensed while spinning. This approach resulted in the production of coatings with higher uniformities when spinning at speeds of up to 4,000 rpm. When coating at such high speeds, the IOL had to be stuck to a piece of Parafilm[®] in order to prevent it from moving away from the chuck. Some of the disadvantages of carrying out the spin coating procedure at high speeds were the spread of the films to the haptics in some of the IOLs and the difficulty to control the deposition of the layers (Figure 6.9). This resulted in a reduction in the reproducibility of the results, which was accentuated by the uncontrolled loss of polymer solution that resulted from spinning at a high speed. Furthermore, while the uniformity of the polymeric films was higher after spinning at these velocities, these structures did not possess the smoothness required for an ophthalmic device. Finally, it was observed that the use of a solution that contained 50% water and 50% methanol as a solvent for pHEMA did not prevent the formation of cracks in the coatings. Thus, it was hypothesised that the use of alternative polymeric systems for the production of these films and the optimisation of the drying process would be the solutions to this problem.

Table 6.2: Summary of the results obtained during the optimisation of the spin coating process.

Speed	Results
800 rpm	The coating was not uniformly spread over the surface
1,000 – 1,800 rpm	The uniformity of the coating improved with an increase in the speed
2,000 – 2,500 rpm	The coating was smoother
4,000 rpm	Highest uniformity observed

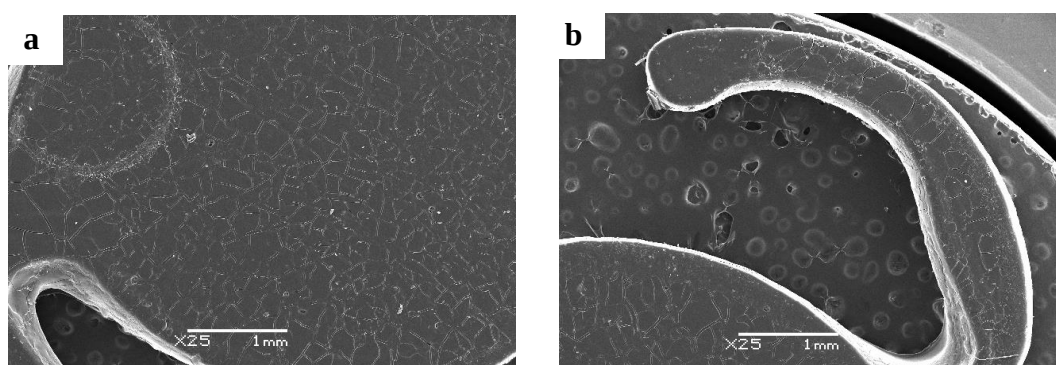


Figure 6.9: SEM micrographs showing the optic part **(a)** and the haptic **(b)** of an IOL coated with three layers of pHEMA at a spinning speed of 4,000 rpm by following the dynamic spin coating method. The scale bars represent 1 mm and the magnification was 25X.

Overall, the experiments described herein showed that the production of coatings with non-crosslinked pHEMA was not a suitable approach for the creation of controlled-release drug delivery systems. This was concluded after observing that the stability of the coatings which resulted from the deposition of this polymer on the surface of the IOLs was not high enough to endure the incubation in an aqueous environment. This was a direct consequence of the high solubility of pHEMA in this medium. The absence of any chemical bonds between the IOL and the polymeric film was another factor that contributed to the low residence time of the polymer on the surface of the IOLs upon immersion in PBS. Furthermore, other issues such as the low uniformity

of the coatings and the formation of cracks on their surface were also encountered in these experiments. Despite the improvements made in the uniformity of the films by optimising the spin coating procedure, the unequal distribution of these coatings over the surface of the lenses and the low reproducibility of the results were still a significant issue. These complications would increase the difficulty in producing drug delivery systems with specific drug loadings and which have the ability to control the release kinetics of the drug. Thus, it was concluded that an alternative method would be required for the production of these devices.

6.4.2 Synthesis of polymer brushes

As the experiments described in the previous section proved that the deposition of pHEMA-based coatings on the printed IOLs would not result in the production of a controlled-release drug delivery system, an alternative methodology was devised. It was hypothesised that an enzyme-responsive drug delivery system could be synthesised in order to achieve a higher degree of control over the release of the therapeutic agent. This polymeric structure would be crosslinked with enzyme-cleavable peptides, which could be conjugated to maleimide-functionalised polymers via Michael addition reaction. In order to increase the durability of the drug delivery system, it was theorised that it could be chemically conjugated to the surface of the printed IOLs. The absence of reactive functional groups on the lenses and the fact that no plasma system was available for use in this project made it necessary to devise a method for the introduction of reactive sites on the surface of the lenses. It was hypothesised that the presence of thiol groups on the surface of the IOLs would enable the chemical conjugation between both systems via Michael addition reaction. Therefore, the objective of the experiments described in this section was the synthesis of thiol-containing polymer brushes on the surface of the printed lenses.

The polymer of choice for the production of the polymer brushes was Ac-PEG-SH. One of the reasons behind this selection was the presence of acrylate groups at one of

their chain ends, which would enable the photo-grafting of these molecules on the surface of photoactivated IOLs. This procedure was based on the physical adsorption of photoinitiator molecules onto the surface of the lenses and the subsequent radical-mediated attachment of acrylate-functionalised polymer chains upon exposure to UV light (Figure 6.10). The protocol developed in this work was an adaptation of a similar methodology reported in the literature for the conjugation of MPC onto the surface of hydrophobic acrylic IOLs²⁰³. The choice of a PEG-based molecule for the experiments described in this chapter was based on the biocompatibility of this polymer and the availability of PEGs functionalised with numerous functional groups. Finally, as previously mentioned, the presence of thiol groups at the terminus of the polymer chains would enable the establishment of chemical bonds with the drug delivery system via Michael addition reaction with the maleimide functionalities present in this polymer.



Figure 6.10: Diagram depicting the synthesis of the polymer brushes composed of Ac-PEG-SH.

The first experiments carried out for the synthesis of the polymer brushes described above were performed by activating the IOLs with a 0.16% (w/v) TPO solution in ethanol, as this was the concentration used in the study that this work was inspired by. The TPO solution was pipetted on the surface of the lenses and incubated for 20 minutes at room temperature prior to the production of the polymer brushes. Since the resulting coating could not be detected by FTIR (Appendix 10), it was hypothesised that a higher concentration of TPO may be required in order to increase the coating efficiency. Thus, the experiment was repeated by using 0.32% (w/v) and 0.64% (w/v) TPO solutions for the first step of the protocol. The coatings generated after making these alterations in the protocol were visible by both eye and FTIR microscopy (Figure 6.11). However, the FTIR spectra did not show any signal from these PEG-based

coatings (Appendix 10). Thus, additional methods were sought for the characterisation of the polymer brushes. These included SEM, water contact angle measurements and Ellman's assay for the detection of the thiol groups.

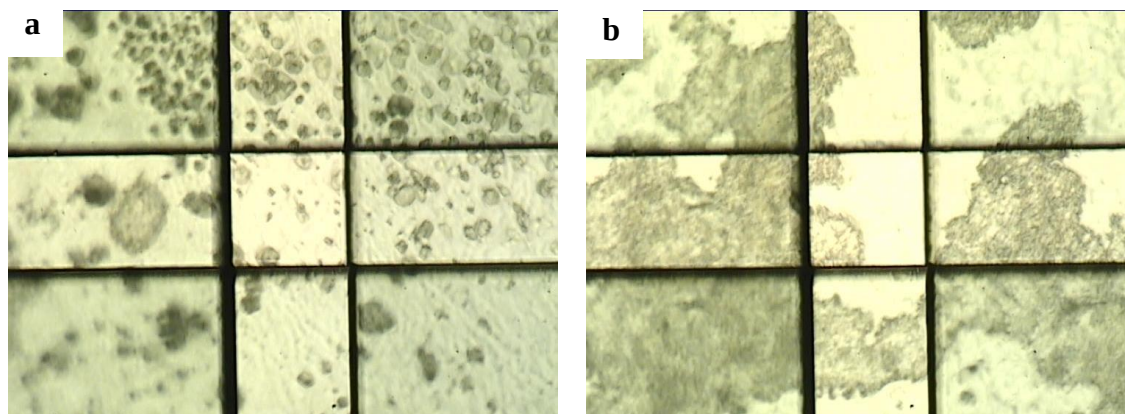


Figure 6.11: FTIR microscopy images of two different locations of a C1 IOL coated with Ac-PEG-SH after being photoactivated with a solution that contained 0.32% TPO. The darker areas correspond to the coating deposited on the surface of the lens.

6.4.2.1 Optimisation of the coating methodology

Having observed in the FTIR microscopy images that the coatings described above were not uniformly distributed over the surface of the lenses, further experiments were carried out with the aim of increasing the efficiency of the photografting process. The concentration of TPO in the activating solution was increased to 10% (w/v) in order to improve the coverage of the surface of the IOLs with Ac-PEG-SH. Moreover, this would increase the feasibility of the reaction of TPO with the acrylate groups from Ac-PEG-SH, which could also react with the thiols present in the same molecule or in other polymer chains. Two different approaches for the adsorption of TPO onto the surface of the lenses were tested in the following experiments. The first method consisted in pipetting 10 μ L of TPO solution on the surface of the lenses, while the second procedure was based on the immersion of the IOLs in 2 mL of this solution. After that, a 20-minute incubation at room temperature was carried out in both cases. The Ac-PEG-SH molecules were subsequently grafted onto the surface-activated

IOLs by following the procedure described on the Materials and Methods section of this chapter. Once the polymer brushes were produced, SEM images and measurements of the water contact angle were taken in order to compare the uniformity and surface coverage of the coatings, as described as follows.

6.4.2.1.1 Scanning Electron Microscopy

SEM images were taken in order to evaluate the degree of coverage and uniformity of the coatings that had been obtained after using a 10% TPO solution to activate the surface of the IOLs. As mentioned above, two methods had been tested for the adsorption of the photoinitiator onto the lenses: pipetting the solution on the surface of the IOLs or incubating the implants in it. The efficiency of each method was assessed by evaluating the degree of coverage and coating density obtained in the films that resulted from each of these two approaches.

As seen in Figure 6.12, the degree of coverage of the IOLs that had been immersed in the TPO solution prior to the attachment of Ac-PEG-SH was higher than in those in which the activating solution had been deposited on their surface. Figures (b) to (d) showed that the polymer brushes which were formed after pipetting the activating solution on the IOLs had a high tendency to accumulate at the centre of the lenses, while the periphery was mostly uncovered. This may be due to the fact that the solution had been dropped in the middle of the optic part of the implants and was then allowed to spread out towards the periphery. On the other hand, Figures (e) to (g) showed that a higher degree of coverage was obtained by immersing the IOLs in the TPO solution prior to photografting Ac-PEG-SH on them. The resulting coating covered almost all the surface of the lens, only leaving the outermost part of the optic portion partly uncovered. While this coating was denser than the polymer brushes obtained after pipetting the activating solution on the IOLs, its uniformity was somewhat lower. This was due to the presence of accumulations of polymeric material which decreased the smoothness of the film. Furthermore, it was observed that the coating was thinner at the periphery than at the centre of the lenses (Figure 6.12, (e)).

These observations indicated that the most efficient approach for the deposition of the photoinitiator on the lenses prior to the production of the polymer brushes was the incubation of the IOLs in the TPO solution. Moreover, further experiments would be required in order to optimise the uniformity and degree of coverage of the coatings obtained with this method.

Given that the thiol groups from the polymer brushes could not be detected in the FTIR analyses performed in previous experiments (Appendix 10), energy dispersive spectroscopy (EDS) was employed to evaluate the presence of sulphur in the coatings. This would provide an indication of the presence of thiol groups on the surface of the lenses. EDS is an analytical technique associated to SEM systems which elucidates the elemental composition of a sample upon detection of the X-rays generated when high-energy electrons interact with the entity subjected to the analysis^{534,535}. Three different locations of a coated IOL were analysed, sulphur being detected in all of them (Figure 6.13). However, spectrums 1 and 2 (obtained from Figure 6.13, (a)) reported the presence of sulphur at lower concentrations than other elements which theoretically were not present in the polymer brushes, such as sodium, phosphine or chlorine. On the other hand, spectrum 1 from Figure 6.13, (b) indicated that the proportion of sulphur in this location was higher than in the previous region. Moreover, sodium and phosphine were not detected, while the proportion of chlorine was 0.74% . Overall, conclusive results with regard to the presence of sulphur from thiol groups could not be obtained from this analysis, as the detection of elements which in theory should not be present in the system suggested a low reliability of the results.

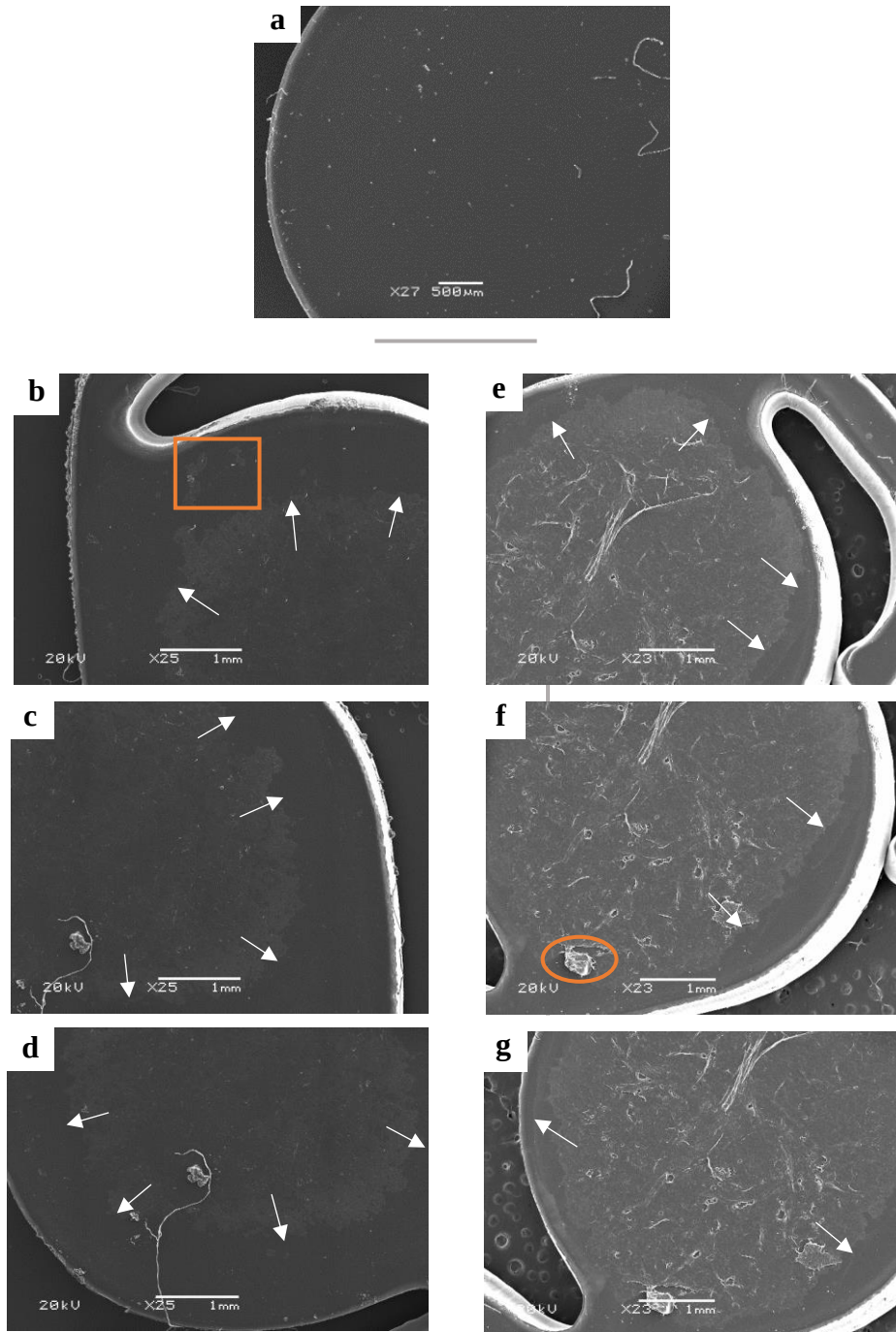


Figure 6.12: SEM images of non-coated C1 IOL (a) and IOLs that had been coated with Ac-PEG-SH after being photoactivated either by pipetting 10 μL of 10% TPO solution on their surface (b-d) or by incubating the IOL in 2 mL of this solution (e-g). The arrows indicate the degree of coverage of the lenses, which was higher when following the second approach. Some of the surface irregularities are highlighted. The scale bar on the image of the non-coated IOL represents 500 μm , while on the rest of the micrographs they represent 1 mm. The magnifications were 25X.

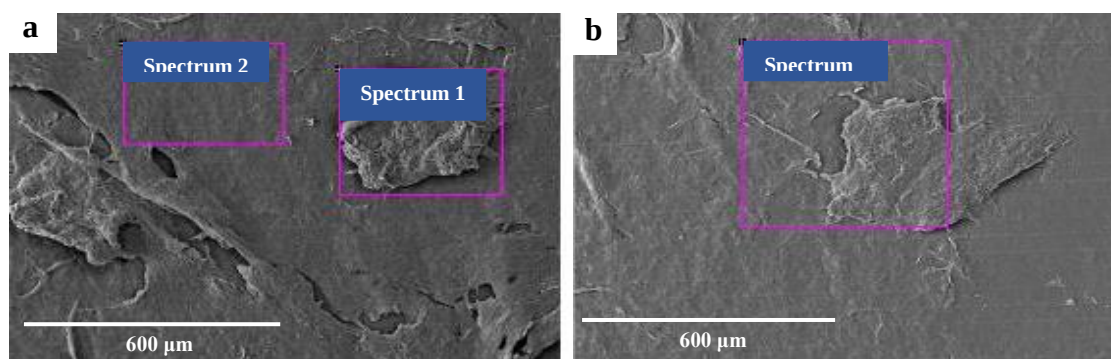


Figure 6.13: SEM micrographs taken on two different locations (**a** and **b**) of an IOL coated with Ac-PEG-SH after being photoactivated via incubation in a 10% TPO solution. The scale bars represent 600 μm and the purple rectangles indicate the areas of the coating that were subjected to EDS analysis.

Table 6.3: Elemental composition of the coatings, as obtained by EDS analysis.

Spectrum	C (%)	O (%)	Na (%)	P (%)	S (%)	Cl (%)	Total (%)
1(a)	35.93	63.29	0.45	-	0.08	0.24	100
2 (a)	41.2	57.09	0.41	0.8	0.31	0.18	100
1 (b)	7.04	90.94	-	-	1.28	0.74	100

6.4.2.1.2 Water Contact Angle

The efficiency of each of the approaches tested for the production of polymer brushes composed of Ac-PEG-SH was also evaluated by measuring the WCA of the coated lenses. The results showed that in both cases the hydrophilicity was higher than in the IOLs which had not been coated (Figure 6.14). This was due to the fact that Ac-PEG-SH is more hydrophilic than the material used in the manufacture of the lenses (C1, described in chapter four). Furthermore, it was observed that the WCA of the IOL that had been coated after incubation in the TPO solution was lower than that of the lens on which this solution had been pipetted prior to photografting with Ac-PEG-SH. This indicated that the surface of the IOL that had been immersed in the photoactivating solution was more hydrophilic, hence suggesting that a higher coating efficiency was achieved. These findings corroborated the conclusions reached upon observation of

the coatings via SEM. However, the WCA should have been measured in at least three samples in order to assess the reproducibility of these results.

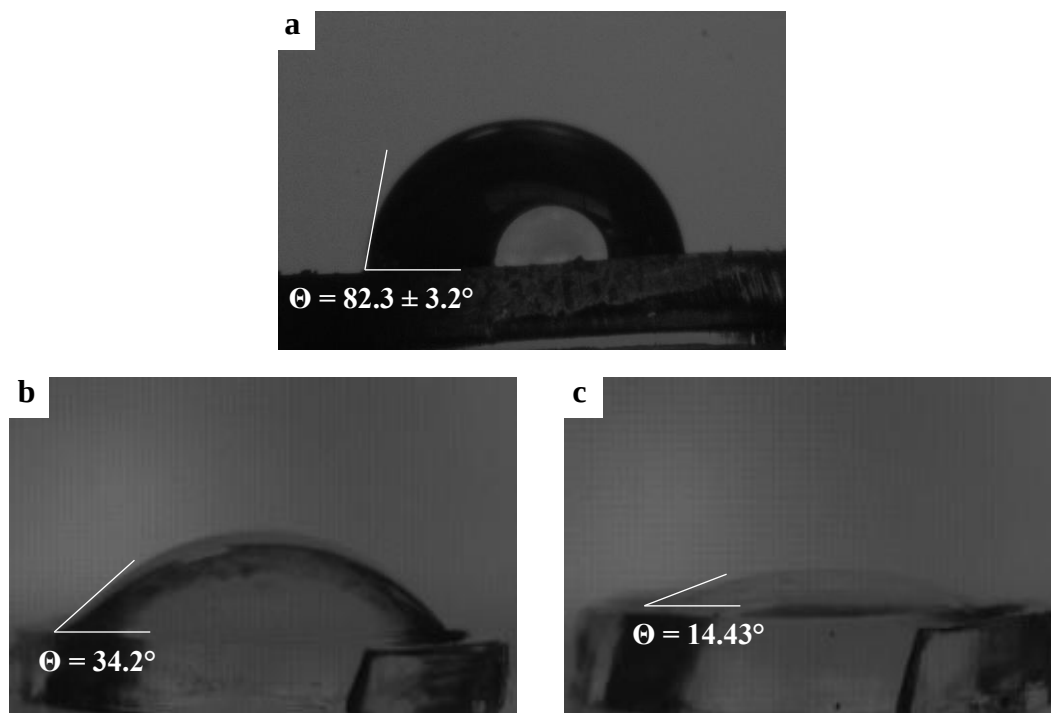


Figure 6.14: WCA of a non-coated C1 IOL **(a)**, an IOL coated with Ac-PEG-SH after being photoactivated by pipetting a 10% TPO solution on its surface **(b)** and an IOL coated with Ac-PEG-SH after incubation in a 10% TPO solution **(c)**. The highest hydrophilicity, and therefore the highest coating efficiency, was achieved when the IOL had been incubated in the photoactivating solution.

6.4.2.1.3 ATR-FTIR

The efficacy of each of the approaches tested for the deposition of TPO on the surface of the lenses was further assessed by ATR-FTIR. Firstly, Ac-PEG-SH and a non-coated IOL were analysed in order to use their spectra as references for the experiment. The signals used for the determination of the coating efficiency included the C-O-C stretch from ethers ($1,100\text{ cm}^{-1}$), the methylene C-H stretch ($2,890\text{ cm}^{-1}$) and the low-intensity signal attributed to the thiol groups ($2,600\text{ cm}^{-1}$). The choice of these bands was based on the fact that they were either present at a higher intensity in the spectrum of Ac-PEG-SH or absent in the non-coated IOLs. The spectra of the

coated IOLs were obtained by analysing four different locations of the coating. The rationale for this procedure was the fact that the polymer brushes were not uniformly distributed over the surface of the lenses. Figure 6.15 shows a comparison between the spectra produced by IOLs that had been photoactivated either by pipetting the TPO solution on their surface or by incubating the implant in it prior to the production of the polymer brushes. The latter showed the highest intensity for the signals generated by Ac-PEG-SH on one of the locations analysed, hence suggesting that this method resulted in the highest coating efficiency. However, the spectra obtained from the analysis of other parts of the sample showed either a similar or a lower intensity in these peaks when compared with those generated by the coating that had been produced after pipetting the photoactivating solution onto the IOL. Therefore, this analysis could not confirm if the highest efficiency had been achieved after incubating the IOLs in the TPO solution. Furthermore, it was concluded that this technique was not suitable for the determination of the coating efficiency, as the results could be biased as a consequence of the non-uniform distribution of the coatings over the lenses and the difficulty to maintain the consistency between the locations analysed in different samples. Thus, the use of alternative techniques such as FTIR microscopy or XPS would have been beneficial in these experiments.

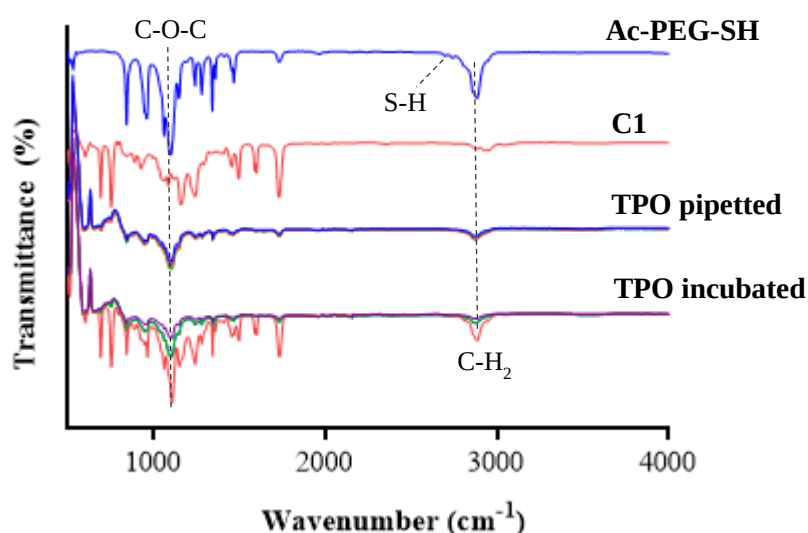


Figure 6.15: ATR-FTIR spectra obtained from the analysis of Ac-PEG-SH (blue), a non-coated IOL (red) and C1 IOLs which had been coated with Ac-PEG-SH after depositing the photoinitiator either by pipetting the 10% TPO solution on their surface or by incubating the implants in this solution. Four spectra were obtained for each of the coated IOLs.

6.4.2.1.4 Optimisation of the concentration of the photoactivating solution

As the characterisation of the polymer brushes described above revealed that the uniformity and surface coverage of these structures was not optimal, further experiments were carried out in order to improve these parameters. This work aimed at increasing the degree of coverage of the coatings by using photoactivating solutions which contained higher amounts of TPO. The concentrations tested in these experiments were 13% and 26% (w/v) TPO in ethanol. The deposition of the photoinitiator was carried out by incubating the implants in these solutions, as the results described in the previous section revealed that this method resulted in the highest coating efficiency. While the resulting coatings were visible by eye when dry, their transparency increased when immersed in water (Figure 6.16). However, the rugosity of their surface had a detrimental effect on the optical quality of the lenses. The efficacy of each of the approaches tested for the production of the polymer brushes was evaluated via SEM imaging, water contact angle measurements, Ellman's assay and FTIR.

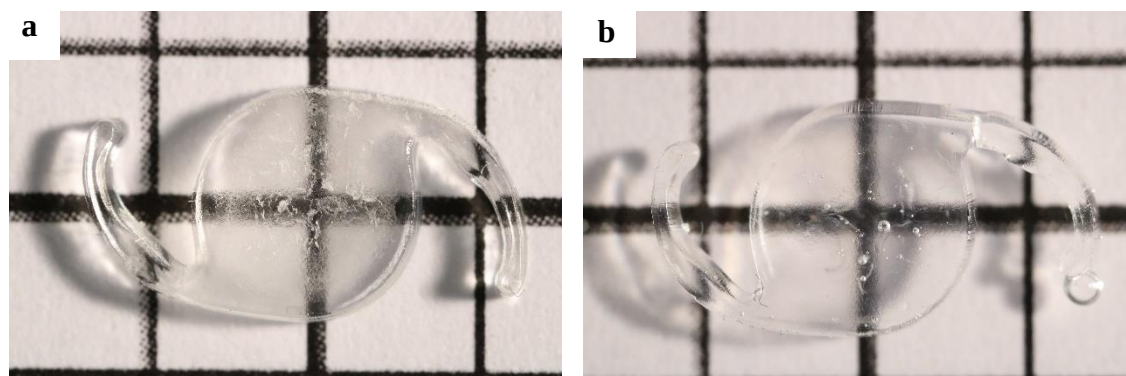


Figure 6.16: Optical images showing a dry IOL coated with Ac-PEG-SH **(a)** and the same implant immersed in water **(b)**.

6.4.2.1.5 Scanning Electron Microscopy

The SEM images of the coated IOLs revealed that the degree of coverage of the surface of the lenses had been slightly improved with respect to the implants that were

coated after the activation of their surface with 10% TPO (Figures 6.12 and 6.17). However, the density of the polymer brushes was noticeably lower after the increase in the concentration of the TPO solutions to 13% and 26% (w/v). This contradicted the observations made when comparing the IOLs that had been photoactivated with either 0.32% or 10% (w/v) TPO solutions (Figures 6.11 and 6.12), which suggested that an increase in the concentration of photoinitiator led to an increase in the density of the polymer brushes. Nevertheless, the comparison between the IOLs that had been pre-coated with either 13% or 26% TPO solutions showed an increase in the density of the coating when the highest amount of photoinitiator had been deposited on the surface of the lenses (Figure 6.17).

With regard to the degree of coverage of each of the coatings obtained in this experiment, it was observed that the polymer brushes created on the IOL that had been photoactivated by incubation in a 13% TPO solution covered most of the surface of the lens (Figure 6.17, (b)-(e)). However, the distribution of this coating was not uniform, as most of the polymer had accumulated at the centre of the IOL. Furthermore, the coating had a high rugosity, which could have a deleterious effect on the optical performance of the lens. This was also the case for the coating that had been produced after the photoactivation of the implant with double the amount of TPO. Moreover, the degree of coverage of this IOL appeared to be slightly lower than that of the lens which had been pre-coated with 13% TPO. Nevertheless, the greater density of the polymer brushes and the higher degree of coverage achieved at the centre of the lens suggested that the coating efficiency had increased after the adsorption of a higher amount of TPO on the surface of the IOL. However, a higher number of implants should have been evaluated in order to reach a more definitive conclusion, as the inconsistencies observed between different experiments may have been caused by a low reproducibility of the results. Furthermore, the use of other analytical techniques was required in order to increase the reliability of the data, as there could be molecules of Ac-PEG-SH attached on the surface of the lenses at such low amounts that they may not have been visible via SEM.

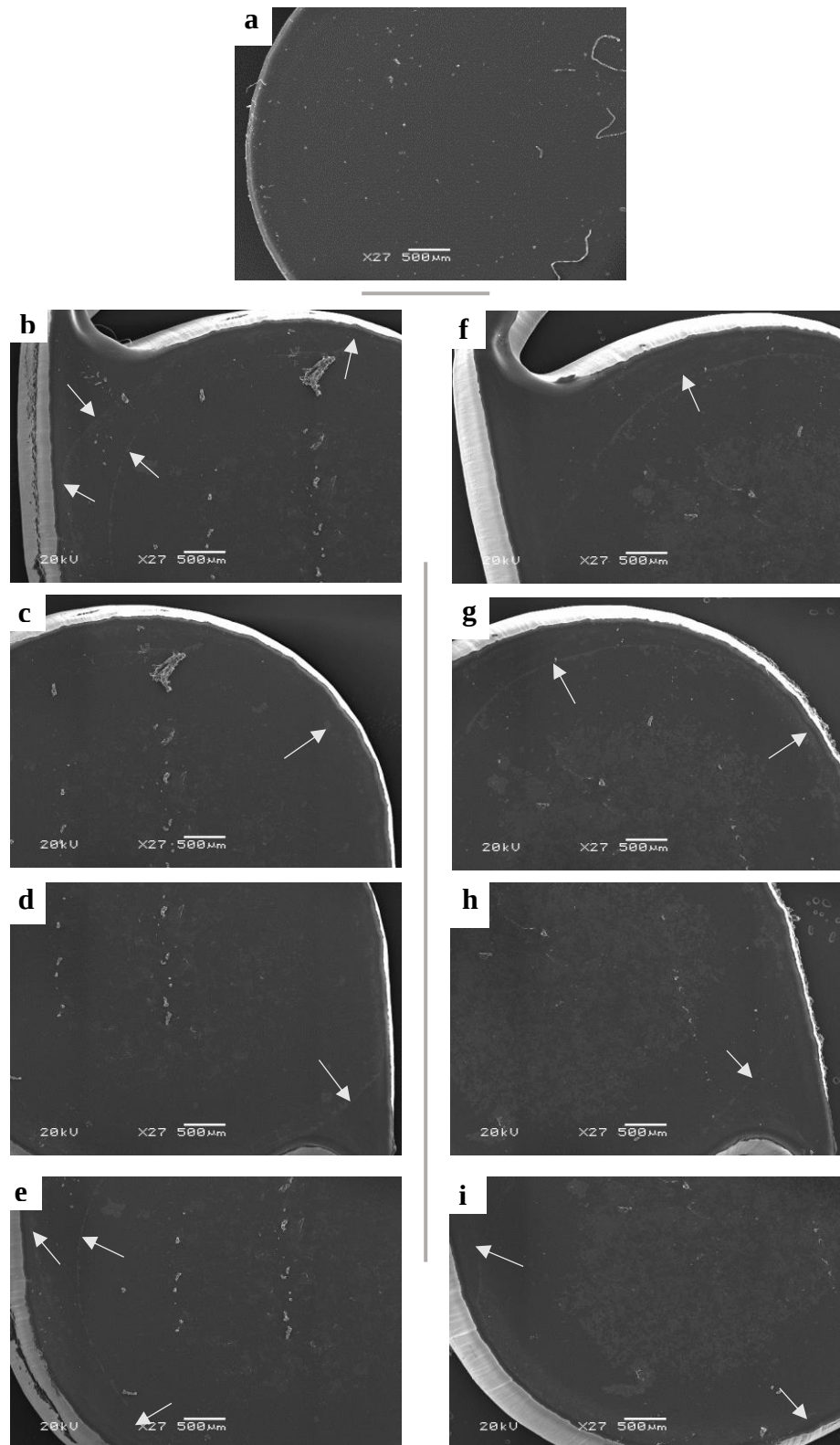


Figure 6.17: SEM images of a non-coated IOL (a) and IOLs that had been coated with Ac-PEG-SH after incubating them in either 13% (b-e) or 26% TPO solutions (f-i). The arrows indicate the degree of coverage of the surface, which was similar in both cases. However, the coating density was higher with 26% TPO. The scale bars represent 500 μm and the magnification is 27X.

6.4.2.1.6 Water Contact Angle

The results obtained from this analysis showed that the surface of the IOLs that had been photoactivated with either 13% or 26% TPO solutions prior to coating was more hydrophilic than in the lenses which had not been coated with Ac-PEG-SH (Figure 6.18). This suggested that the lenses had been successfully coated with Ac-PEG-SH. Furthermore, the WCA of the IOL that had been incubated in a 26% TPO solution was lower than that of the implant which had been photoactivated with half the concentration of photoinitiator (Figure 6.18, (b) and (c)). Therefore, this suggested that the increase in the amount of TPO adsorbed on the lenses prior to the conjugation of Ac-PEG-SH led to a greater coating efficiency. However, when comparing the WCA of the IOL that had been incubated in 13% TPO to that of the lens which had been submerged in a 10% solution of photoinitiator, it was observed that the latter had a higher hydrophilicity (WCA = 14.43°) and therefore a higher coating density. Thus, it was concluded that the methodology followed for the production of the polymer brushes should be improved in order to increase the reproducibility of the results. Furthermore, the WCA of at least three IOLs from each experimental group should have been analysed in order to increase the reliability of this data.

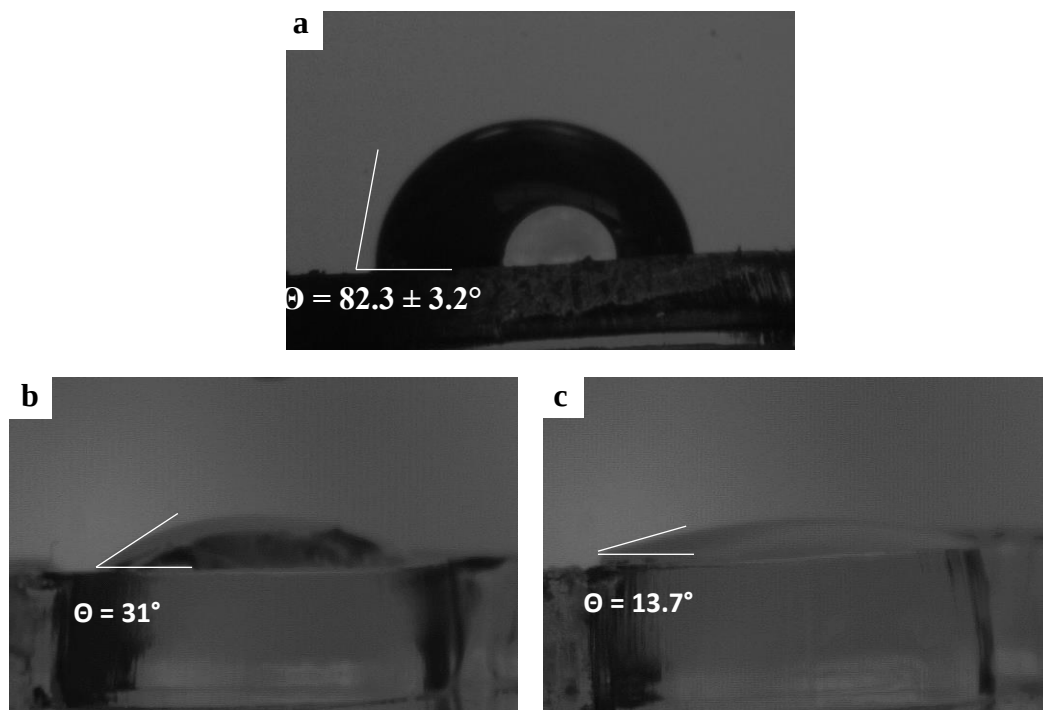


Figure 6.18: WCA of a non-coated IOL **(a)** and IOLs coated with Ac-PEG-SH after being incubated in a solution containing either 13% **(b)** or 26% TPO **(c)**. The coating efficiency, inferred from the increase in the hydrophilicity of the surface, was achieved when photoactivating the IOLs with a 26% TPO solution.

6.4.2.1.7 Ellman's assay

An Ellman's assay was performed in order to evaluate the efficiency of the formation of Ac-PEG-SH polymer brushes on the surface of the IOLs by assessing the presence of thiol groups on the coated implants. This procedure is based on the reaction between a thiol group and 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), which results in the production of a mixed disulfide and one molecule of 5-thio-2-nitrobenzoic acid (TNB^{2-}). The latter produces an intense yellow colour, as it absorbs light with a wavelength of 412 nm (Figure 6.19). This permits the quantification of the number of TNB^{2-} anions formed in the reaction, which, due to the stoichiometry of the process, is the same as the number of thiol groups present in the analyte⁵³⁶.

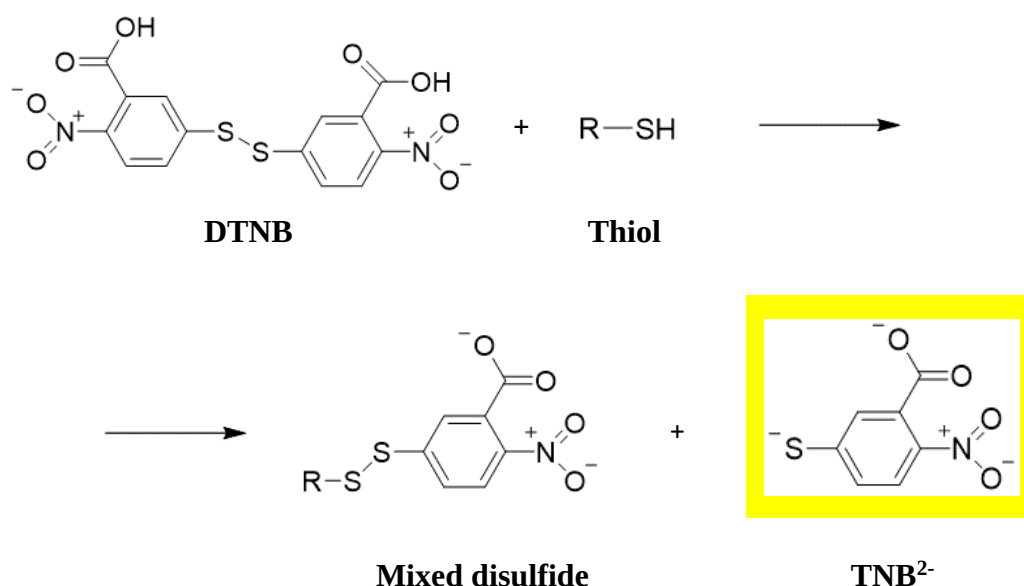


Figure 6.19: Reaction scheme of the Ellman's reaction. The reaction between DTNB and a thiol group results in the production of TNB²⁻, which absorbs light with a wavelength of 412 nm and, consequently, has an intense yellow colour.

The IOLs analysed in this assay included three implants which had been coated with Ac-PEG-SH after photoactivation with 13% TPO and three lenses that had been submerged in a 26% TPO solution instead. In addition, three IOLs that had not been coated were used as a negative control group. The IOLs were incubated in the Ellman's reagent solution, as explained in the Materials and Methods section of this chapter. After that, the absorbance of the incubation medium was measured in order to elucidate the concentration of thiol groups present in the sample. This was calculated by using the value provided on the protocol for the molar extinction coefficient of TNB²⁻ in the reaction buffer used in this assay (14,150 cm⁻¹). The results suggested that the IOLs that had been coated with Ac-PEG-SH after incubation in a 26% TPO solution had the highest amount of thiol groups, which was a direct indication of a higher coating efficiency with respect to the other experimental group (Table 6.5). A one-way ANOVA test was performed in order to evaluate the statistical significance of the differences detected between the experimental groups included in this assay. The result of this test indicated that the differences in the concentration of thiols between the different groups was statistically significant ($p < 0.0001$). A

Tukey's multiple comparisons test was then performed in order to compare the difference between the samples which had been treated with 13% TPO and those in which 26% TPO had been used. These results confirmed that the concentration of thiol groups was significantly higher in the latter.

Table 6.4: Values of absorbance and concentration of thiol groups obtained from the analysis of the coated IOLs via Ellman's assay. The results show that the coated IOLs which had photoactivated with a 26% TPO solution had the highest concentration of thiol groups.

Sample	Absorbance (average)	Concentration (M)
Negative control	0.002 ± 0.003	$1.5 \cdot 10^{-7} \pm 1.6 \cdot 10^{-7}$
13% TPO	0.153667 ± 0.032	$1.086 \cdot 10^{-5} \pm 2.23 \cdot 10^{-6}$ M
26% TPO	0.248333 ± 0.034	$1.76 \cdot 10^{-5} \pm 2.41 \cdot 10^{-6}$ M

6.4.2.1.8 ATR-FTIR

The spectra obtained from the ATR-FTIR analysis of IOLs which had been coated with Ac-PEG-SH after incubation in either 13% or 26% TPO are shown in Figure 6.6. Ac-PEG-SH and an IOL that had not been coated were also analysed in order to use their spectra as references for the experiment. The peaks evaluated for the determination of the coating efficiency included the C-O-C stretch from ethers ($1,095 \text{ cm}^{-1}$), the methylene C-H stretch ($2,880 \text{ cm}^{-1}$) and the low-intensity signal attributed to the thiol group (approximately $2,600 \text{ cm}^{-1}$). The results suggested that the highest coating efficiency had been obtained after the incubation of the IOLs in 13% TPO, as this spectrum showed the greatest intensity in the peaks produced by Ac-PEG-SH. However, as previously discussed, this result could be biased by the fact that the coatings were not equally distributed over the surface of the lenses. In addition, it contradicted the results obtained from the WCA measurements and the Ellman's assay, which reported a higher coating efficiency in the IOLs that had been photoactivated with 26% TPO. Thus, the results obtained from the FTIR analysis were not used for the determination of the coating efficiencies obtained when using different concentrations of photoactivating solution. However, the spectra confirmed

the presence of polymer brushes composed of Ac-PEG-SH on the surface of the lenses.

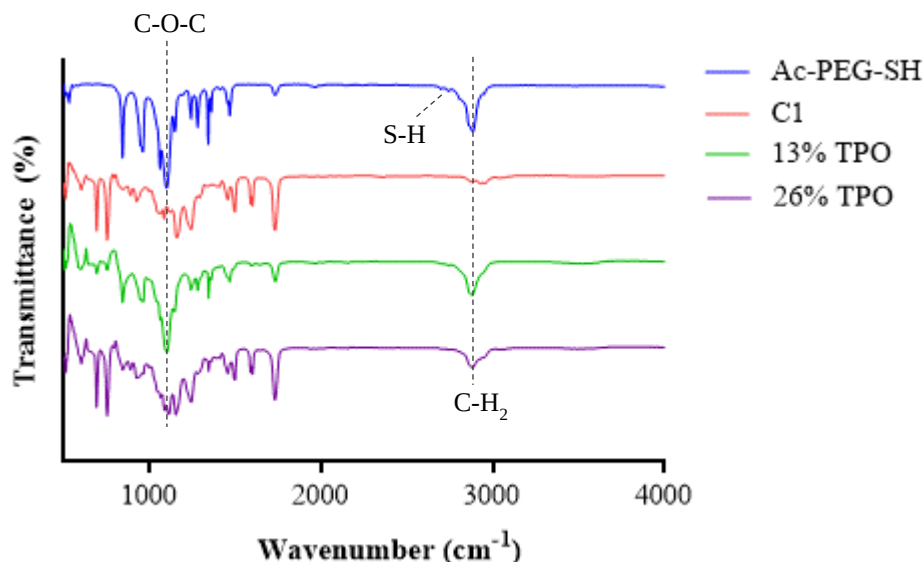


Figure 6.20: ATR-FTIR spectra obtained from the analysis of Ac-PEG-SH (blue), a C1 IOL that had not been coated (red) and IOLs which had been coated with Ac-PEG-SH after incubation in either 13% TPO (green) or 26% TPO solutions (purple). The higher intensity in the peak produced by the C-H₂ bond in the coated samples with respect to the non-coated IOL showed that the polymer brushes were present on the surface of the lenses.

6.5 Discussion

The work described in this chapter aimed at attaching a hydrophilic drug delivery system to the surface of the printed IOLs which resulted from the experiments described in chapter four. This polymeric system could entrap drug molecules and modulate their release upon implantation in the capsular bag. The use of hydrophilic polymers would facilitate the release of the drug when swollen in this aqueous environment. Since the coatings deposited on the surface of ophthalmic devices must be smooth and uniform in order to prevent any light scattering phenomena that could compromise their optical quality, it was hypothesised that spin coating would be an ideal technique for the production of these polymeric films. The polymer of choice for these experiments was pHEMA, as this is a biocompatible material with excellent

optical properties which has been extensively used in the IOL manufacturing industry, as well as in the production of ophthalmic drug delivery systems^{302,537}. As the drug release kinetics are directly influenced by the molecular weight of the polymer used as a delivery system, RAFT polymerisation was the method of choice for the synthesis of pHEMA. This provided a high degree of control over the molecular mass of the material, which would increase the consistency between the release kinetics obtained with different systems produced by using this technique.

As the deposition of polymers by spin coating requires the solubilisation of the material before carrying out this procedure, the polymer used in these experiments could not be chemically crosslinked. This had a detrimental effect on the stability of the resulting coatings when the IOLs were incubated in an aqueous environment, as the polymer was readily solubilised in this medium. While an increase in the molecular weight of the polymer would have delayed this process, it would not have guaranteed the long-term durability of the coatings. The low residence time of the polymeric films on the surface of the IOLs compromised the ability of these devices to serve as controlled-release drug delivery systems. Furthermore, the formation of cracks on the coatings and the pink coloration of these structures as a consequence of the presence of a coloured RAFT agent in the polymer had a deleterious effect on the optical properties of these devices. Consequently, the polymeric coatings described herein were deemed unsuitable for use as ophthalmic controlled-release drug delivery systems.

Another problem encountered during the deposition of the polymeric films on the IOLs was the difficulty to spread the coatings uniformly over the surface of the lenses. This occurred as a consequence of the differences in hydrophilicity between pHEMA and the material used in the manufacture of the IOLs. Furthermore, the absence of chemical interactions between both polymers contributed to the low stability of the resulting coatings. While the uniformity of the films was improved by increasing the spinning speed, this caused a substantial loss of polymer solution which led to a decrease in the reproducibility of the results. Furthermore, an equal distribution of the

material could not be achieved in any of the experiments. Thus, these issues made it highly desirable to seek alternative techniques for the synthesis of the drug delivery system and its subsequent conjugation to the printed IOLs.

As the experiments described above showed that the spin-coated polymeric films would not be suitable for use as ophthalmic controlled-release systems, an alternative drug delivery device was designed. This system would contain enzyme-cleavable peptide crosslinkers in order to ensure that the drug is released only when the MMP enzymes that are overexpressed at the onset of PCO are present in the aqueous humour. Thus, this polymeric structure could be synthesised via Michael addition reaction between 4-arm PEG maleimide and MMP-cleavable peptide crosslinks. It was hypothesised that the chemical conjugation of this system to the surface of the IOLs would prevent the detachment of the polymeric films from the lenses upon incubation in an aqueous environment. As the IOLs did not possess any chemical groups that could establish covalent interactions with the drug delivery system, the functionalisation of the implants would be an essential requirement to achieve this objective. The most common technique employed in the surface modification of medical devices is plasma treatment. However, this method was not available for use in the present project. Therefore, an alternative technique was devised in order to functionalise the surface of the lenses prior to the attachment of the drug delivery system. As this enzyme-degradable polymer contained maleimide functionalities, it was theorised that if the hydrogel was synthesised in such a way that part of these functional groups remained unreacted, these could be employed to establish covalent bonds with IOLs whose surfaces had been functionalised with Michael addition donors. Therefore, this required the conjugation of thiol groups to the surface of the lenses prior to the attachment of the drug delivery system.

The methodology devised for the conjugation of thiol-containing polymer chains to the surface of the IOLs was based on the physical adsorption of photoinitiator molecules on the lenses and the subsequent attachment of Ac-PEG-SH. This protocol was based on a methodology developed by Huang *et al.* for the conjugation of MPC

to the surface of hydrophobic acrylic IOLs²⁰³. It was theorised that the adsorption of photoinitiator molecules on the surface of the lenses would provide attachment sites for the acrylate-functionalised polymer chains upon UV irradiation. Moreover, the benzyl rings present in the structure of the photoinitiator could establish aromatic interactions with the phenyl groups from the polymer used in the manufacture of the IOLs. This would increase the affinity between the lenses and the polymer brushes attached to their surface. The rationale behind the use of Ac-PEG-SH was the presence of acrylate and thiol groups at its chain ends, which would lead to the establishment of chemical interactions with both the photoactivated surface of the IOL and the drug delivery system. The lowest molecular weight available (M_n 2000 Da) was selected in order to minimise the steric hindrance caused by longer polymer chains, as this would hamper the conjugation reaction. Furthermore, the use of a PEG-based molecule with similar properties to those of the drug delivery system would prevent the loss of optical quality that would result from any disparities between the refractive indices of these polymeric structures. Finally, the high biocompatibility of PEG made it suitable for use in the manufacture of implantable ophthalmic devices.

Once the polymer brushes composed of Ac-PEG-SH were synthesised, various characterisation techniques were employed in order to assess the efficiency of the method described herein. The images obtained via SEM revealed that the resulting coatings were not equally distributed over the surface of the lenses. Furthermore, these images showed that the reproducibility of the process should be improved, as the density and degree of coverage of the coatings varied considerably between different experiments. Nevertheless, the WCA measurements suggested that the efficacy of this method was high enough to cause a considerable increase in the hydrophilicity of the surface of the IOLs. Moreover, the results of these analyses revealed that this increase was proportional to the density of the polymer brushes created on the surface lenses. However, a higher number of samples should have been analysed in order to obtain more conclusive results and to assess the reproducibility of the technique. The observations made when measuring the WCA of the coated IOLs were corroborated by the Ellman's assay. This experiment confirmed the presence of thiol groups on the surface of the lenses and showed that they were more abundant in denser polymer

brushes. While this analysis was carried out using triplicates for each experimental group, the use of a larger sample size would have increased the power of the statistical analyses performed after the experiment. It is important to note that these techniques were more efficient than FTIR for the determination of the coating efficiency, since the non-uniform distribution of the polymer brushes over the surface of the lenses caused a bias in the results obtained from this method. Overall, the use of different characterisation techniques confirmed the successful formation of the polymer brushes on the surface of the IOLs. The results obtained from these analyses provided a good indication of the efficiency and reproducibility of the technique used in their production. However, the use of additional techniques such as XPS or AFM would have provided more information with regard to the chemical composition and structure of the resulting coatings.

It is important to note that the coated IOLs maintained their transparency upon incubation in an aqueous medium. However, the long-term stability of the polymer brushes in this environment should have been analysed. The fact that the coatings were still attached to the IOLs after a three-hour incubation in water indicated that the stability of these polymer brushes was higher than that of the films produced by spin coating. Nevertheless, it would be essential to evaluate the strength of the interactions established between Ac-PEG-SH and the IOLs under physiological conditions, as the increase in temperature could affect the stability of the relatively weak aromatic interactions that may have been established between the IOLs and the photoinitiator molecules adsorbed on their surface.

Finally, an evaluation of the ability of the polymer brushes to establish chemical bonds with the drug delivery system should have been carried out. As mentioned above, the chemical conjugation between the coatings and the drug delivery system could be attained by performing a Michael addition reaction. However, this could not be tested as a consequence of the time constraints encountered after the development and evaluation of the drug delivery system, which was the main priority of the project.

6.6 Conclusions

The experiments described in this chapter aimed at conjugating a drug delivery system to the surface of the printed IOLs developed in this project. The results of this work proved that spin coating could be used to deposit polymeric films on the surface of the lenses. These coatings could potentially entrap a drug and modulate its release when swollen in water. However, the low uniformity of their surface compromised the optical quality of the IOLs. Moreover, the high solubility of these films in water, coupled with the absence of chemical bonds between the IOLs and the films, led to the rapid loss of these coatings once immersed in an aqueous environment. As this would compromise the ability of the polymeric films to control the release of the drug, it was concluded that an alternative system should be designed in order to overcome this problem.

An enzyme-responsive hydrogel was developed in order to increase the ability of the system to control the release of the drug entrapped within the polymer. The synthesis and evaluation of this system is described in detail in the following chapter. In order to ensure the successful conjugation of this polymer to the surface of the lenses, a methodology was developed for the production of polymer brushes that could establish covalent bonds with the drug-loaded system. Ac-PEG-SH was thus conjugated to the lenses by following a “grafting-to” approach. The subsequent evaluation of these structures revealed the success of this technique in the production of detectable coatings on the surface of the lenses. However, it was observed that the surface coverage was not complete and that the polymer brushes were not evenly distributed over the surface of the IOLs. Therefore, further experiments would be required in order to improve these characteristics of the coatings. One approach that could be taken in this endeavour is the optimisation of the concentration of the photoactivating and coating solutions, composed of TPO and Ac-PEG-SH, respectively. Moreover, the use of Ac-PEG-SH molecules that could not be completely solubilised in the solvent used for coating may have contributed to the relatively low efficiencies detected in some of the experiments. Thus, it would be important to use polymer molecules that have a higher solubility in the solvent of choice.

Finally, the conjugation of the polymer brushes to the enzyme-responsive drug delivery system should have been tested in order to validate the functionality of these coatings. In addition, the long-term stability of these films under physiological conditions should have been carefully evaluated. Another parameter that should have been analysed is the optical quality of the coated lenses, for which measurements of the refractive index should have been taken.

Overall, it was concluded that the surface of the IOLs was successfully modified with the polymer brushes. This was confirmed by an increase in the hydrophilicity of the surface of these implants, which would facilitate the conjugation of hydrogel-based drug delivery systems. However, further optimisation would be required in order to improve the surface coverage and uniformity of these coatings. Finally, the ability of these polymer brushes to establish covalent interactions with the drug delivery system should be evaluated in the future by performing a Michael addition reaction. If these experiments were successful, the drug loading efficiency and release kinetics of this system should be evaluated *in vitro*.



CHAPTER 7

Conclusions and Future Directions



7. Conclusions and Future Directions

7.1 Conclusions

The aims of this thesis were the fabrication of functional IOLs by using additive manufacturing technologies and the functionalisation of these lenses in order to allow the attachment of a drug delivery system to their surface. These were accomplished through the completion of the work described in previous chapters. The conclusions reached after each of the stages of the project are described as follows.

7.1.1 Additive manufacturing of IOLs

The techniques currently employed in the manufacture of IOLs do not allow the production of these implants in research laboratories or clinical settings. This impairs the research required for the development of novel IOLs with improved biocompatibility and the application of personalised medicine in the treatment of cataract. The use of 3D-printing technologies could be the solution to these problems. Therefore, the work presented in this thesis focused on the employment of additive manufacturing techniques for the production of functional IOLs. As these implants can be made with a wide variety of polymeric materials, this research explored the production of lenses with different types of polymers. Acrylic materials were chosen due to the positive results reported in the clinic after the implantation of IOLs made with these formulations. Moreover, implants made with this type of polymers could be produced by stereolithography, which was the system employed in this project. Both hydrophilic and hydrophobic acrylic IOLs were manufactured in this work and their functionality was assessed by evaluating their physico-chemical properties and biological performance *in vitro*.

The copolymer developed for the production of the hydrophilic acrylic IOLs was based on a formulation reported in a patent that describes the manufacture of this type of lenses. This polymer was mainly composed of HEMA and contained a smaller proportion of MMA in order to decrease the equilibrium water content of the material.

However, this formulation could not be used in the production of IOLs by stereolithography due to the slow polymerisation rate of the methacrylate monomers in the system used in this project. Therefore, the methacrylate monomer units were replaced by their acrylate counterparts, which due to their higher polymerisation rate allowed the production of IOLs by stereolithography. The resulting lenses were optically clear and flexible when dry, which indicated that they could potentially be used for implantation in the capsular bag. However, these implants lost their transparency and mechanical strength upon incubation in an aqueous environment. Furthermore, their high swelling ratio caused a significant increase in their size when subjected to these conditions. Therefore, these IOLs were deemed unsuitable for implantation in the capsular bag via phacoemulsification, as they could not be injected through a small incision without being substantially damaged. In addition, the increase in their size when submerged in an aqueous medium would cause the stretching of the capsular bag, with the subsequent damage of this tissue. Another issue encountered in the manufacture of these IOLs was the low resolution achieved when printing the lenses with the hydrophilic formulation described herein. This resulted in a high incidence of dimensional inaccuracies and a low fidelity with respect to the commercial IOL employed as a model.

Given the problems encountered when manufacturing hydrophilic IOLs, the next part of this project focused on the production of hydrophobic lenses with physico-chemical properties that could prevent the issues described above. A monomer containing a phenyl group was chosen as the main constituent of the hydrophobic material, as this functionality increases the rigidity of the resulting polymer. Furthermore, the presence of phenyl groups in this material would lead to an increase in the refractive index, with the subsequent improvement in the optical quality of the polymer. The additive layer manufacturing of the hydrophobic IOLs using the build platform resulted in the production of lenses that possessed poor mechanical properties and a high incidence of dimensional inaccuracies. Therefore, an alternative method was developed in order to produce these IOLs without making use of the platform. This led to a significant improvement in the resolution and mechanical strength of the implants. The functionality of the hydrophobic IOLs that had been produced by following this

method was assessed by evaluating their physico-chemical properties and biological performance. The results obtained from these analyses showed that the lenses possessed an appropriate optical quality, flexibility and mechanical strength. The biocompatibility of these implants was subsequently confirmed by performing a cytotoxicity test *in vitro*. Finally, an injection test was carried out using a human capsular bag model *ex vivo* in order to evaluate the suitability of the printed IOLs for implantation through a microincision. This test proved that the lenses possessed the mechanical strength and flexibility required for them to withstand the mechanical stress that they were subjected to during implantation.

While the printed lenses complied with the minimum requirements established by the regulatory bodies for any IOL that is to be used in the clinic, a number of improvements should be made before these implants would be suitable for use in cataract surgery. Firstly, the surface of the lenses must be polished in order to remove any artefacts that may affect the transparency of the IOLs and which could cause abrasion in the surrounding tissues. As can be seen in the images shown in previous chapters, the surface of the optic portion of the printed implants was not completely smooth, which made them translucent rather than transparent as a consequence of the scattering phenomena caused by the roughness of the surface. Moreover, the methodology followed for the manufacture of these IOLs did not allow the production of convex or concave lenses that could improve the quality of vision of the patients. Finally, the optic design could also be modified in order to correct optical aberrations such as astigmatism and to mimic the accommodation reflex that occurs in the natural crystalline lens.

With regard to the resolution achieved when printing these lenses, this was high enough to allow the production of the optic and haptic structures of the IOLs. However, some dimensional inaccuracies and a distortion in the shape of the optic-haptic junction were detected. Furthermore, as the prints were carried out without using the build platform, the thickness of the lenses could not be controlled. This

resulted in the production of lenses which had a higher thickness than the CAD model, which would affect the optical properties of the IOLs.

7.1.2 Optimisation of the 3D-printing resolution

The dimensional inaccuracies and distortions in the design observed in the printed IOLs made it desirable to increase the resolution achieved in the manufacture of these lenses. It was hypothesised that the addition of a light absorber to the photopolymerisable resin would lead to a decrease in its cure depth, hence increasing the Z-resolution of the system. Therefore, a light-blocking agent was added to the hydrophobic acrylic formulation in order to increase the resolution of the IOLs produced with this material. Firstly, an experiment was performed in order to evaluate the effect of the concentration of light absorber on the cure depth of the resin. The results showed that a significant decrease in the cure depth of this formulation was caused by the addition of increasing amounts of absorber. This confirmed the positive effect that the addition of this compound had on the Z-resolution of the printed structures. After that, the manufacture of IOLs was carried out with this formulation. The IOLs that resulted from these experiments had a higher fidelity with respect to the AcrySof[®] IOL that was used as a model. All the design features of this implant were accurately replicated and the dimensional accuracy had increased considerably. This increase in the resolution allowed the production of novel IOL prototypes with structural elements that could improve the bioactivity of these implants. Therefore, the addition of a light absorber to the printable formulations ensured that the IOLs possessed all the design features that control the cellular responses which lead to the development of PCO after cataract surgery, hence contributing to the prevention of this syndrome.

The physico-chemical properties of these IOLs were analysed and compared to those of the lenses that had been printed in the absence of the light absorber. These tests revealed that the T_g of the polymer used in the manufacture of the IOLs was affected by the addition of the light blocker. As this would have an effect on the mechanical

properties of the material, special attention should be given to the amount of this compound that is added to the photopolymerisable resin. Furthermore, an optimisation of the formulation may be required in order to compensate for the effects of the absorber on the mechanical strength of the material. Another characteristic that was affected by the addition of the light absorber was the optical quality of the lenses, which was substantially reduced. Nonetheless, it is important to note that the production of the IOLs that contained the absorber was carried out layer by layer, while the lenses that had been produced before adding the light blocker had been printed in only one layer. The multiple boundaries that existed between the constitutive layers may have caused light scattering phenomena, hence compromising the optical quality of the lenses that contained the absorber. Moreover, the roughness of their surface may have contributed to this phenomenon. Thus, an unbiased judgement could only be made after polishing the surfaces of the implants in order to eliminate these artefacts from the lenses. It is worth noting that UV and visible light absorbers are commonly used in the manufacture of IOLs without causing any major compromises in the optical performance of the polished lenses. Hence, it was concluded that while the addition of a light absorber to the resin caused a reduction in the optical quality of the printed lenses, the presence of layer boundaries within these structures could have contributed to this effect.

With regard to the biocompatibility of the lenses that contained the light absorber, cytotoxicity studies should be performed in the future in order to assess the potential toxicity of this material. The absorbers employed in the IOL manufacturing industry are covalently linked to the polymer network, hence preventing the leaching of toxic molecules of this compound into the intraocular environment. However, the characterisation of the light absorber used in the present project revealed that this molecule did not contain any chemical groups that could establish covalent interactions with the polymer. Thus, the potential leaching of these molecules into the aqueous humour could compromise the biocompatibility of the 3D-printed IOLs.

7.1.3 Surface modification of IOLs

The attachment of a drug delivery system to the 3D-printed IOLs would require the functionalisation of the surface of the lenses in order to facilitate this process. The spin coating experiments showed that the deposition of polymeric films via physical adsorption resulted in the formation of unstable coatings that detached from the surface of the lenses upon incubation in an aqueous medium. Therefore, a method was developed for the functionalisation of the surface of the IOLs in order to facilitate the attachment of the drug delivery system. This technique was based on the production of polymer brushes composed of Ac-PEG-SH on the surface of the lenses. It was hypothesised that the thiol groups from these molecules could react with any free maleimide functionalities present in a drug delivery system composed of 4arm PEG-maleimide. While not described in this thesis, an enzyme-responsive drug delivery system was envisaged which would release Axitinib[®] upon cleavage by MMP2, which is one of the enzymes that are overexpressed during PCO development. Therefore, the creation of polymer brushes containing thiol groups on the surface of the IOLs would open up the possibility of creating covalent bonds between these linkers and the drug delivery system via Michael addition reaction.

The results showed that Ac-PEG-SH had been successfully attached to the optic portion of the IOLs and that the resulting coatings did not diminish the transparency of the lenses when these were submerged in water. The creation of these polymer brushes resulted in an increase in the hydrophilicity of the surface of the IOLs, which would facilitate the deposition of a hydrophilic drug delivery system on these implants. Moreover, the presence of oxygen atoms bearing lone pairs of electrons in the chemical structure of Ac-PEG-SH would allow the formation of hydrogen bonds between these molecules and the amines present in the drug delivery system. Thus, this would increase the stability of the interactions established between the surface of the IOLs and the delivery system, hence ensuring the durability of this device.

The attachment of Ac-PEG-SH to the IOLs had been achieved by adsorbing photoinitiator molecules on the surface of the lenses in order to create radicals that would react with the acrylate groups from the polymer brushes. The adsorption of the

photoinitiator onto the IOLs was mediated by physical interactions. The presence of covalent bonds between the lens and the polymer brushes would have increased the stability of the system. However, the material used in the manufacture of the IOLs did not contain any functional groups that could be easily conjugated to other chemical functionalities. While a plasma treatment would have facilitated the covalent attachment of the drug delivery system to the IOLs, this approach was not available for use in this project. Therefore, the methodology described herein provided the highest stability achievable with the materials and equipment available. Further experiments could be performed in order to test the plasma functionalisation of the surface of the IOLs in order to create covalent bonds between the lenses and the drug delivery system.

7.1.4 General conclusions

The work presented in this thesis provided evidence that additive manufacturing could be utilised to fabricate functional IOLs that possessed all the design features that these implants are formed of. The application of this technology in industrial, research and clinical settings would enable the personalisation of the implants and it would aid the research required for the creation of bioactive IOLs with an improved functionality. This is of particular importance for the prevention of PCO, a syndrome that causes the relapse of cataract after surgery. The production of IOLs with materials and structural elements that can thwart the cellular responses which cause the development of PCO is one of the most effective approaches for the prevention of this disease. Thus, the work conducted in the present project focused on the optimisation of the formulations in order to maximise the resolution of the stereolithography system, hence enabling the production of IOLs which contained all the design features required for the prevention of PCO.

The administration of therapeutic agents can also contribute to the prevention of secondary cataract. The use of drug delivery systems would increase the efficacy of these treatments by controlling the release of the drug. Furthermore, the employment of an IOL as a drug delivery vehicle would provide a localised delivery of the

therapeutic agent, hence decreasing the incidence of systemic side effects. Therefore, part of the work described in this thesis focused on the functionalisation of the printed IOLs in order to facilitate the attachment of a drug delivery system to their surface. While the experiments conducted in this project resulted in the successful functionalisation of the lenses, further work would be required in order to increase the efficiency of the methodology described herein.

Overall, the introduction of 3D-printing technologies in the manufacture of IOLs enabled the production of these implants at a lower cost than the techniques currently employed and over shorter time periods. Moreover, the use of these technologies facilitated the fabrication of IOLs in a research environment, hence increasing the availability of these implants for the development of novel drug delivery systems that can prevent the recurrence of cataract. It is envisaged that the introduction of 3D-printing in the production of these lenses would have a positive impact on the treatment of cataract by allowing the creating of personalised implants and improving the therapeutic effect of these devices.

7.2 Future directions

The work presented in this thesis proved that additive manufacturing could be used for the production of functional IOLs. However, a number of improvements must be made before these implants can be translated to the clinic. As previously mentioned, the lenses should be polished in order to obtain the transparency required for their utilisation as ocular implants. The standard procedure for the polishing of IOLs is based on tumble finishing. This method consists in the introduction of the IOLs into glass barrels filled with a slurry and the subsequent rotation at a predetermined speed in order to increase the smoothness of the surfaces.

The dimensional accuracy of the IOLs is another parameter that requires further optimisation. While a significant increase in the resolution was achieved with the

addition of a light absorber to the photopolymerisable resin, the lenses were still thicker than expected. As the thickness of a lens has a direct effect on its optical properties, this parameter should be more accurately controlled. Hence, the use of printers which allow the production of implants with a higher resolution would be required in order to achieve a higher dimensional accuracy, which would in turn improve the functionality of the IOLs. Systems such as microstereolithography or two photon polymerisation would be ideal for the production of these devices, as they allow the fabrication of structures at the micro and nanoscale, respectively.

The physico-chemical characterisation techniques performed for the evaluation of the functionality of the printed IOLs showed that they possessed the adequate mechanical strength to endure the implantation through a microincision. However, further mechanical testing should be performed in order to assess the strength of the haptics, as it is fundamental to ensure that these structures can provide enough support once the IOL is implanted in the capsular bag. This would prevent the decentration of the implant and the subsequent loss of optical quality. Acrylic IOLs are often composed of copolymers of acrylate and methacrylate monomers. The presence of methacrylate units in the formulation increases the stiffness of the IOLs, thus conferring the haptics with enough mechanical strength to maintain the IOL in a fixed position within the capsular bag. However, the printable materials developed in this project were comprised entirely of acrylate monomers, as the stereolithography system used in these experiments did not allow the polymerisation of methacrylate units at relatively high concentrations. Therefore, a comprehensive mechanical characterisation would be required in order to assess the stability of the printed IOLs once they are implanted in the capsular bag. Further optical testing would also be mandatory before evaluating the performance of these implants *in vivo*. Such examinations should include measurements of the refractive index, dioptric power and spectral transmittance of the lenses.

The cytotoxicity tests performed *in vitro* demonstrated that the materials used in the production of the IOLs were not toxic. However, other aspects of the biocompatibility of the implants, such as sensitisation and tissue tolerance, should be evaluated *in vivo*.

As well as their biocompatibility, the ability of the lenses to prevent PCO is another characteristic that should be thoroughly evaluated. These studies can be performed using human capsular bag models *in vitro* or by carrying out *in vivo* studies. It is important to note that numerous refinements in the manufacturing process should be made before testing the printed IOLs *in vivo*. The materials used in the production of the lenses should possess a higher purity, while the final product should be thoroughly sterilised. Moreover, the use of clean room facilities would reduce the risk of contamination during the production and functionalisation of the IOLs.

Depending on the results obtained from the tests described above, further improvements may be required in order to optimise the mechanical and optical performance of the lenses. The addition of nanoparticles into the printable formulations would be an interesting avenue that could potentially lead to significant improvements on these parameters. For example, the mechanical strength of the polymer could be significantly improved by adding nanofibers or nanoparticles to the printable formulation^{538,539}. Furthermore, an increase in the refractive index of hydrophilic materials has been previously reported after the addition of TiO₂ nanoparticles⁵⁴⁰. Therefore, this could be an effective way of improving the optical performance of the printed lenses. It is important to note that the production of nanocomposites via stereolithography would require adjustments in the process parameters, as these particles would cause a decrease in the cure depth and an increase in the viscosity of the resin⁵⁴¹.

While the square edge of an IOL contributes to the prevention of PCO, the complete inhibition of this disease may require the creation of nanoscale features that provide a higher degree of control over the responses of the cells that interact with the implant. The rationale for this is based on the fact that the extracellular matrix proteins that establish interactions with cells have nanoscale dimensions, so the bioactivity of the IOLs would be significantly increased upon addition of nanoscale features on their structure. The creation of nanotopographies on the surface of the haptics is one of the approaches that could be taken in order to achieve this objective, since these structures can modulate the adhesion and migration of lens cells^{506,507}. The production of these

lenses would require the utilisation of additive manufacturing techniques based on two photon polymerisation, since this method allows the production of prototypes at nanoscale resolution. The use of additive manufacturing technologies will also facilitate the evaluation of the effect that different materials have on the development of PCO. The stiffness of the biomaterial has been shown to have an effect on cell differentiation. Thus, it would be of particular interest to assess the potential effect that this parameter would have on the transdifferentiation of LECs into fibroblastic cells, which is one of the main responses that lead to the development of PCO.

The IOLs produced in this project did not have the ability to mimic the accommodation reflex of the natural crystalline lens or to correct optical aberrations such as corneal astigmatism. Therefore, there is scope for improving the functionality of these lenses by adding design features on the optic part of the implants in order to create multifocal or toric IOLs that can solve these issues. Furthermore, the employment of additive manufacturing technologies in the production of IOLs would facilitate the development of novel prototypes that are engineered to mimic the accommodative reflex of the crystalline lens, hence improving the biomimicry of these implants.

The solution to the limited biomimicry achieved with current methodologies may lie in the development of regenerative approaches. A novel surgical technique based on the performance of a smaller capsulorhexis in order to preserve the LECs present in the anterior lens capsule has been recently tested in paediatric patients^{542,543}. The results showed that the lens had begun to regenerate three months after the surgical procedure due to the regenerative potential of LECs. Moreover, after eight months it was observed that the regenerated lens had the same thickness as the natural crystalline lens and, most importantly, it showed accommodative power. While this could be achieved in juvenile patients, the loss of the LEC's regenerative potential in the ageing lens would pose a challenge in the application of this technique in older patients. A possible solution to this problem would be the use of biomaterials to promote the regenerative capacity of lens cells by mimicking the natural extracellular

matrix⁵⁴⁴. The controlled deposition of nanofibers could be advantageous for this purpose, as they have comparable structural similarities to the crystalline proteins that form the natural crystalline lens. Furthermore, the engineering of stimuli-responsive nanomaterials that respond to the pressure exerted by the ciliary muscles on the lens would open up the possibility of creating accommodating lenses which would closely resemble the natural lens.

With regard to the functionalisation of the IOLs, there are various aspects of the methodologies used for this purpose that could be further optimised. Firstly, the surface of the lenses could be treated with plasma in order to allow the covalent conjugation to the system. This would result in a greater stability of the attachment of the drug delivery system to the IOL, hence ensuring a high durability. Furthermore, more experiments would be required in order to assess the efficiency of the polymer brushes in increasing the hydrophilicity of the surface of the implants. This would include the analysis of a higher number of IOLs which have been functionalised with Ac-PEG-SH and a control group on which the photoinitiator has been adsorbed but which has not been subjected to the attachment of the polymer brushes. Finally, it would be desirable to perform further analysis on the functionalised IOLs with techniques such as XPS, as it would provide more information about the chemical composition of the surface of the implants.

To conclude, a drug delivery system should be synthesised and evaluated in order to increase the therapeutic efficiency of the IOLs towards the prevention of PCO. As previously mentioned, it was hypothesised that the creation of an enzyme-responsive system would increase the efficacy of this approach by providing a higher degree of control over the release of the therapeutic agent. Hence, future work should focus on the creation of a system which would ensure that the drug is released only when PCO starts to develop. Finally, the covalent conjugation of this smart delivery system to the surface of the functionalised IOLs should also be explored. It is important to note that this procedure should result in the formation of coatings that are uniformly distributed in order to negate any detrimental effect on the optical quality of the lenses. Once this

is achieved, the transparency and durability of the coated IOLs should be evaluated in conditions that mimic the intraocular environment.

8. Bibliography

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9. Appendixes

Appendix 1

Chapter 3

Manufacture of IOLs

Dimensional accuracy along the Z-axis of the IOLs printed with a hydrophilic acrylic formulation

Table 9.1: Thickness of the 3D-printed IOLs and dimensional accuracy with respect to the CAD model.

IOL	Thickness of the CAD model (mm)	Thickness of the printed IOL (mm)	Error (%)
1	1.2	1.78	+ 48.33
2	0.8	1.25	+ 55.75
3	0.9	1.4	+ 55.56
Average	0.97 ± 0.21	1.48 ± 0.27	$+ 53.21 \pm 4.23$

Appendix 2

Chapter 3

Thermogravimetric Analysis

TGA poly(methyl acrylate)

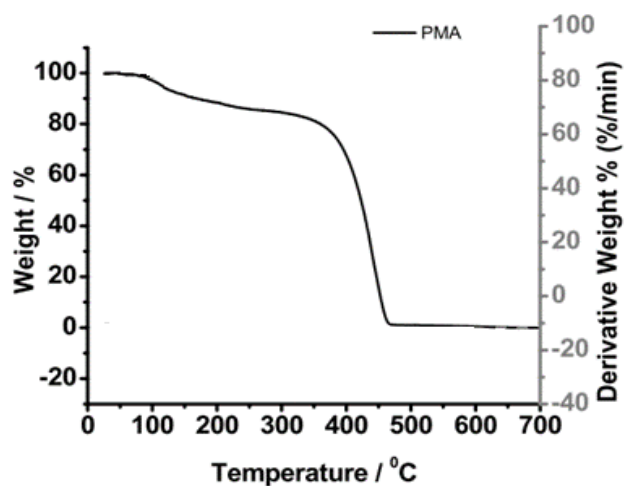


Figure 9.1: Thermogram obtained from the thermogravimetric analysis of pMA. Adapted from Brostow et al., 2008⁴⁸⁵.

TGA poly(hydroxyethyl acrylate)

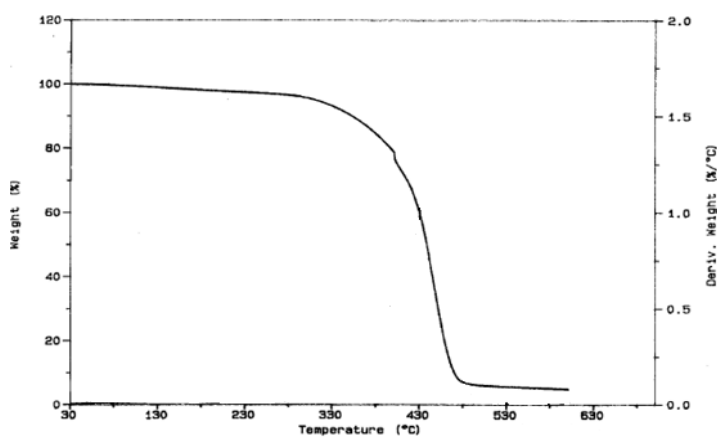


Figure 9.2: Thermogram obtained from the thermogravimetric analysis of pHEA⁵⁴⁵.

Appendix 3

Chapter 4

Manufacture of IOLs

Dimensions of IOLs printed with formulations C1 and C2

**The IOL created on the CAD software had an optic diameter of 6 mm and a thickness of 0.9 mm.*

Table 9.2: Dimensions of the IOLs printed with formulation C1.

IOL	Optic diameter (mm)	C1 IOLs	
		Thickness (mm)	Haptic-to-haptic length (mm)
1	6.305	1.41	11.38
2	6.355	1.49	10.75
3	6.335	1.55	11.65
Average	6.33 ± 0.03	1.48 ± 0.07	11.26 ± 0.46

Table 9.3: Dimensions of the IOLs printed with formulation C2.

IOL	Optic diameter (mm)	C2 IOLs	
		Thickness (mm)	Haptic-to-haptic length (mm)
1	6.31	1.84	12.74
2	6.115	1.77	12.91
3	6.31	1.78	13.25
Average	6.25 ± 0.11	1.796 ± 0.03	12.97 ± 0.26

Appendix 4

Chapter 4

Analysis of the efficiency of the post-curing protocol by ^1H -NMR

Analysis performed using IOLs that were printed with formulation C2

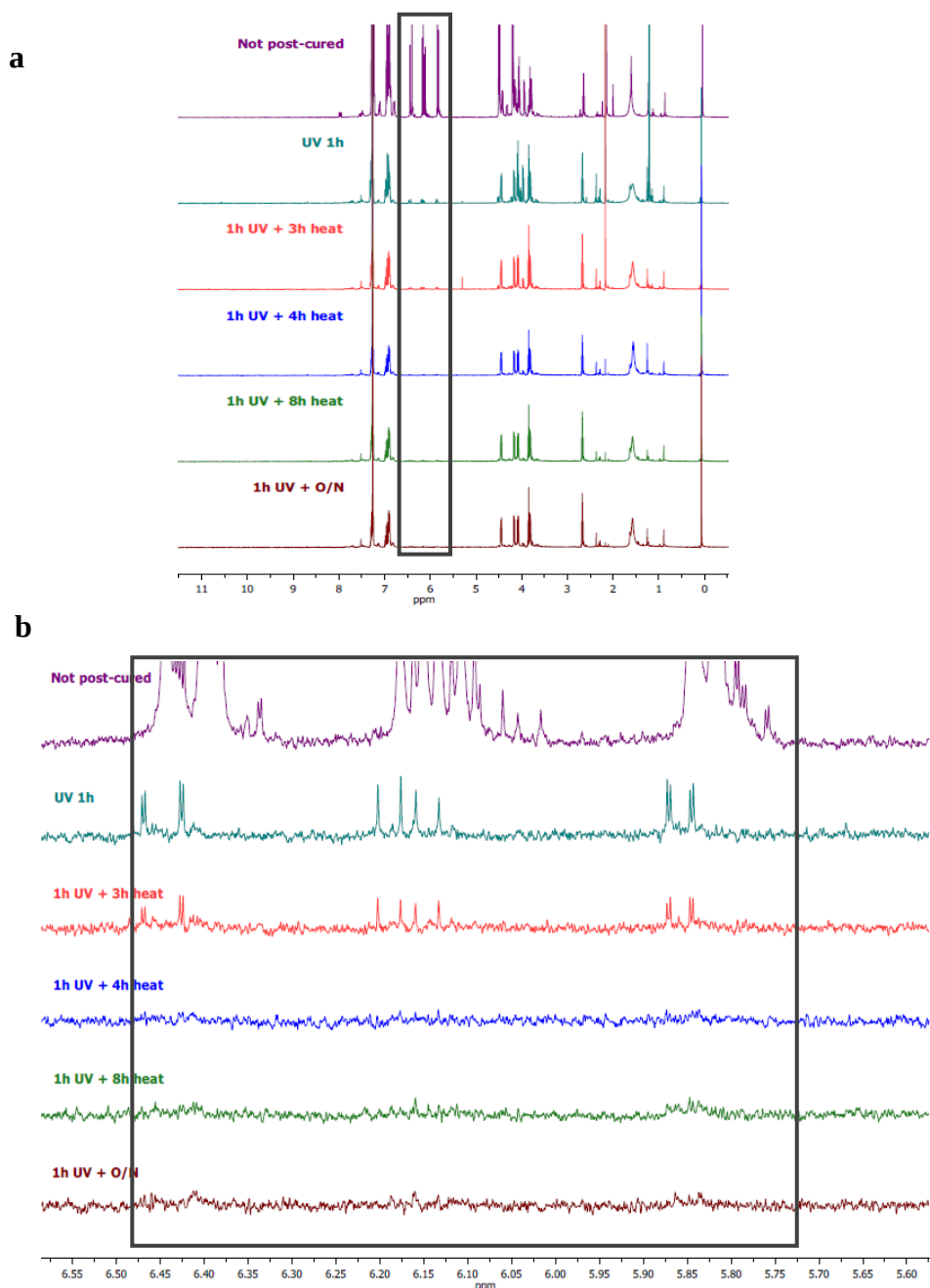


Figure 9.3: ^1H -NMR spectra obtained after the analysis of IOLs printed with formulation C2 and which had been subjected to different stages of the post-curing process (UV irradiation only or complemented with different heating times) or had not been post-cured. A reduction in the intensity of the signal produced by the acrylate groups (6.47 – 5.84 ppm) was observed with longer times of post-curing (a). An enlarged view of the acrylate signals is shown (b).

Appendix 5

Chapter 4

Analysis of the efficiency of the curing and post-curing protocols by ATR-FTIR

Analysis performed using IOLs that were printed with formulation C2

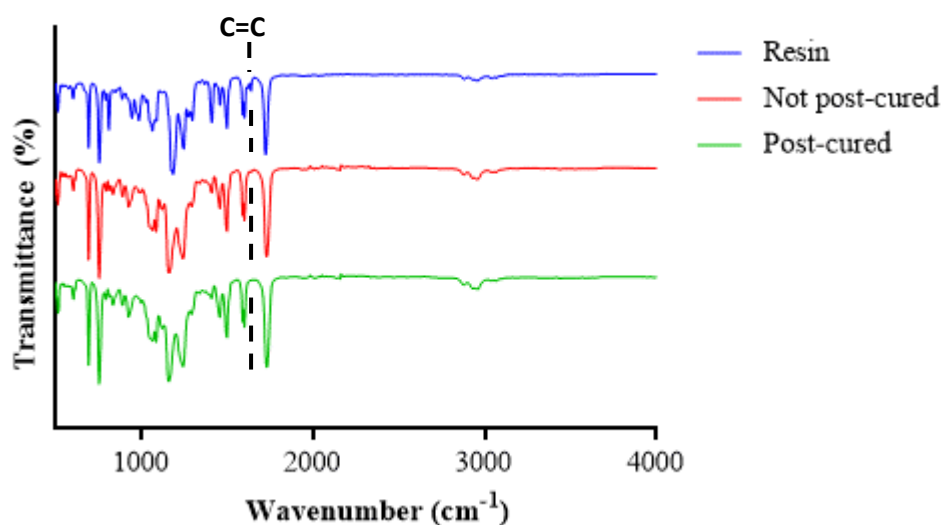


Figure 9.4: Spectra obtained from the analysis of the resin used for the production of C2 IOLs (blue), the printed IOLs before post-curing (red) and the post-cured IOLs (green). A reduction in the intensity of the signal produced by the C=C bonds was observed after each step of the production process.

Appendix 6

Chapter 4

Additive manufacturing of hydrophobic acrylic IOLs using the stereolithography system Form 2

Photos of IOLs printed on the build platform and without supports

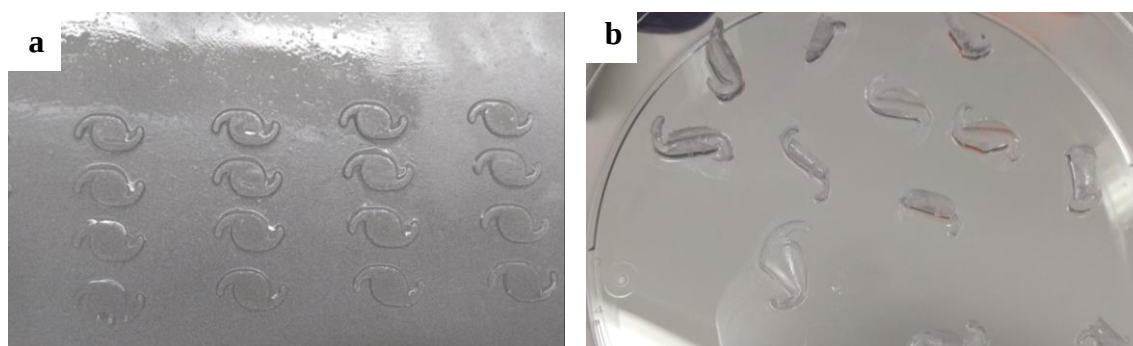


Figure 9.5: (a) IOLs printed with formulation C1 on the build platform of the SLA system and (b) same IOLs once they had been detached from the platform.

Appendix 7

Chapter 5

UV absorption spectrum of Eusorb® UV-1995

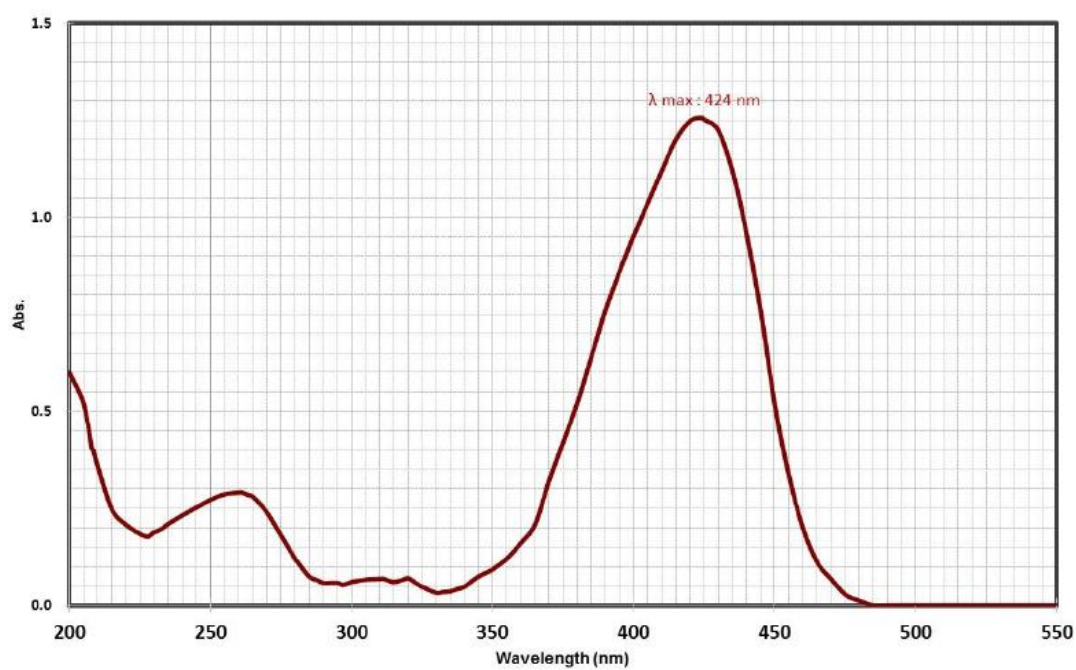


Figure 9.6: UV absorption spectrum of Eusorb® UV-1995.

Appendix 8

Chapter 5

ATR-FTIR

ATR-FTIR analysis of formulation C1 + 0.012% Eusorb UV-1995

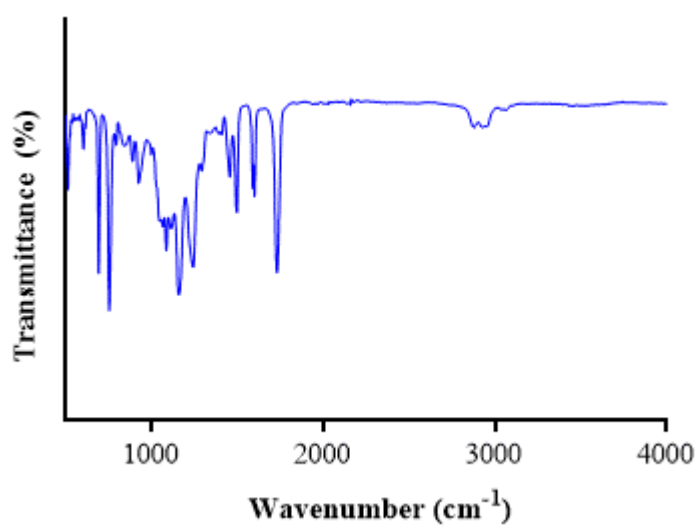


Figure 9.7: ATR-FTIR spectrum obtained from the analysis of an IOL that was composed of formulation C1 + 0.012% Eusorb UV-1995.

Appendix 9

Chapter 5

Cure depth measurements

Thickness of the cylinders produced with each formulation

Table 9.4: Thickness of the cylinders obtained with each of the formulations which contained increasing concentrations of Eusorb UV – 1995.

Thickness (mm) with different concentrations of light absorber (%)					
Cylinder	0 % Eusorb	0.006% Eusorb	0.012% Eusorb	0.024% Eusorb	0.048% Eusorb
1	2.8	1.2	0.68	0.32	0.11
2	2.9	1.18	0.73	0.37	0.15
3	3.3	1.24	0.7	0.34	0.2
Average	3 ± 0.26	1.21 ± 0.03	0.7 ± 0.03	0.34 ± 0.03	0.15 ± 0.05

Manufacture of IOLs

Dimensions of the IOLs printed with 0.006% and 0.012% Eusorb UV-1995

Table 9.4: Dimensions of the IOLs printed with formulation C1 + 0.006% Eusorb UV – 1995.

C1 + 0.006% Eusorb			
IOL	Optic diameter (mm)	Thickness (mm)	Haptic-to-haptic length (mm)
1	5.795	1.27	14.61
2	6.285	1.27	14.14
3	6.17	1.34	14.33
Average	6.08 ± 0.26	1.29 ± 0.04	14.36 ± 0.24

Table 9.5: Dimensions of the IOLs printed with formulation C1 + 0.012% Eusorb UV – 1995.

C1 + 0.012% Eusorb			
IOL	Optic diameter (mm)	Thickness (mm)	Haptic-to-haptic length (mm)
1	6.165	1.166	14.13
2	6.145	1.156	14.57
3	6.13	1.166	14.97
Average	6.15 ± 0.02	1.16 ± 0.01	14.56 ± 0.42

Appendix 10

Chapter 6

Synthesis of polymer brushes

ATR-FTIR spectra of IOLs that had been coated with Ac-PEG-SH polymer brushes produced after the photoactivation of the lenses with TPO solutions of different concentrations

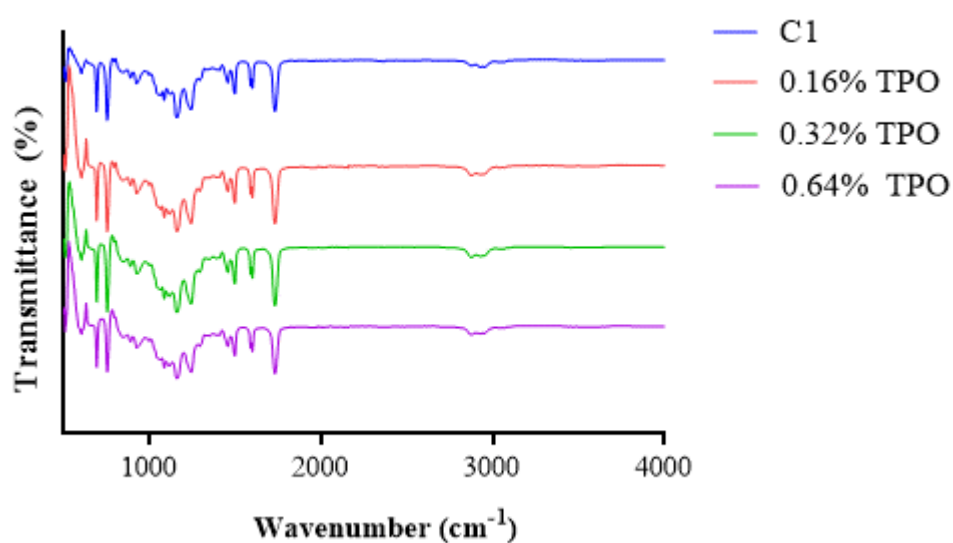


Figure 9.8: ATR-FTIR spectra obtained from the analysis of non-coated C1 IOLs (blue) or IOLs which had been coated with Ac-PEG-SH polymer brushes which had been produced after the photoactivation of the lenses with either 0.16% (red), 0.32% (green) or 0.64% (purple) TPO solutions. No additional signals were detected on the spectra that could be attributed to the coating.

Appendix 11

FTIR

Table 9.6: IR group frequencies. Adapted from Coates, 2000⁴⁹⁵.

Assignment	Wavenumber (cm ⁻¹)
C=C stretch	1,680 – 1,620
=CH-H bend	1,420 – 1,410
O-H	3,570 – 3,200
C=O	1,750 – 1,725
C-O-C	1,150 – 1,050
Methylene C-H stretch	2,850 – 2,935
Methyl	2,970 – 2,860
Alkyne C-H stretch	3,320 – 3,310
Alkyne C-H bend	680 - 610
Aromatic C-H ring in-plane bend	1,225 – 950 (several)
Aromatic C-H out-of-plane bend	900 – 670 (several)
Aromatic ring stretch (C=C-C)	1,615 – 1,450 (pair of bands)
Aromatic C-H stretch	3,130 – 3,070
Aromatic C-C bend	1,690
Monosubstituted aromatic C-H bend	770 – 710

