Design and Synthesis of Biodegradable Thermoplastic Polyurethanes for Tissue Engineering

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B. App. Sci. (Chem) (Hons)

A Thesis Submitted for the Degree of Doctor of Philosophy
Swinburne University of Technology
2005
Abstract

The aim of this study was to design and synthesise thermoplastic biodegradable and biocompatible polyurethanes for tissue engineering applications. A secondary aim was to tailor a range of degradation rates of the polyurethanes to suit a broad spectrum of tissue engineering applications.

Various factors were systematically investigated in order to provide a means of controlling mechanical, thermal and degradation properties of the polyurethanes. The factors investigated included variation of the hard segment percentage, the diisocyanate, the soft segment macrodiol as well as the chain extender. Soft segment macrodiols were synthesised for this study including a poly(γ-butyrolactone) macrodiol which has been used to make biodegradable aliphatic poly(ester-urethane) for the first time. A novel range of degradable chain extenders was also developed and has been reported.

The polymers were characterised using Gel Permeation Chromatography (GPC), Instron tensile testing, Differential Scanning Calorimetry (DSC) and Shore hardness. Cell culture testing was performed as was a three-month degradation study which showed the polyurethanes to be biocompatible and biodegradable respectively.

Selected materials were shown to be suitable for scaffold fabrication using Fused Deposition Modelling (FDM), and the scaffolds made were further shown to support primary fibroblast growth in vitro.
Acknowledgments

I thank the Division of Molecular Science, CSIRO (Commonwealth Scientific and Industrial Research Organisation) where all the polymer work was carried out for kindly granting me permission to work as a student in their laboratories under the supervision of Dr. Thilak Gunatillake. I also thank Dr. Thilak Gunatillake for his invaluable guidance and advice. I also especially thank Dr. Raju Adhikari of Molecular Science, CSIRO / PolyNovo Biomaterials Pty Ltd for his advice and friendship during my time at CSIRO.

A big thankyou to Dr. Tony Barton for his assistance and advice throughout the project especially for his help with the cell culture studies that were carried out at Swinburne University of Technology. Many thanks to both Associate Professor Bob Laslett and Dr. Tony Barton for coming out to CSIRO a number of times for thesis meetings, and for reading through and offering advice on thesis drafts.

I would like to acknowledge the work done by Dr. Andro Lau (Industrial Research Institute Swinburne) who operated the FDM and also by Mr. Heng Taing (CSIRO, Molecular Science Division) who assisted by doing some hydroxyl number and acid number determinations, which I very much appreciate. Mr. Heng Taing also assisted in the synthesis of three of the soft segment macrodiols that were used in this study under my direction – again many thanks. Thanks also to Dr. Alf Uhlherr (CSIRO, Molecular Science Division) who wrote the transesterification computer program.

Thanks to both Dr. Mike O'Shea and Mr. Gary Peeters for their help and advice in teaching me how to operate the extruders at CSIRO.

Thankyou to PolyNovo Biomaterials Pty Ltd for allowing me access to their laboratory for the last part of my experimental work.

Finally, I’d like to thank my family and fiancée Dalena for making this post-graduate degree possible by way of their support and understanding throughout my studies.
Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma, except where due reference is made in the text of the thesis. To the best of my knowledge, this thesis contains no material previously published or written by another person except where due reference is made in the text of the thesis.

Signature of Candidate: _______________________________
Timothy Moore

Dated: ________________________________
Research Publications and Awards

Journal Articles

T.Moore, R.Adhikari and P.Gunatillake, Chemosynthesis of bioresorbable poly(γ-butyrolactone) by ring opening polymerisation: A review, Biomaterials, 26: 3771-3782, 2005

Conference Proceedings (Oral Presentations)


Patents


R.Adhikari, P.A.Gunatillake and T.Moore, Biodegradable polyurethane and polyurethane ureas based on degradable chain extender, Draft divisional patent filed September 2005

Awards

CSIRO, Molecular Science Divisional Strategic Action Plan Award for Innovation, 2004. This was a monetary team award presented to recipients for the development of technologies leading to the formation of PolyNovo Biomaterials, Pty Ltd.

The Australian Society for Biomaterials Inc., Student Travel Award. This was towards travel costs in attending the World Biomaterials Congress in May 2004 and was awarded based on quality of abstract.
Table of Contents

Abstract ii
Acknowledgments iii
Declaration iv
Research Publications and Awards v
Table of Contents vi
List of Figures xiii
List of Tables xv
List of Abbreviations xvi

1. Introduction 1

1.1. Tissue Engineering 1

1.2. Scaffold Fabrication Methods for Tissue-Engineering 3

1.2.1. Non-woven Mesh 4

1.2.2. Porogen Leaching 4

1.2.3. Solvent Casting 5

1.2.4. Foams / Emulsions / TIPS 6

1.2.5. Other Methods of Scaffold Fabrication 7

1.2.6. Rapid Prototyping 8

1.2.6.1. Fused Deposition Modelling 10

1.3. Biodegradable Polymers for Tissue Engineering 15
1.5.3. Poly(lactic acid)  

1.5.4. Poly(lactic acid-co-glycolic acid)  

1.5.5. Poly(γ-butyrolactone)  

1.6. Experimental Outline and Objectives of the Study  

2. Effect of Diisocyanate and Hard Segment Percentage  

2.1. Methods  

2.1.1. Synthesis of Polyurethanes from Series 1  

2.1.2. Synthesis of Polyurethanes from Series 2  

2.1.3. Compression Moulding  

2.1.4. Instron Tensile Tests  

2.1.5. Differential Scanning Calorimetry (DSC)  

2.1.6. Gel Permeation Chromatography  

2.1.7. Shore Hardness Indentor  

2.2. Results and Discussion  

2.3. Summary and Conclusion  

3. Synthesis and use of Macrodiols  

3.1. Methods  

3.1.1. Method - General Synthesis of PGA Macrodiols  

3.1.2. General Synthesis of PLA, PLGA Macrodiols  

3.1.3. Synthesis of Poly(γ-butyrolactone) Macrodiol  

3.1.4. Acid Number Titration  

3.1.5. Hydroxyl Number
3.1.6. Synthesis of Polyurethanes from Series 3 and 4  62
3.1.7. Compression Moulding  62
3.1.8. Instron Tensile Tests  62
3.1.9. Differential Scanning Calorimetry (DSC)  63
3.1.10. Gel Permeation Chromatography  63
3.1.11. Shore Hardness Indentor  63
3.1.12. Nuclear Magnetic Resonance  63
3.2. Results and Discussion  64
3.2.1. Soft Segment Synthesis  64
3.2.2. Macrodials Synthesised For This Study  69
3.2.3. Synthesis of Polyurethanes with Varying Soft Segments  74
3.3. Summary and Conclusion  80
4. Polyurethanes with Degradable Chain Extenders  81
4.1. Methods  81
4.1.1. Synthesis of Novel Chain Extenders  81
4.1.1.1. GA-EG Degradable Chain Extender  81
4.1.1.2. LA-EG Degradable Chain Extender  82
4.1.1.3. GA-1,3-PD Degradable Chain Extender  83
4.1.1.4. EG-Suc-EG Degradable Chain Extender  83
4.1.1.5. EG-Seb-EG Degradable Chain Extender  83
4.1.1.6. EG-Fum-EG Degradable Chain Extender  84
4.1.2. Synthesis of Polyurethanes from Series 5 and 6  84
4.1.3. Crosslinking of the EG-Fum-EG Polyurethane 85
4.1.4. NMR – GA-EG Transesterification Kinetics 85
4.1.5. Melt Flow Index 85
4.1.6. Nuclear Magnetic Resonance 86
4.1.7. Compression Moulding 86
4.1.8. Instron Tensile Tests 86
4.1.9. Differential Scanning Calorimetry (DSC) 86
4.1.10. Gel Permeation Chromatography 87
4.1.11. Shore Hardness Indentor 87

4.2. Results and Discussion 88
4.2.1. Degradable Chain Extenders 88
4.2.2. Kinetics and Effect of Transesterification in Dimer Syntheses 95
4.2.3. Theoretical Modelling of the Transesterification Reaction 99
4.2.4. Refinement of the Degradable Chain Extender 101
4.2.5. GA-EG Degradable Chain Extender 104
4.2.6. LA-EG Degradable Chain Extender 111
4.2.7. GA-1,3-PD Degradable Chain Extender 113
4.2.8. EG-Suc-EG Degradable Chain Extender 115
4.2.9. EG-Fum-EG Degradable Chain Extender 120

4.3. Polyurethanes Synthesised Using Degradable Chain Extenders 122

4.4. Summary and Conclusion 129
5. Degradation and Cell Growth Studies 130

5.1. Materials and Methods 130

5.1.1. Cell Culture Biocompatibility Testing 130

5.1.2. Accelerated Degradation study 131

5.1.3. Detailed Degradation study 131

5.1.4. Scanning Electron Microscopy 131

5.1.5. Light Microscopy 132

5.2. Results and Discussion 133

5.2.1. Accelerated Degradation study 133

5.2.2. Detailed Degradation Study 136

5.2.3. Cytocompatibility 141

5.2.3.1. Cell Growth Study – Polymer Degradation 144

5.3. Summary and Conclusion 146

6. FDM Scaffolds 147

6.1. Materials and Methods 147

6.1.1. Brabender Extruder 147

6.1.2. Mini-Extruder 147

6.1.3. FDM 148

6.1.4. Cell Growth on FDM Scaffolds 148

6.2. Results and Discussion 149

6.3. Cell Colonisation of FDM Scaffolds 154

6.4. Summary and Conclusion 157
7. Conclusions and Recommendations 158
8. Appendices 162
9. Bibliography 178
List of Figures

Figure 1 – SEM of foamed polyurethane scaffolds (thin sections) ................................................................. 6
Figure 2 – Photograph of FDM head and building platform ........................................................................... 10
Figure 3 – The Stratasys® FDM Modeler and controlling computer ................................................................. 11
Figure 4 – Schematic diagram showing the process of FDM ........................................................................ 12
Figure 5 – Photograph of the FDM head showing rollers .............................................................................. 12
Figure 6 – A roll of 1.7mm diameter filament being fed into the back of the FDM ........................................ 13
Figure 7 – Structure of poly(glycolic acid) .................................................................................................... 16
Figure 8 – Formation of urethane linkage .................................................................................................... 17
Figure 9 – Formation of a urethane crosslink (triol initiated) ...................................................................... 18
Figure 10 – Formation of a urethane crosslink (trisocyanate initiated) ...................................................... 18
Figure 11 – Formation of a urethane linkage from amine and cyclic carbonate reactants .......................... 19
Figure 12 – Formation of allophosphonate links .......................................................................................... 20
Figure 13 – Formation of an amide linkage from an isocyanate and a carboxylic acid .............................. 20
Figure 14 – Reaction of disocyanate with water to form cross-links ............................................................ 22
Figure 15 – Degradation of a urethane link .................................................................................................. 24
Figure 16 – Formation of a poly(urethane-urea) .......................................................................................... 25
Figure 17 – Hydrogen bonding between polyurethane chains ................................................................... 26
Figure 18 – Structure of the degradable chain extender used by G.A.Skarja and K.A.Woodhouse134 .... 30
Figure 19 – Ring opening polymerisation of ε-caprolactone with ethylene glycol initiator ..................... 35
Figure 20 – Polymerisation of γ-butyrolactone with ethylene glycol to PyBL ........................................... 38
Figure 21 – Dimensions of the 1mm thick Instron tensile test specimens ................................................. 44
Figure 22 – Stress-strain curve for TM1-2 .................................................................................................. 48
Figure 23 – Diagram of tensile failure showing characteristic cold-drawing of TM1-3 .............................. 49
Figure 24 – Stress-strain curve for TM1-3 .................................................................................................. 49
Figure 25 – Comparison of stress-strain curves for TM1-1 and TM1-2 ...................................................... 50
Figure 26 – Comparison of stress-strain curves for TM1-3 and TM1-4 ...................................................... 50
Figure 27 – Stress-strain curve for TM2-9 .................................................................................................. 53
Figure 28 – Stress-strain curve for an oriented specimen of TM2-9 ............................................................ 53
Figure 29 – Stress-strain curves for TM2-9 showing effect of premature failure ...................................... 54
Figure 30 – Effect of varying the ratio of hard segment to soft segment — thermal properties of Series 1 56
Figure 31 – Structures of the synthesised macrodiols ................................................................................ 69
Figure 32 – 1HNMR of PGA-macrodiols of different molecular weights initiated with EG ........................... 71
Figure 33 – Structure of poly(γ-butyrolactone) initiated using ethylene glycol ....................................... 73
Figure 34 – 400MHz 1HNMR of poly(γ-butyrolactone) of 291 molecular weight ..................................... 73
Figure 35 – Chain length varying with macrodiol composition (similar molecular weights) .................... 74
Figure 36 – Series 4: Thermal properties (Differential Scanning Calorimetry) ............................................. 77
Figure 37 – Stress-strain curve for TM4-2 .................................................................................................. 78
Figure 38 – Stress-strain curve for TM4-6 .................................................................................................. 78
Figure 39 – Polyurethane hard segment composed of hexamethylene diisocyanate and ethylene glycol... 88
Figure 40 – Proposed degradable chain extender (hydroxyacetic acid-2-hydroxyethylster) ...................... 89
Figure 41 – Hydrolysis of GA-EG degradable chain extender ..................................................................... 89
Figure 42 – Introduction of an ester group to the hard segment using GA-EG diol .......................................... 89
Figure 43 – Incorporation of the GA-EG chain extender into the hard segment .......................................... 90
Figure 44 – Some expected degradation products of GA-EG chain extender and HDI hard segment ..... 90
Figure 45 – Approximate length of a 70% hard segment of HDI and EG with PCL400 as soft segment 91
Figure 46 – GPC after esterification of EG and GA (1:1) for 12 hours ......................................................... 93
Figure 47 – Transesterification of a PGA-macrodiol with EG (coordination/insertion mechanism) ........ 94
Figure 48 – Oligocyclisation of glycolic acid by condensation and transesterification ............................... 94
Figure 49 – Transesterification of GA-EG to give EG and GA-EG-GA ........................................................ 95
Figure 50 – 1HNMR spectra of GA-EG over time at 150°C in deuterated DMSO .................................... 96
Figure 51 – GA-EG dimer and ethylene glycol – choice of protons for monitoring transesterification .... 97
Figure 52 – Formation of EG during transesterification of GA-EG dimer at various temperatures ............ 97
Figure 53 – First-order rate plots of EG formation during transesterification of the GA-EG dimer .......... 98
Figure 54 – Eyring plot for the transesterification reaction of GA-EG .......................................................... 98
Figure 55 – Graph of expected yield of dimer vs fraction of glycolic acid in feed .................................... 101
Figure 56 – Hard segment composed of HDI and GA-EG degradable chain extender ............................ 102
List of Tables

Table 1 – Comparison of linear biodegradable polyester properties .................................................................................................................. 15
Table 2 – Comparison of linear biodegradable polyesters with methyl substituents .................................................................................................. 15
Table 3 – Approximate price comparison of the cyclic and linear forms of the α-hydroxy acids .................................................................................. 36
Table 4 – Composition of polyurethanes from Series 1 ................................................................................................................................. 42
Table 5 – Composition of polyurethanes from Series 2 ................................................................................................................................. 43
Table 6 – Series 1: Composition of polyurethanes varying diisocyanate and soft segment length ........................................................................ 46
Table 7 – Series 1: Physical properties of polyurethanes varying diisocyanate and soft segment length ..................................................................... 46
Table 8 – Series 1: Molecular weights of polymers varying diisocyanate and soft segment length .......................................................................... 47
Table 9 – Series 2: Varying the ratio of HDI hard segment to soft segment – mechanical properties ................................................................. 52
Table 10 – Mechanical properties of cold-drawn TM2-9 (75% hard segment) .................................................................................................................. 54
Table 11 – Series 2: Molecular weights of polymers varying hard segment percentage .......................................................................................... 55
Table 12 – Relationship between Effective Mn, Effective Xn and Acid number in the absence of diol .............................................................. 65
Table 13 – Polyesterification data, assuming K = 0.1 ............................................................................................................................................... 65
Table 14 – Polyesterification data, assuming K = 1.0 ............................................................................................................................................... 66
Table 15 – Literature values of acid number for degradable macrodiols ............................................................................................................... 68
Table 16 – Characterisation of soft segment macrodiols used in Series 3 and 4 ........................................................................................................ 69
Table 17 – Molecular weight change during transesterification for PLA-352 ........................................................................................................ 71
Table 18 – Molecular weight of oligo-γ-butyrolactone over time ...................................................................................................................... 72
Table 19 – Comparison of ~400 Mw macrodiols ............................................................................................................................................... 75
Table 20 – Series 3: Changing the Soft Segment - Composition .......................................................................................................................... 75
Table 21 – Series 3: Effect on physical properties of changing the soft segment .................................................................................................. 76
Table 22 – Series 4: Mechanical properties of polymers with different soft segments ............................................................................................ 76
Table 23 – Series 4: Molecular weights of polymers with different soft segments ............................................................................................... 79
Table 24 – Effect of hard segment % and chain length on the number of consecutive urethane linkages ............................................................... 91
Table 25 – Composition of glycolic acid / ethylene glycol oligomeric diols ...................................................................................................... 100
Table 26 – Computer generated data for predicted products of EG / GA esterification .......................................................................................... 100
Table 27 – Formula and melting point of common diacid monomers ............................................................................................................... 117
Table 28 – Series 5: Physical properties of polyurethanes containing degradable chain extenders ........................................................................... 122
Table 29 – Series 5: Molecular weight of polyurethanes containing degradable chain extenders ........................................................................... 122
Table 30 – Melt Flow Index of selected polymers from series 5 .................................................................................................................................... 128
Table 31 – Composition of polyurethanes used in preliminary degradation study ................................................................................................... 134
Table 32 – Series 3: Mass loss and GPC molecular weights before and after degradation ................................................................................... 135
Table 33 – Series 1: Degradation results (PBS buffer pH 7.4, 37°C, 3 months) ....................................................................................................... 136
Table 34 – Series 2: Degradation results (PBS buffer pH 7.4, 37°C, 3 months) ....................................................................................................... 137
Table 35 – Series 4: Degradation results (PBS buffer pH 7.4, 37°C, 3 months) ....................................................................................................... 137
Table 36 – Series 5: Degradation results (PBS buffer pH 7.4, 37°C, 3 months) ....................................................................................................... 138
Table 37 - Series 6: Degradation results (PBS buffer pH 7.4, 37°C, 3 months) ....................................................................................................... 139
Table 38 – Cell study results (relative number of cells each day from 0-5) ........................................................................................................... 143
Table 39 – Acute toxicity data for ethylene glycol from the literature .................................................................................................................. 164
Table 40 – Acute toxicity data for 1,6-hexanediame (the degradation product of HDI) .......................................................................................... 164
Table 41 – Acute toxicity data for various degradation products ....................................................................................................................... 164
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>[ ]</td>
<td>Concentration</td>
</tr>
<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>ASTM</td>
<td>American Standard Testing Method</td>
</tr>
<tr>
<td>BCMO</td>
<td>3,3-bis(chloromethyl)oxacyclobutane</td>
</tr>
<tr>
<td>CAD</td>
<td>Computer Aided Design</td>
</tr>
<tr>
<td>CL</td>
<td>ε-Caprolactone</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>CU</td>
<td>Cutaneous</td>
</tr>
<tr>
<td>DABCO</td>
<td>1,4-diazo(2,2,2)bicyclo-octane</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle’s Medium</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethyl formamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>EG</td>
<td>Ethylene glycol</td>
</tr>
<tr>
<td>EG-Fum-EG</td>
<td>But-2-enedioic acid bis-(2-hydroxy ethyl) ester</td>
</tr>
<tr>
<td>EG-Suc-EG</td>
<td>Succinic acid bis-(2-hydroxy ethyl) ester</td>
</tr>
<tr>
<td>e-LDI</td>
<td>Lysine ethyl ester diisocyanate</td>
</tr>
<tr>
<td>GA</td>
<td>Glycolic acid</td>
</tr>
<tr>
<td>GA-1,3-PD</td>
<td>Hydroxy-acetic acid 3-hydroxy-propyl ester</td>
</tr>
<tr>
<td>GA-EG</td>
<td>Hydroxy-acetic acid 2-hydroxy-ethyl ester</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas Chromatography – Mass Spectroscopy</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel Permeation Chromatography</td>
</tr>
<tr>
<td>HDI</td>
<td>Hexamethylene diisocyanate</td>
</tr>
<tr>
<td>HFIP</td>
<td>Hexafluoroisopropanol</td>
</tr>
<tr>
<td>HNMR</td>
<td>$^1$Hydrogen Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>IM</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>IP</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared spectroscopy</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>LA</td>
<td>Lactic acid</td>
</tr>
<tr>
<td>LA-EG</td>
<td>2-Hydroxy-propionic acid 2-hydroxy-ethyl ester</td>
</tr>
<tr>
<td>LD$_{50}$</td>
<td>Dose lethal to 50% of test animals</td>
</tr>
<tr>
<td>MLD</td>
<td>Minimum Lethal Dose</td>
</tr>
<tr>
<td>m-LDI</td>
<td>Lysine methyl ester diisocyanate</td>
</tr>
<tr>
<td>Mn</td>
<td>Number average molecular weight</td>
</tr>
<tr>
<td>MP</td>
<td>Peak Molecular Weight Average (GPC)</td>
</tr>
<tr>
<td>Mw</td>
<td>Weight average molecular weight</td>
</tr>
<tr>
<td>NMIM</td>
<td>N-Methylimidazole</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>NOEL</td>
<td>No Observable Effect Limit</td>
</tr>
<tr>
<td>OR</td>
<td>Oral</td>
</tr>
<tr>
<td>P3HB</td>
<td>Poly-3-hydroxybutyrate</td>
</tr>
<tr>
<td>P4HB</td>
<td>Poly-4-hydroxybutyrate</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>PCL</td>
<td>Poly-ε-caprolactone</td>
</tr>
<tr>
<td>PD</td>
<td>Propane diol</td>
</tr>
<tr>
<td>PED</td>
<td>Precision Extruding Deposition</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PGA</td>
<td>Poly(glycolic acid)</td>
</tr>
<tr>
<td>PLA</td>
<td>Poly(lactic acid)</td>
</tr>
<tr>
<td>PPA</td>
<td>Polyhydroxypropionate</td>
</tr>
<tr>
<td>PPG</td>
<td>Polypropylene glycol</td>
</tr>
<tr>
<td>PVL</td>
<td>Poly-δ-valerolactone</td>
</tr>
<tr>
<td>SC</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>Sn-Mont</td>
<td>Tin ion-exchanged Montmorillonite</td>
</tr>
<tr>
<td>Tₙ</td>
<td>Glass Transition Temperature</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMBDA</td>
<td>Tetramethylbutanediameine</td>
</tr>
<tr>
<td>γ-BL</td>
<td>γ-Butyrolactone</td>
</tr>
<tr>
<td>AGp</td>
<td>Gibbs Free Energy of Polymerisation</td>
</tr>
<tr>
<td>ε-CL</td>
<td>ε-Caprolactone</td>
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</table>
1. Introduction

1.1. Tissue Engineering

Tissue engineering is a growing field worldwide with enormous medical potential. Skin grafts are one example of a tissue-engineered product that is currently available\(^1,2,3\) and more products are likely to come into the marketplace over the next decade. Tissue-engineered products are being investigated to replace or regenerate human tissue include bone\(^4\), cartilage\(^5\), smooth muscle\(^6\), tendon\(^7\), cardiovascular components\(^8\), eye\(^9\) and nerve\(^10\).

The underlying principle of tissue engineering is that living cells are grown on temporary scaffolds into functional tissue for replacement or augmentation of body parts. Some examples of when body parts may need replacing are when tissue is damaged or missing such as in the case of severely burnt skin, damaged or removed meniscal cartilage, calcified heart valves and occluded blood vessels.

Tissue engineering has been well expressed by D.F. Williams, 2004 as “…tissue engineering is the persuasion of the body to heal itself, achieved by the delivery to the appropriate site of cells, biomolecules, and supporting structures.”\(^11\) The supporting structures are usually polymeric scaffolds, which are designed to degrade to monomers once their purpose of directing and supporting cell growth is fulfilled. There are many biodegradable materials used for tissue engineering scaffolds yet almost all share the major disadvantage that they are difficult to fashion into scaffolds of defined porosity in the complicated shapes required for tissue engineering of three dimensional tissues. The meaning of the word ‘biodegradable’ in this context is that the material will degrade \textit{in vivo} and be safely resorbed or excreted over time until it is entirely gone. In this way the scaffold should lead to the formation of cell-derived structures that can undergo remodelling into native-like tissue. Biodegradability of tissue engineering scaffolds is generally regarded as a necessity since cells must be supported while they grow and lay down their own supporting framework called extra-cellular matrix which is primarily composed of collagen.

There are numerous examples of tissue engineering in the literature; one well-known example of tissue engineering is the human ear growing on the back of a mouse.\(^12\) More recent work by the same group\(^13\) (J.P. Vacanti, 2004) produced scaffolds in the shape of
a human ear by solvent-casting and particulate-leaching of polymers in hand-made clay moulds. These polymer scaffolds were seeded with chondrocytes and implanted into the backs of athymic mice. The polymer scaffold fabrication method was quite crude (set in a hand-made clay mould) and has a number of potential problems such as being difficult to reproduce or mass-produce since they were hand-made, uses toxic solvents including chloroform during the solvent casting step, has a possible source of contamination from the clay mould, and the whole process is very labour-intensive. This is by no means an uncommon situation and many tissue-engineering groups have resorted to producing scaffolds by similarly crude means due to polymer processing limitations and the lack of an alternative.

Tissue-engineering methods have often involved mechanical stress on the growing tissues. Mechanical stress is used in order to form the correct tissue types, orientation, and strength which would not always form without such stress. The growth of cartilage (intended for load-bearing applications) is an example where mechanical stress has been used. Another such example is in the tissue engineering of heart valves which have been cultured under pulsatile flow conditions. The scaffolds required for tissue engineering in such an environment must be robust enough to withstand the dynamic conditions until the cells can provide their own support structures by way of extracellular matrix. The requirements of a scaffold depend largely upon the application and may necessitate rigidity or elasticity, fast or slow degradation, from very strong and tough polymers to very weak hydrogels. Due to this range of requirements, the methods for scaffold fabrication and the polymers that have been commonly used in tissue engineering scaffolds are reviewed in the following sections.
1.2. Scaffold Fabrication Methods for Tissue-Engineering

A biodegradable scaffold for tissue-engineering is a temporary structure that is designed to degrade over an appropriate timeframe and be replaced by living tissue. The scaffold must be biocompatible as must its degradation products. The degradation products should ideally be known to resorb into or be excreted from the body without any harmful effects. For this reason there has been a large amount of research into using biopolymers such as collagen, starch, chitin and polyhydroxy alkanoates. However, having a biocompatible and biodegradable material is only half of the requirement of a successful scaffold, the other half being the shape (both macroscopic and microscopic), which can aid or hinder tissue formation. It is generally recognized that scaffolds should be made porous rather than solid in order to promote cell colonisation, ingrowth and proliferation. This porosity leads to a weaker structure and it can also accelerate degradation due to the larger surface area. Having a porous structure allows nutrients to flow in to the cells in the inner regions of the scaffold and for waste to be removed. The ideal pore size and pore morphology of the scaffold depends on the intended purpose of the scaffold but the most commonly investigated pore size range in the literature is between 100–500µm diameter, and tending more towards the higher end of the range than the lower end. It has also been claimed that the optimal pore size is between five and ten times the diameter of the cell i.e.100-300µm. There have been a number of reviews of scaffolds for tissue engineering applications and the number of different materials, as well as methods of production of scaffolds is continuously increasing. The following section is a short review of the common fabrication methods and the materials that are frequently used, including the advantages and disadvantages of each.
1.2.1. Non-woven Mesh

A non-woven mesh is a random mesh of fine fibres that are typically heat or solvent-welded together. The most commonly used materials for biodegradable non-woven mesh are poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and copolymers thereof.\(^{27,28,29,30,31,32}\) However, other materials have also been used such as collagen\(^ {19}\) and poly(3-hydroxybutyrate) (P3HB).\(^ {33}\)

There are two major advantages of non-woven mesh. Firstly the diameter of the fibres obtained by this method can be in the nano range (~1-100nm), and secondly the porosity of the scaffolds is generally very high (>90%). It is generally thought to be advantageous to have smaller diameter fibres for a number of reasons such as increased degradation rate due to higher accessible surface area, and easier cell infiltration, entrapment and adhesion.

One disadvantage of non-woven mesh is that in order to form a fibre the polymer must be of reasonably high molecular weight, which is a limitation that thermal processing such as injection moulding does not share where wax-like materials can be used. Another disadvantage is that the mesh is random so there is little or no control over the pore size or orientation in the mesh and also the mesh is usually pressed as a sheet making 3D objects difficult or in some cases impossible to fabricate.

1.2.2. Porogen Leaching

Porogen leaching is a simple way to make a porous scaffold and involves dissolving out solid particles such as salt from the polymer matrix leaving pores of the same dimensions as the salt. Highly controlled and uniform pore sizes can be obtained through careful and selective preparation of the porogen, however the orientation of the pores is not easy to control in most instances. A ‘porogen’ in this case is a solid particle which can be added to the polymer scaffold (either when the polymer is melted or dissolved in a solvent), and then leached out (typically with water) to leave a pore, or hole. Unless sufficient porogen is used there may be difficulty in leaching it from the polymer, and at high porogen loading the mechanical properties of the polymer are substantially reduced. With insufficient loading of porogen one can end up with a “Swiss cheese” effect, where there are discrete holes in the polymer but no
interconnectivity. Interconnectivity is usually achieved by adjacent porogen particles touching each other, similar to a close-packed arrangement of spheres. As a rough guide approximately 70-80% porosity is required for the pores to be interconnected however this is dependent upon the shape and dimensions of the porogen particles. There are variations on this theme where solvents have been used to partially dissolve the walls between porogen molecules to achieve interconnectivity\textsuperscript{34} and this is called solvent merging. A large number of groups have made PLA, PGA and PLGA (Poly(lactide-co-glycolide)) porous by porogen leaching. The average porogen size range used is 100-840µm diameter with most between 300-500µm.\textsuperscript{34,35,36,37,38,39,40,41,42,43,44,45,46,47} The most common porogen is NaCl which is why the technique is often named ‘salt-leaching’ rather than porogen leaching, however glucose and other leachable solids have also been used. Often the solid porogen is mixed with the polymer by dissolving the polymer in a solvent which is usually cytotoxic and must be removed fully before implantation. The possible presence of residual solvent is one of the major disadvantages of this method of scaffold fabrication. The pore size is usually quoted as being the same as the size of the porogen particles that were used which in turn is given as a range between two sieve sizes. However, this is not strictly accurate for two reasons. Firstly, a particle may have one dimension larger than the sieve size and still be included in this range, and secondly: although the porogen may be 500µm the ‘hole in the wall’ between two adjacent particles is considerably smaller. Both of these factors depend heavily on the morphology of the particle that is used – whether cuboid, spheroid, prismatic, irregular etc. At one extreme it may be possible to have a close-packed arrangement of identical spherical porogen particles which only just touch each other, hence when they are leached out there is only a very small hole joining any two particles. This does not facilitate adequate cell ingrowth until the polymer degrades sufficiently to undergo mass loss by which time mechanical properties are already lost.

1.2.3. Solvent Casting

Solvent casting is rarely used alone for scaffolds since unless a porogen is added the polymer will be nonporous. Solvent-casting in simple terms consists of dissolving polymer in a suitable solvent, pouring the solution onto a flat non-stick surface (often
Solvent casting is a useful method to prepare uniform samples for tensile testing or degradation studies but is rarely used for scaffolds. The properties of a collagen-based scaffold material have been investigated by using a solvent casting technique. In this case water-soluble or acetic acid-soluble collagen was dried onto a coverslip and then crosslinked trialling two different crosslinking agents to obtain a stable film.

Solvent casting is similar to dip coating where both form a non-porous two-dimensional film. The difference with dip coating is that it can be performed on 3D objects.

1.2.4. Foams / Emulsions / TIPS

Scaffolds created by foams, emulsions and TIPS (Thermally Induced Phase Separation) are similar to porogen leached scaffolds but rather than a solid porogen they rely on a liquid or gaseous phase that can be removed leaving a porous scaffold. The most commonly used polymers are linear PLA and PLGA.

Figure 1 shows two polyurethane scaffolds produced by the addition of controlled amounts of water to the reactants to cause evolution of CO$_2$(g) which creates the pores that are evident in the figure. The scaffolds in this case were generally closed-pored which is less desirable than an open-porous structure. The scaffolds shown in Figure 1 are from unpublished work by the author.
The only real advantage of these methods is the very high porosities that can be attained when compared to most other methods. Common values for percentage porosity are between 93% and 97% porosity.\textsuperscript{52,53,54,55,56,57}

The disadvantage of using foams is that there is less control over the pore size and distribution than methods such as rapid prototyping and porogen leaching. An additional disadvantage of foaming is that it requires a mould and often requires organic solvents, which aren’t ideal due to toxicity and difficulty in removal.

1.2.5. Other Methods of Scaffold Fabrication

There are many other methods of producing biodegradable scaffolds for tissue engineering that are not as widely exploited as the previous methods.

Porous scaffolds have been made by ablating fine holes in thin sheets of polyurethane materials using an excimer laser and these scaffolds were designed for use in cardiovascular tissue engineering applications.\textsuperscript{136} The disadvantage is that the scaffolds cannot be easily made with three-dimensional architectures using laser ablation due to the inherent limitations of this system – the laser only acts on the exposed surface hence it cannot create three-dimensional porosity.

Bacterially derived poly(3-hydroxybutyrate), (P3HB), has been made into thin films on silicon wafers using a spin-coater in order to form a reproducible coating to investigate surface properties.\textsuperscript{58} This is a good method for forming an appropriate coating for cell studies \textit{in vitro} but has not been shown useful for \textit{in vivo} studies. One would expect difficulties in removing the polymer from the silicon substrate without a loss of structural integrity of the film. For cell studies the silicon wafer can be advantageous since some polymers (especially when porous) can be less dense than the cell medium causing them to float to the surface. The silicon wafer anchors the film down and prevents this from occurring.

There are an increasing number of photopolymerisable materials used for scaffold production. One advantage of photopolymerisable polymers is that they can be modified to suit \textit{in situ} repairs or augmentations by way of an injectable material that can be cured on demand. One problem with photopolymerisable materials \textit{in vivo} is that the degradation products are difficult to predict due to the nature of free radical polymerisations and they have not been shown to be non-toxic. One of the more widely
published and better-known systems involves crosslinking through the alkene of fumaric acid, forming a carbon-carbon covalent linkage.\textsuperscript{59,60,61,62,63} This system has been evaluated both \textit{in vitro}\textsuperscript{64} and \textit{in vivo}\textsuperscript{65} by Fisher et al. and is reported to be biodegradable and non-toxic.

Another photopolymerisable material that has been investigated is PEG-diacrylate (acrylate-terminated poly(ethylene glycol)) which was crosslinked using UV light.\textsuperscript{66} The resulting material was a hydrogel that had little mechanical strength. A similar system based on PLA-PEG-acrylate copolymers has been used to create supposedly biocompatible films, however fibroblast adhesion was only \textasciitilde{}10\% or less when compared to fibroblasts on glass.\textsuperscript{67,68} The authors attribute decreasing cell adherence to increasing amounts of PEG in the network.

Knitted or woven structures are not commonly reported as scaffolds, mainly due to PGA and PLA being difficult to draw into fibres and a lack of flexibility. Hepatocytes have been grown on a woven mesh of poly(ethylene terephthalate), (PET), which was coated with PLGA.\textsuperscript{69} This does not really qualify as a biodegradable scaffold and the authors name it a “partially degradable film/fabric”. Polyglactin, i.e. poly(glycolic acid-co-lactic acid) has been knitted and woven into fabrics as an entirely biodegradable material.\textsuperscript{70}

\subsection*{1.2.6. Rapid Prototyping}

Rapid Prototyping is an attractive technology for the fabrication of scaffolds since the scaffold shape can be altered relatively easily unlike other types of fabrication, which usually necessitate a mould. The rapid prototyping of biodegradable scaffolds can be done using a number of different technologies. Some involve extrusion of thermoplastics, while others may involve solvents, free radical polymerisation or laser sintering.

Some groups have previously explored the extrusion of fine thermoplastic biodegradable fibres with varying degrees of success. Arguably the most successful outcome was achieved using poly(\textepsilon\text{-caprolactone}) fibres extruded using Fused Deposition Modelling (FDM).\textsuperscript{77,78,79,80,81} Recently poly(\textepsilon\text{-caprolactone}) has been extruded successfully in a similar manner but using “Precision Extruding Deposition” (PED), and this was shown to achieve very good resolution of \textasciitilde{}250\textmu\text{m} diameter
fibres.\textsuperscript{71} PED works in the same way as a small conventional extruder with a rotating screw arrangement and fed by a continuous supply of pellets. There have been other groups that have used similar technologies to FDM such as SolidScape, Inc. Model Maker II 3D printer which has been utilised to construct the inverse of a scaffold by depositing molten polysulfonamide and molten wax to create a solid mould, layer by layer.\textsuperscript{72} The wax was then removed by melting it and/or by washing in suitable solvent systems leaving a mould for casting a biodegradable material, in this case PLA. They called this an ‘indirect solid free form fabrication’ technique.

The extrusion of ceramic-loaded materials through fine diameter nozzles has been investigated and shown possible down to \textasciitilde100µm diameter.\textsuperscript{73} This did not however involve a thermoplastic polymer extrusion but instead a wax/stearic acid/ceramic blend was used, formulated to ensure low melt viscosity. The extrudate contained 55\% by weight of zirconia and was forced out by 350kPa air pressure in the melt but did not weld strand-to-strand very well. The authors suggested as a conclusion that extrusion free forming could also be extended to bone substitute material.

It has been shown that poly(L-lactic acid) can been used to make scaffolds for bone tissue engineering via precise extrusion at 160°C with a deposited filament width of 500µm.\textsuperscript{74} However rapid prototyping polymer extrusion has not always been accomplished by high temperatures. There has been work on solvent-based systems where 15\% poly(L-lactic acid)/15\% tricalcium phosphate/70\% dioxane (solvent) was extruded to create 3D scaffolds at 0°C.\textsuperscript{75} The resulting scaffold had large macropores between the deposited rows around 300-500µm and small micropores around 5µm.

One of the problems with rapid prototyping to date is that while the scaffolds have very well controlled architecture they lack the high resolution that some other methods can attain. The smallest features created by rapid prototyping methods are typically in the order of 300-1000µm diameter. There is a resolution limit that is material-dependent, and this lower limit could be reduced through the development of more suitable materials.
1.2.6.1. Fused Deposition Modelling

Fused deposition modelling is a rapid prototyping system that builds three-dimensional polymer models in a layer-by-layer manner. The polymer is melt extruded from a fine nozzle and deposited onto a flat substrate and the extrusion head is moved in the XY plane; once one plane is finished, the flat support substrate (light blue platform in Figure 2) is moved down in an incremental fashion and the next layer is deposited upon the first. The shape of the object is dictated by a CAD (Computer Aided Design) model. Some work has been done to produce CAD models directly from CT (Computed Tomography), eg. J.R.Meakin et al.76

![Figure 2 – Photograph of FDM head and building platform](Image)

There is a number of competing rapid prototyping technologies, each with their own advantages and disadvantages. Some of the other rapid prototyping technologies available are: Laminated Object Manufacturing (LOM™) by Cubic Technologies, 3D plotting by Solidscape Inc., Direct Shell Production Casting by Soligen, Selective Laser Sintering (SLS) by DTM Corporation, Solid Ground Curing by Cubital and Stereolithography by 3D Systems of Valencia to name a few.
The Fused Deposition Modeller described in this chapter was manufactured by Stratasys® and is an older model of machine that first became available in the early 1990’s (see Figure 3).

![Figure 3 – The Stratasys ® FDM Modeler and controlling computer](image)

In simple terms FDM can be thought of as an extruder that can move around depositing strands of molten polymer layer by layer controlled by a computer design. The force for extrusion in FDM is provided by two counter-rotating rollers that push a 1.7mm diameter filament into the extruder where it melts and is forced out of a smaller diameter die (see Figure 4). This force is transmitted directly from the rollers to the filament and relies on the filament’s own mechanical strength to transmit the force to the melt at the die orifice rather than using a conventional rotating screw arrangement. For this reason the filament must possess sufficient rigidity and strength to transmit the force applied by the rollers.
Figure 4 – Schematic diagram showing the process of FDM

Figure 5 – Photograph of the FDM head showing rollers

Soft materials that lack rigidity (approximated by either Young’s modulus or flexural modulus) bend or warp in the unsupported region between the rollers and the heating zone since they are not stiff enough to overcome the friction experienced in the heating zone.
zone and this becomes a limiting factor in designing materials to be used in FDM. On the other hand, the maximum stiffness of a material is limited if it needs to be run continuously from a spool since it has to bend in order to be fed into the rollers. The FDM is fed by a continuous spool of polymer filament situated at the rear of the machine (see Figure 6). If the material is too brittle and inflexible then it is likely to snap rather than unwind off the spool.

![Figure 6 – A roll of 1.7mm diameter filament being fed into the back of the FDM](image)

Stiffness of the material is just one of two key properties that a material needs in order to be suitable for FDM. The other is melt viscosity. Melt flow index (MFI) is a measure of the viscosity of a polymer and can be used to compare how easily different materials can be extruded and this is particularly useful parameter for polymers that are being prepared for FDM. Determination of MFI avoids the time-consuming process of preparing polymer filament and FDM trials. (It is difficult to prepare a polymer for FDM as it needs to be exactly 1.7mm diameter, free of defects and gels and a sufficient quantity of polymer to undergo the necessary stages). The MFI is dependent on the viscosity of a given material at a given temperature, the higher the value of MFI, the easier it extrudes and hence the lower the melt viscosity. The units of MFI are given as grams of material extruded through a standard die over a ten-minute period at a specified temperature and a specified weight (2.16kg) pressing down on it (ASTM D 1238), (American Society for Testing Materials). The MFI value for a material depends on the temperature at which it is measured. In general, the MFI increases with increasing temperature, but there is a limitation on the upper temperature due to degradation of the material. The MFI is inversely proportional to the molecular weight.
of a polymer, that is, the higher the molecular weight the lower the MFI – a low molecular weight polymer extrudes more easily than a high molecular weight polymer. The value of melt flow index gives a reasonable indication as to the suitability of a material for FDM, however the rigidity of the material must also be taken into account. To date there has only been one biodegradable polymer in the literature that has been successfully used for FDM, namely poly(ε-caprolactone), (PCL)\textsuperscript{77,78,79,80,81}. PCL has a number of disadvantages for tissue engineering that include relative hydrophobicity\textsuperscript{82} compared to the shorter polyesters such as poly(glycolic acid), a long degradation time (about 2 years\textsuperscript{83}) and “set properties”, i.e. the properties of the material cannot be changed by altering the chemistry since it is a homopolymer. PCL scaffolds made by FDM are typically not highly regular due to some difficult processing characteristics of PCL on FDM such as die-swell.
1.3. Biodegradable Polymers for Tissue Engineering

All of the scaffold fabrication techniques described in section 1.2 can use a linear polymer and many require a linear polymer. The most common polymers found in the literature for tissue-engineering scaffolds are from the linear biodegradable polyester family. The biodegradable polyesters consist mainly of poly(lactic acid), (PLA), poly(glycolic acid), (PGA), and poly(ε-caprolactone), (PCL), with the occasional use of poly(3-hydroxybutyrate), P3HB, and poly(4-hydroxybutyrate), (P4HB). Table 1 and Table 2 give details of the some common linear polyesters, PGA, PCL, PLA being more commonly used, and the others rarely (if ever) used – poly(3-hydroxypropionic acid), P3HP, degrades to 3-hydroxypropionic acid which is toxic, P4HB and poly(5-hydroxy valerate), P5HV, are both difficult to synthesise and P3HB is usually bacterially-derived. In Table 2, PLA is separated as poly(L-lactic acid), (PLLA) and poly(D,L-lactic acid), (DLPLA), to distinguish between the poly-L-enantiomer and the racemate.

![Chemical structure of PGA](image)

Table 1 – Comparison of linear biodegradable polyester properties

<table>
<thead>
<tr>
<th>m</th>
<th>Polyester</th>
<th>Melting Point (ºC)</th>
<th>Tg (ºC)</th>
<th>Tensile Strength (MPa)</th>
<th>Young’s Modulus (MPa)</th>
<th>Elongation at Break %</th>
<th>Properties</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PGA</td>
<td>223-233</td>
<td>46.5</td>
<td>100</td>
<td>6300</td>
<td>1.5</td>
<td>Hard and brittle</td>
<td>84, 85, 86</td>
</tr>
<tr>
<td>2</td>
<td>P3HP</td>
<td>77</td>
<td>-19</td>
<td>103</td>
<td>1590</td>
<td>500-600</td>
<td>Toxic</td>
<td>87, 88, 89, 90, 91</td>
</tr>
<tr>
<td>3</td>
<td>P4HB</td>
<td>53</td>
<td>-51</td>
<td>50</td>
<td>70</td>
<td>1000</td>
<td>Soft &amp; plastic</td>
<td>83, 92</td>
</tr>
<tr>
<td>4</td>
<td>P5HV</td>
<td>57</td>
<td>-55</td>
<td>12.5</td>
<td>570</td>
<td>150-200</td>
<td>Soft, plastic</td>
<td>88, 93</td>
</tr>
<tr>
<td>5</td>
<td>PCL</td>
<td>60</td>
<td>-65</td>
<td>37</td>
<td>216</td>
<td>746</td>
<td>Soft, plastic and hydrophobic</td>
<td>94, 95, 96</td>
</tr>
</tbody>
</table>

Table 2 – Comparison of linear biodegradable polyesters with methyl substituents

<table>
<thead>
<tr>
<th>Polyester</th>
<th>Melting Point (ºC)</th>
<th>Tg (ºC)</th>
<th>Tensile Strength (MPa)</th>
<th>Young’s Modulus (MPa)</th>
<th>Elongation at Break %</th>
<th>Properties</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLPLA</td>
<td>Amorphous</td>
<td>50-53</td>
<td>45</td>
<td>2200</td>
<td>2.6</td>
<td>Very hard</td>
<td>97, 98</td>
</tr>
<tr>
<td>PLLA</td>
<td>175-178</td>
<td>60-65</td>
<td>48</td>
<td>3100</td>
<td>2.8</td>
<td>Very hard</td>
<td>97</td>
</tr>
<tr>
<td>P3HB</td>
<td>178</td>
<td>4</td>
<td>43</td>
<td>3500</td>
<td>5</td>
<td>Small processing window between melting and thermal degradation</td>
<td>99, 100</td>
</tr>
</tbody>
</table>

15
Unfortunately the biodegradable polyester family is limited in terms of breadth of mechanical properties and most of the polyesters currently in use have high glass transition temperatures which cause the polymers to be inflexible at physiological temperatures. PCL is a notable exception in the family of biodegradable polyesters and is the only one in general usage that has a glass transition below ambient temperature. PCL is relatively hydrophobic and slow to degrade making it unsuitable for many applications.

Additionally, almost all of the polyesters suffer the same drawback of being difficult to fabricate into scaffolds with controlled three dimensional networks of interconnected pores which is generally regarded as essential for supporting cell ingrowth.\textsuperscript{101} For example, poly(glycolic acid) (see Figure 7) is insoluble in most solvents, has a high glass transition temperature which causes it to be brittle at ambient and physiological temperatures, and cannot be easily melt-processed since the melting point is $\sim$225°C where thermal degradation begins to occur.

![Figure 7 – Structure of poly(glycolic acid)](https://example.com/figure7.png)

Given these limitations, it would be valuable to have a range of polymers for scaffold fabrication that can be tailored to exhibit desired properties and is suitable for use in thermal processing applications such as FDM. Biodegradable polyurethanes are a class of polymers that have the potential for tailoring to such a purpose since the properties of polyurethanes can be controlled through their chemistry as outlined in the following section.
1.4. Polyurethane Chemistry

Polyurethanes are an exceptionally versatile class of polymers that can be tailored to range from a biostable material\textsuperscript{102} to one that rapidly degrades\textsuperscript{103}. The term ‘polyurethane’ in this thesis includes segmented polyurethanes, which are sometimes described instead as poly(ester-urethanes) or poly(ether-urethanes) depending on the nature of the included substituents. The physical properties of urethanes can be varied from soft thermoplastic elastomers to hard, brittle and highly cross-linked thermosets. There are numerous non-degradable or biostable polyurethanes available such as: Tecoflex®; Tecothane®; Tecophilic®; Tecoplast®; Pellethane®; Cardiothane®; Cardiomat®; Bionate®; Elasthane®; Elast-Eon® and Biospan®. The reason for this diversity of properties is the large number of variables that can be altered in the chemistry by incorporating a wide variety of chemical classes from the easily degraded ester family to the biostable siloxanes. The chemistry of polyurethane polymerisation in its simplest form is the one-step reaction of a diol and a diisocyanate (see Figure 8) however two-step polymerisation is also common where a diol and diisocyanate are reacted to form a prepolymer (a low molecular weight polymer, typically 10,000 Mw or less) before adding a chain extender (a difunctional compound that links two chains, e.g. ethylene glycol) to complete the polymerisation. In cross-linked urethanes a trifunctional (or greater) monomer is employed which causes branching (see Figure 9 and Figure 10).

\[ \text{HO-R-OH} + \text{OCN-R'-NCO} \rightarrow \text{OCN-R-O-R'-OCN} + \text{R-OH} \]

\textbf{Figure 8} – Formation of urethane linkage
By changing the monomers one can control the properties of the resulting polymer. For example polyester macrodiols are commonly used in degradable polyurethanes (sometimes called poly(ester-urethane)) because of their susceptibility to hydrolysis. The term ‘macrodiol’ will be used in this study (interchangeably with ‘soft segment’) and refers to an oligomer terminated by two hydroxyl groups, typically greater than 300Mw. Macrodiols are often also referred to as ‘polyols’ however this nomenclature is ambiguous and has other meanings such as for a compound containing many hydroxyls rather than just two, and hence ‘polyol’ will not be used. The properties of a segmented polyurethane depend upon the nature of the components for example poly(ε-caprolactone) is relatively hydrophobic and degrades more slowly than poly(glycolic acid) and these properties are carried through to some degree to the poly(ester-urethane) copolymer.

While the reaction of diisocyanate and diol encompasses the vast majority of polyurethane syntheses, it is possible to make polyurethanes by other means, such as by the reaction of diamines with cyclic carbonates.104
The corresponding urethane is hydroxy-functionalised which makes it an interesting intermediate for a variety of applications and has been referred to as being a poly(hydroxyurethane). In the case where \( R \) (see Figure 11) contains a second cyclic carbonate and \( R' \) contains a second amine it has been possible to form poly(hydroxyurethane)s with mass average molecular weight up to 24,000.

Phase separation can occur in segmented polyurethanes and the two phases are generally referred to as the ‘hard segment’ composed of the diisocyanate and chain extender, and the ‘soft segment’ that is due to a more amorphous macrodiol. In order to vary the properties of polyurethanes, it is simply a matter of changing the ratio of hard segment to soft segment which can be done by varying the ratio of diisocyanate, polyester macrodiol and chain extender. The number of hydroxyl groups in the starting monomers must equal the number of isocyanate groups in order to achieve a complete polymerisation; too few isocyanates will result in unreacted hydroxyl groups making a sticky polymer of low molecular weight, whereas excess isocyanate causes cross-linking and formation of allophonate linkages (see Figure 12).
The ratio of isocyanate groups in the starting monomers to the total hydroxy groups (soft segment macrodiol and chain extender) is called the isocyanate index. In some cases an excess of isocyanate up to 1.05 is added in order to improve the molecular weight and extent of reaction of the polyurethane. The problem with having an isocyanate index greater than 1.00 is the cross-linking and allophonate linkages that occur due to the excess isocyanate cause gels, making the polymer more difficult or impossible to process by conventional melt-processing such as extrusion and injection moulding. Allophonate linkages are reversible at high temperatures (too high for degradable polyurethanes since thermal degradation will occur) but reform when cooled.

A complication that can occur in polyurethane synthesis is the formation of amide linkages due to the presence of carboxylic acids (see Figure 13). The carboxylic acids are often present when making polyurethanes that contain hydroxy-acid monomers such as lactic acid and this is discussed at length in chapter 3.
The biostability of a polyurethane depends upon the chemical nature of its bonds. K. Gorna and S. Gogolewski (2002) \(^{115}\) identified a general trend: poly(ether urethane urea)s and poly(ether urethane dialkylsiloxane) copolymers are more stable \textit{in vivo} than poly(ether urethane)s and poly(ester urethane)s. Therefore poly(ether urethane)s and poly(ester urethane)s are more suitable for degradable applications than their more stable siloxane and urethane-urea counterparts which are used in biostable materials.

\subsection*{1.4.1. Polyurethane Elastomer Synthesis}

There are two main strategies of using diisocyanates in the synthesis of polyurethanes. One strategy \(^{107,108,109,110}\) involves using the diisocyanate as a major component of the polymer giving rise to the hard segment and the other strategy \(^{111,112,113,114}\) involves the diisocyanate as a minor component simply to link two macrodiols. By the use of two macrodiols with differing properties, such as poly(ethylene glycol), (PEG), and PCL \(^{115}\), one can still achieve phase separation and elastomeric properties after linking the two with a diisocyanate. Polymers that have a high proportion of urethane compared to hydrolysable ester groups are generally relatively slow to degrade due to the stability towards hydrolysis of the urethane linkage. This may be an advantage or disadvantage depending on the intended application. In bulk polymerisation (as opposed to solution polymerisation) the length of the oligomer that can be used is limited in size since the polymerisation must occur in the liquid state. For example, PCL-macrodiol of higher molecular weight than 1000 Daltons is solid at room temperature whereas low molecular weight oligomers, such as the commonly used MW 530, are liquid. Solution polymerisation is one way to get around this problem, where a non-participating solvent is used and removed post-synthesis, however the solvents can be difficult to remove completely. Another common way is simply to heat the reactants into a melt (usually 70°C), which also serves to shorten the reaction time. One of the most critical factors in polyurethane syntheses is to ensure that all the reagents are dry because any water present will cause cross-linking and foaming due to evolution of CO\(_2\)\(_{(g)}\) (see Figure 14).
This is usually achieved by heating the reagents under vacuum to degas the solutions and remove the more volatile water. In some cases foam is desirable and water is purposefully added in controlled quantities to the reactants to achieve this. Such instances include the formation of porous scaffolds for tissue engineering.\textsuperscript{116} The effect of adding water is to form urethane-urea linkages that give rise to quite different properties than are shown by urethane bonds.

The molecular weight of polymers greatly affects properties such as thermal and tensile properties and polyurethanes are no exception. An increase in molecular weight increases both the tensile properties as well as the melting point. For polyurethanes this occurs only up to around MW 50,000-80,000. Increasing the molecular weight above this point generally has a relatively small effect on these properties.
1.4.1.1. One-Step Synthesis

In a one-step bulk polymerisation a diisocyanate is added to a mixture of diols to initiate polymerisation. A catalyst is usually used to speed up the polymerisation and to ensure that high molecular weights are attained.

The polymerisation reaction is exothermic and while temperatures of reactants are often quoted in the range of 50-70°C the actual temperature reached upon gelation is very much higher and can exceed 200°C if care is not taken. This makes scaling-up the reaction challenging since thermal degradation does occur at temperatures greater than 200°C.

One-step syntheses are used less commonly than two-step syntheses since the method does not give as reproducible results.

1.4.1.2. Two-Step Synthesis

Two-step syntheses are more commonly used than one-step syntheses since they typically yield a polymer with a higher and better-controlled polyurethane structure.

There is more than one way to carry out a two-step synthesis but all methods involve making a prepolymer in the first step followed by chain-extension or linking as a second step.

The same reactions take place in a two-step synthesis as in a one-step synthesis – a diol plus a diisocyanate forms a urethane linkage. The difference in this case is that the diisocyanate is typically reacted in excess with the amorphous macrodiol to form an isocyanate terminated prepolymer in the first step followed by chain extension with a low molecular weight diol in the second step.

1.4.2. Diisocyanates Used in Biodegradable Polyurethanes

A major limitation on the type of diisocyanate that can be used in biodegradable polymers for tissue engineering applications is the toxicity of the degradation products. Diisocyanates such as methylene diphenyl diisocyanate (MDI) offer attractive properties and have been used in many types of polyurethane, yet MDI is unsuitable for
biodegradable polymers due to toxicity of its aromatic degradation products, hence researchers have been primarily focussing on the aliphatic diisocyanates. Polyurethanes do not degrade to give the original monomers (since the isocyanate group is highly reactive), but rather degrade via hydrolysis to the corresponding amine, alcohol and CO₂ (see Figure 15).

![Figure 15 – Degradation of a urethane link](image)

The following section describes some of the diisocyanates that have been used in the synthesis of biodegradable polyurethanes and the merits of each.

### 1.4.2.1. Butane Diisocyanate (BDI)

BDI: 1,4-butane diisocyanate) or (1,4-diisocyanatobutane)

BDI is a diisocyanate that is suitable for biodegradable polyurethanes due to the biocompatibility of its degradation product – putrescine (1,4-butanediamine) which is a natural metabolite. BDI is considerably more expensive than other diisocyanates, an order of magnitude more expensive than 1,6-hexamethylene diisocyanate (HDI) and for this reason it is not commonly used.

BDI has been used to make tough biodegradable polymers with tensile strength of 44 MPa, Young’s Modulus of 62 MPa and elongation at break of 560%. These figures show the polymer is strong and quite soft.

The in vitro degradation of BDI-based polyurethane has been investigated and this work has been carried on to in vivo trials in dogs for knee joint meniscus. The soft segment in both cases was a 50/50 copolymer macrodiol of PCL/PLA, MW 2000.

BDI has been used as a monomer in biodegradable poly(ester-urethanes) for cardiovascular applications due to the elastomeric properties it imparts to the
synthesised polyurethanes. Poly(ε-caprolactone) macrodiols MW 1250 and MW 2000 were used as soft segment and chain extension was performed with either lysine ethyl ester or putrescine. This gives a poly(urethane-urea), (See Figure 16).

\[
\text{OCN-R-NC} + \text{HO-R'} + \text{H}_2\text{N-R''} \\
\text{Urea} \quad \rightarrow \quad \text{Urethane}
\]

Figure 16 – Formation of a poly(urethane-urea)

Tensile strength of the synthesised polymers varied between 9.2MPa and 29MPa with elongation at break between 660 to 895%.

1.4.2.2. Hexamethylene Diisocyanate (HDI)

HDI: 1,6-hexamethylene diisocyanate or 1,6-diisocyanatohexane

HDI is the most commonly used diisocyanate in biodegradable polyurethanes. It is relatively inexpensive when compared to BDI or e-LDI (ethyl-lysine diisocyanate, see section 1.4.2.4) and degrades to give 1,6-hexanediame (1,6-hexamethylenediamine) which is generally regarded as being a biocompatible degradation product in tissue engineering applications.\(^{121}\) The amount of polymer used in tissue engineering applications is typically quite small and of high porosity; the diisocyanate makes up only a small proportion of the total and the diamine is released over a substantial period of time.

A toxicity study\(^{121}\) has shown 1,6-hexamethylenediamine to be substantially less toxic than other tested reactants including lactic acid. There are claims in the literature that it is ‘more or less toxic’\(^{122,123,124}\) when compared with amino acid-based isocyanates however these are based on speculation. The LD\(_{50}\) of 1,6-hexanediame is shown later in Table 40 (Appendix, page 164), and is orally ~1g/kg which is not particularly toxic.
1,6-hexamethylenediamine has also been shown to be relatively non-toxic in a bacterial model when compared with a range of other degradation products.\textsuperscript{125}

HDI-based polyurethanes are characteristically very strong and tough when compared to other biodegradable polymers. The hard segment is somewhat akin to the polyamide nylon where there is strong hydrogen bonding between the amine of one chain and the carbonyl of an adjacent chain (see Figure 17).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{hydrogen_bonding.png}
\caption{Hydrogen bonding between polyurethane chains}
\end{figure}

HDI-based thermoplastic polyurethanes display the tendency to cold-draw, which explains their high elongation at break values in conjunction with high tensile strength. Values of tensile strength for HDI-based biodegradable polyurethanes have been quoted as high as 63MPa\textsuperscript{106} with a corresponding elongation at break of 580% which shows it to be extremely tough. In another paper from the same group (Gorna, K. et al.)\textsuperscript{126}, they claim up to a tensile strength of 60 MPa with elongation to 950%. These HDI-based polyurethanes are the strongest of all the aliphatic degradable polyurethanes that have been published. Since these polyurethanes can be cold-drawn, much stronger fibres can be formed by orienting the chains through controlled drawing. However, there has not been much research into cold-drawing of biodegradable polyurethanes. This may be due in part to longer degradation times for oriented materials which is not usually desirable. HDI has been used as a chain linker in a PLA-PEG-PLA tri-block copolymer and implanted into dogs.\textsuperscript{127} While the HDI comprised only a minor part of the polymer it was well tolerated by the host.

P3HB macrodiol 2100-4400Mw and PCL macrodiol 1080-5800Mw were chain extended with HDI to form high molecular weight polymers.\textsuperscript{113} Melting points were all between 126-148°C and the maximum tensile strength of the polymers was 30.5 MPa.
The effect of PCL content in a P(CL/LA) soft segment has been investigated (chain-linking was performed with HDI). Maximum tensile strength was 48 MPa. An excess of HDI was used to chain extend the macrodiols and the authors measured a significant amount of gel (crosslinked polyurethane) up to 18.6%. A similar macrodiol based on poly(LA-co-CL) but with butane diol as chain extender was shown to attain 47MPa. This shows that the diisocyanate does not have a large effect on the physical properties when it is being used for linking chains. The effect is much more pronounced in segmented polyurethanes of the type synthesised in this thesis.

A soft segment series containing HDI with varying ratios of PCL and PEG has been published. The molecular weights of the PCL that were investigated were 530, 1250 and 2000, the molecular weights of PEG were 600 and 2000. The highest tensile strength achieved was 48.6 MPa, however the mechanical properties varied more with molecular weight of the polymer than with composition. Comparison of degradation times was by mass loss – the fastest was about 30 weeks to complete mass loss at 37°C \textit{in vitro}. However, mass loss results can be somewhat deceiving since a low molecular weight hydrophilic polymer can be water soluble and appear to degrade quite quickly compared with a higher molecular weight polymer, hence it is often a function of molecular weight rather than material composition. Degradation time for polyurethanes is proportional to the percentage of hard segment; the higher the hard segment percentage, the longer the degradation time. This is because the urethane bond in the hard segment is less easily hydrolysed than esters in the soft segment. However, polyurethanes containing aliphatic diisocyanates such as HDI have been shown to degrade under composting conditions more quickly than polyurethanes containing aromatic diisocyanates.

1.4.2.3. Isophorone Diisocyanate (IPD)

IPD (Isophorone diisocyanate)
Isophorone diisocyanate has been used\textsuperscript{131,132} for biodegradable tissue engineering applications although it is not widely regarded as being suitable despite being aliphatic. The irregular, non-linear structure does not give very good properties to the resulting polymers compared to the linear diisocyanates such as HDI and BDI. A comparison of HDI and IPD showed that IPD-based polyurethanes did not achieve as high molecular weights as HDI-based polyurethanes.\textsuperscript{106} The authors attributed this to the isocyanates of IPD being less reactive than those of HDI.

1.4.2.4. Lysine Diisocyanate (LDI)

e-LDI (lysine diisocyanate ethyl ester) and m-LDI (lysine diisocyanate methyl ester)

\[
\begin{align*}
\text{e-LDI:} & \quad \text{O} \quad \text{C} = \text{C} - \text{C} - \text{C} - \text{C} - \text{NCO} \\
\text{m-LDI:} & \quad \text{O} \quad \text{C} = \text{C} - \text{C} - \text{C} - \text{C} - \text{NCO}
\end{align*}
\]

There has been an increasing amount of interest in LDI-based urethanes over the past few years due to the bioresorbability of the material. The degradation products of m-LDI are methanol, carbon dioxide and the amino acid lysine. The degradation products of e-LDI are perceived to be somewhat more biocompatible than those of m-LDI considering that ethanol rather than methanol is produced upon degradation but e-LDI has not been commercially available until very recently and hence has been less commonly used.

Polyurethanes made using lysine diisocyanate are more amorphous than polyurethanes made from a linear diisocyanate due to the effect of the methyl/ethyl ester side chain that prevents neatly aligned inter-chain hydrogen-bonding. The resulting polyurethanes are less strong or tough than their HDI or BDI counterparts and are typically quite rubbery rather than plastic. The maximum tensile strength of LDI-based polyurethanes found in the literature is 26 MPa.\textsuperscript{133} In this case phenylalanine was used as a chain extender so rather than polyurethane it was really a polyurethane-urea. Crosslinked polyurethanes containing LDI have shown higher tensile strengths up to 62 MPa which is not surprising.
1.4.2.5. Trimethylhexamethylene diisocyanate (TMDI)

TMDI (2,2,4-trimethylhexamethylene diisocyanate)

This diisocyanate is relatively rare in the literature and gives properties that are comparable to LDI-based polyurethanes – relatively soft and having low tensile strength due to disruption of the hard segment by the methyl substituents. The commercially available degradable scaffolds, DegraPol™ (Swiss Federal Institute of Technology), have often (not always) included TMDI as the diisocyanate in the formulation. The materials lack the strength that HDI and BDI polyurethanes have and are also quite soft materials, for example typical published values are a Young’s modulus of 46MPa, maximum tensile strength of 16MPa, and elongation at break of 1250%.

1.4.3. Chain Extenders Used in Biodegradable Polyurethanes

Chain extenders in thermoplastic polyurethanes are typically low molecular weight diols. In the case of an isocyanate-terminated prepolymer a diol is required for extension to occur but in the case of a hydroxy-terminated prepolymer a diisocyanate is required. The chain extender in either case is typically a low molecular weight difunctional monomer such as ethylene glycol or 1,4-butane diol. For this study the term ‘chain extender’ refers to a low molecular weight diol.

1.4.3.1. Degradable Chain Extenders

There are some degradable chain extenders that have been previously developed which incorporate amino acids. These degradable chain extenders are diamines (see Figure 18 for example).
There are two obvious problems with using this for a chain extender – firstly it is a diamine which will form urethane-urea linkages which hydrolyse less easily than simple urethane bonds, and secondly the large and bulky size (Mw 438) could significantly disrupt the hard segment and this limits the range of properties that can be achieved using it.

1.4.4. Soft Segments Used in Biodegradable Polyurethanes

The soft segment must be greater than monofunctional with respect to groups that are reactive toward isocyanates otherwise termination of chains will occur. Macrodials are used for linear polyurethanes as both ends react with isocyanate to form urethane linkages. For biodegradable polyurethanes for tissue engineering applications it is common to use polyester macrodials as the degradation and toxicity properties are well known. Hydroxy-acid oligomers have also been used but these form urethane-ureas. Degradation in poly(ester-urethane)s typically begins in the soft segment; hence the properties of the soft segment dictate the rate of degradation. There are a number of mechanisms by which poly(ester urethane)s degrade but the single most important is hydrolytic chain scission.\textsuperscript{137} Degradation by hydrolysis depends on the nature of the chemical links and increases with the hydrophilicity of the material. Polyurethanes that incorporate differing ratios of the more hydrophobic ε-caprolactone to the more hydrophilic lactic acid were shown to degrade by hydrolysis faster with higher lactic acid content.\textsuperscript{129} There are a limited number of oligomers that have been used in biodegradable polyurethane block copolymers, and the main ones are detailed in the following sections.
1.4.4.1. Poly(ε-caprolactone) (PCL)

PCL-macrodiols used in biodegradable polyurethane are typically in the order of 530 to 3000 molecular weight although a few papers use higher molecular weights up to 7300. ε-caprolactone is relatively hydrophobic compared with the shorter degradable esters and correspondingly has a longer degradation period and for this reason it is often copolymerised with other esters. Some of the copolymers and blends with ε-caprolactone in the soft segment are: lactic acid polyethylene glycol, glycolic acid, valerolactone and 3-hydroxybutyrate. Advantages of using PCL in polyurethanes are the low glass transition that it imparts, high strength and percentage elongation (commonly 500%-800%), and the ease of macrodiol synthesis from the lactone. Random copolymers of ε-caprolactone (CL) and lactic acid have lower glass transition temperatures with increasing CL content from -2°C in P(LA70/CL30) urethane to -26°C in P(LA50/CL50) urethane and to -45°C in P(LA30/CL70) urethane. This shows that the glass transition temperature can be controlled by chemical composition of the soft segment.

1.4.4.2. Poly(lactic acid) (PLA)

Lactic acid macrodiols are used in biodegradable polyester-urethanes due to their degradable and biocompatible attributes. One problem exists with pure lactic acid-based polyurethanes and this is the glass transition temperature. Hiltunen et al. made a polyurethane with 100% PLA as the soft segment and it displayed a glass transition temperature of 53°C. While this is suitable for some applications it will tend to be hard and brittle which limits its range of uses. Brittle polymers are rarely an advantage for biodegradable applications (bone is the only tissue in the body that has similar hardness), hence it is desirable to have a glass transition temperature below that of physiological temperatures ~37.5°C. Macrodiols of LA/CL of ratio 94:6 show a glass transition of 33°C due to the lower crystallinity of the soft segment and this is relatively plastic at physiological temperatures and contains only a minimum of ε-caprolactone. As the proportion of caprolactone increases, the glass transition temperature decreases.
For this reason lactic acid is usually used in co-oligomers rather than homo-oligomers.\textsuperscript{111,129,143,144,145}

It should also be mentioned that the properties of lactic acid-based soft segments depend upon the tacticity. L-lactic acid is the natural form and polymers formed purely of L-lactic acid are highly crystalline and have been said to degrade in as much as 5 years, whereas the DL-Lactic degrades in a much shorter timeframe.

### 1.4.4.3. Poly(glycolic acid) (PGA)

PGA is commonly used as a biodegradable material in medical applications, however PGA-based urethanes are not as common as PLA or PCL based urethanes. The reason for this is that glycolic acid macrodiols are not as easy to synthesise as ε-caprolactone and lactic acid macrodiols due to PGA having a much higher melting point (>200$^\circ$). Oligomers of glycolic acid of greater than $\sim$300MW are solid at room temperature which makes it difficult to carry out useful syntheses since most syntheses require macrodiols of greater than 500MW. Glycolic acid macrodiols are particularly difficult to work with since they are also insoluble in most solvents, the exception being highly fluorinated solvents such as hexafluoroisopropanol (HFIP). Unfortunately HFIP is not suitable to use in the synthesis of these macrodiols since it causes chain termination via esterification with carboxylic acids.

For biodegradable polyurethanes it would be advantageous to use GA macrodiols since the corresponding polyurethane would be expected to exhibit greater hydrophilicity and degradability as is shown in the corresponding PGA homopolymer, however there does not appear to be any made to date in the literature. There are copolymers that contain glycolic acid moieties but not the homo-oligomer.

Crosslinked polyurethanes have been made that contain glycolic acid in a copolymer however these are not thermoplastics and hence cannot be melt processed.\textsuperscript{122} The diisocyanate used in this case was eLDI which resulted in an elastic material.
1.4.4.4. Poly(hydroxybutyrate), (P3HB and P4HB)

Poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) have been used in biodegradable polyurethanes\textsuperscript{113,142} however there are not currently any biodegradable polyurethanes made from the poly(4-hydroxybutyrate) homopolymer. Chain linking of P(GA-co-CL) macrodiols with P(3HB-co-4HB) has resulted in degradable elastomers that have been marketed under the trade name DegraPol\textsuperscript{TM,135} There are a number of other blends and copolymers that are marketed under the same name and they all appear to involve chain-linking with a diisocyanate. P3HB and P(3HB-co-4HB) are commercially available polyhydroxyalkanoates (for example, BioPol\textsuperscript{TM} which is owned by Metabolix, Inc.) and can be used as precursors to make macrodiols for poly(ester-urethanes) via transesterification.

1.4.4.5. Poly(ethylene glycol) (PEG)

Poly(ethylene glycol) has been used extensively in polyurethane chemistry due to the availability of macrodiols with a wide range of controlled molecular weights. While the ether linkage is not particularly degradable, PEG is water-soluble and hence able to be excreted from the body up to certain molecular weights. PEG is very hydrophilic and this is desirable in degradable polyurethanes; the more water in contact with the polyurethane the better since the major degradation pathway is hydrolysis. Thermoplastic polyurethanes containing PEG are typically strong and have a high percentage elongation at break.

1.4.5. Catalysts Used in Biodegradable Polyurethanes

There are two competing considerations when considering catalysts – one is suitability in terms of efficacy of catalysis and the other is suitability in terms of non-toxicity. The most widely used catalysts are stannous compounds; stannous octoate and dibutyl tin dilaurate are common examples that are effective, but also have a degree of toxicity. Stannous octoate and dibutyl tin dilaurate have both been shown to inhibit cell growth more than some alternative catalysts such as 1,4-diazo(2,2,2)bicyclo-octane (DABCO) and tetramethylbutanediamine (TMBDA).\textsuperscript{146} The LD\textsubscript{50} (oral, rat) for DABCO and
TMBDA is 1700 and 750 mg/kg respectively, whereas stannous octoate and dibutyl tin dilaurate are 90.7 and 175 mg/kg showing them to be relatively more toxic. The amount of catalyst used is typically in the range of 0.1% to 0.01% by mass of the polyurethane. The weight of a biodegradable medical implant is not expected to exceed a few grams of polymer, and only 0.1-1mg of catalyst is used per gram of polymer. It is also expected that this would leach out over a much longer period of time than an oral dose. Hence a lethal dose of polymer due to catalyst in a normal 80kg man (assuming the same LD$_{50}$ as for an oral dose) would be $\sim$7.2-72kg of polymer implant which is orders of magnitude greater than would be considered for use.

G. Wegener et al. has comprehensively described the current state of catalysis in the manufacture of polyurethanes.

Among the catalysts that have been used, the most effective are ferric acetyl acetonate, dibutyl tin dilaurate and zinc octoate, next most effective are stannous octoate and manganese 2-ethyl hexanoate, and least effective is magnesium methoxide.
1.5. Macrodial Synthesis

1.5.1. Introduction

The chemistry of segmented polyurethanes depends heavily upon the macrodiols and diol chain extenders which usually make up more than half the weight of the polyurethane. For example, polyurethane made from the soft and slow-degrading PCL macrodiol should logically be expected to exhibit different properties to one made from the much harder and faster-degrading PGA macrodiol.

Poly(ε-caprolactone) macrodiol and poly(ethylene glycol) macrodiol are available commercially but there is not currently a commercial source for the other macrodiols that would be useful for poly(ester-urethane) syntheses such as macrodiols of PLA and PGA. Much of the literature in the past has dealt with polyurethane-amides which are formed when a diisocyanate is reacted with a hydroxy-acid, for example with a lactic acid (hydroxy-acid) oligomer. This isn’t an ideal situation since the amide bond is less degradable than the urethane bond and the presence of higher molecular weight amides may lead to problems in vivo. In order to avoid this problem, macrodiols must be synthesised with a low percentage of carboxylic acid functionalised end groups. This can be achieved through reaction with a low molecular weight diol such as ethylene glycol to give a macrodiol product (see Figure 19).

![Figure 19 – Ring opening polymerisation of ε-caprolactone with ethylene glycol initiator](image)

\[
\text{H-O-} + 2n \overset{\text{2n}}{\text{O}} \rightarrow \text{H-[O-} + \text{O-} + \text{O-} + \text{O-} + \text{H-}}
\]
The macrodiols have very different properties to each other and hence require different methods of synthesis. For example, poly(γ-butyrolactone) cannot be made by condensation polymerisation unlike the other hydroxy-acids due to thermodynamic reasons and must instead be made by ring-opening polymerisation. Poly(glycolic acid) melts in the order of 225°C where it also undergoes thermal degradation, whereas poly(ε-caprolactone) melts around only 60°C making it a much easier prospect to synthesise in the melt.

The most common way of synthesising controlled molecular weight macrodiols is to use glycolide (1,4-dioxane-2,5-dione) or lactide (3,6-dimethyl-1,4-dioxane-2,5-dione) rather than glycolic acid and lactic acid. This eliminates the difficult task of water removal during condensation since no water is formed during the ring-opening reaction.

The ring-opening of glycolide and lactide is the more common method of synthesis but the cost can be prohibitive when compared to their respective acids (see Table 3).

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Source</th>
<th>AUD$ per kilogram (Approx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolide</td>
<td>Purac</td>
<td>1,034</td>
</tr>
<tr>
<td>Glycolic Acid</td>
<td>Sigma-Aldrich</td>
<td>200</td>
</tr>
<tr>
<td>Lactide</td>
<td>Sigma-Aldrich</td>
<td>2,405</td>
</tr>
<tr>
<td>Lactic Acid (90% in water)</td>
<td>Sigma-Aldrich</td>
<td>46</td>
</tr>
</tbody>
</table>

*Table 3 – Approximate price comparison of the cyclic and linear forms of the α-hydroxy acids*

The following sections detail some of the prior art in the area of macrodiol synthesis.

**1.5.2. Poly(glycolic acid)**

There are not currently any published references where PGA has been used alone in a polyurethane, however it has been used in a copolymer with lactic acid (PLGA).

The most likely reason for this is that polyurethanes are typically synthesised from macrodiols in the range of 500-2,000 Daltons and PGA in this range is solid up to about 200°C. The high molecular weight homopolymer melts ~ 225-230°C.

One might suggest a solution polymerisation as a means to overcome this but as has been previously mentioned PGA dissolves only in hexafluoroisopropanol which is not a good solvent for urethane polymerisations.
Synthesis of the poly(glycolic acid) macrodiol by condensation polymerisation is difficult due to the high melting point and it has not been reported to date. It is easier to simply synthesise the hydroxy-acid oligomer rather than the macrodiol although control of molecular weight is much more difficult without the diol (ethylene glycol) present.

1.5.3. Poly(lactic acid)

Poly(lactic acid) macrodiol is an easier material to make than poly(glycolic acid) macrodiol since it is soluble in a number of solvents and it is has a lower melting point, only 175-178°C compared with 225-230°C for poly(glycolic acid). There has been a large volume of research over the past decade into trying to make high molecular weight PLA by polycondensation and there have been a number of different approaches that have been tried with varying degrees of success. These include direct melt polycondensation, melt/solid polycondensation, melt/solid polycondensation, chain extension, microwave mediated synthesis, solution polymerisation combined with azeotropic distillation, and enzymatic. The highest molecular weights attained for direct polycondensation of lactic acid have been by solution polymerisation with azeotropic distillation through molecular sieves to remove water and molecular weights of 300,000 have been obtained by this method. However, this is not what is needed for polyurethane synthesis. What is needed is a low-molecular weight macrodiol with a low acid number and fully hydroxy-functionalised.

1.5.4. Poly(lactic acid-co-glycolic acid)

Copolymers of lactic acid and glycolic acid tend to display intermediate properties between those of the two homopolymers. There have also been many claims in the literature of increased degradation rates for the copolymers due to a less crystalline structure. This makes PLGA macrodiols attractive for situations where rapid degradation is desired.
1.5.5. Poly(γ-butyrolactone)

Synthesis of poly(γ-butyrolactone), (Pγ-BL), has been little reported to the present and when it has been reported it has usually been said to be unpolymerisable. For this reason, a detailed literature review was written by the author of this thesis and has been attached as an appendix (T.Moore et al.). It details the history of poly(γ-butyrolactone) synthesis as well as other information such as biocompatibility. As a result, this section contains only a brief summary.

For a long time γ-butyrolactone was thought unpolymerisable, however quite recently (1999), γ-butyrolactone has been shown to undergo ring-opening polymerisation under relatively benign conditions to give useful macrodiols of poly(γ-butyrolactone), (see Figure 20). These macrodiols are of low molecular weight and while they are of little value in their own right, they are extremely valuable for polyurethane syntheses.

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\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{OH} \\
\text{H} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
n & \quad \text{H}
\end{align*}
\]

Figure 20 – Polymerisation of γ-butyrolactone with ethylene glycol to PγBL

Poly(γ-butyrolactone) is a valuable soft segment for use in biodegradable polyurethanes since it is quite similar to poly(ε-caprolactone) and has been reported to have similar physical properties such as a low glass transition temperature and yet is stronger and faster degrading.

A major disadvantage of PCL is the slow rate of hydrolysis and fortunately Pγ-BL degrades more quickly. This places Pγ-BL, in a unique and highly desirable position in the degradable polyester family where it lies halfway between poly(glycolic acid) which is hard and brittle, and poly(ε-caprolactone).
The final degradation products of poly(γ-butyrolactone) are γ-butyrolactone and 4-hydroxybutyrate which are a naturally-occurring metabolites found extensively in the mammalian body and have been shown to be rapidly eliminated from the body and non-toxic. High concentrations have been shown to induce pharmacological effects\textsuperscript{161} but these concentrations are well above the amounts expected from polymer degradation.

1.6. Experimental Outline and Objectives of the Study

The overall objective of this study is to create a range of biodegradable polyurethanes for tissue engineering that can be tailored to suit different requirements such as degradation rates and mechanical properties. In order to create such a range of biodegradable polyurethanes there are a number of factors that could be systematically investigated to provide a better understanding as well as a broader range of these polymers than has been previously available. The general factors that were investigated were split into separate chapters as follows:

- **Chapter 2** – Choice of diisocyanate, soft segment length and hard segment percentage
- **Chapter 3** – Effect of changing the chemical composition of the soft segment (including synthesis of the soft segment)
- **Chapter 4** – Effect of introducing degradable chain extenders into the hard segment (including synthesis of the chain extenders)
- **Chapter 5** – Cell growth and \textit{in vitro} degradation experiments
- **Chapter 6** – Scaffold fabrication

These are broad summaries of the chapters in this thesis but it can be seen that they take the polyurethanes ‘from cradle to grave’. In other words, this thesis investigates the synthesis of components that are used in the polymers, the synthesis of the polyurethanes, the properties of the synthesized polymers, the use of the polymers (scaffold fabrication and cell growth) and the degradation of the polymers. It was considered important in the planning of this experimental work that all of these aspects should be considered. The reasoning was that if the materials were not biocompatible and biodegradable then the aim of this work would not have been achieved since the
materials could not be used for tissue engineering. Furthermore, if the materials were unsuitable for scaffold fabrication then the biocompatibility and biodegradability would be irrelevant since not only the composition but also the architecture of the polymer is very important for successful tissue engineering.

The order of the work in chapters 2, 3 and 4 is a logical progression of polyurethane synthesis building upon what was learnt in each preceding chapter and chapters 5 and 6 involve the use of the finished polymers in scaffold fabrication, cell growth and degradation studies.
2. Effect of Diisocyanate and Hard Segment Percentage

2.1. Methods

2.1.1. Synthesis of Polyurethanes from Series 1

In order to compare the effect of mLDI and HDI and the effect of different lengths of soft segment, four polyurethanes were made by the following method:

Materials: PCL macrodiol (molecular weight 500) from ERA Polymer Pty Ltd was dried at 90°C for 4 hours under vacuum (0.1 torr). Ethylene glycol (Aldrich) was degassed at 90°C under vacuum (0.1 torr) for three hours and m-LDI and HDI (Aldrich) were distilled before use. Stannous octoate (Aldrich) was kept moisture-free and used as received.

A mixture of PCL macrodiol and EG and dibutyl tin dilaurate was placed in a 100 ml predried polypropylene beaker, covered with aluminium foil and heated to 70°C under nitrogen in a laboratory oven. The diisocyanate was weighed in a separate wet-tared predried polypropylene beaker and added to the PCL/EG/dibutyl tin dilaurate beaker and stirred manually until gelation occurred (3 ½ to 6 minutes), at which time the viscous mixture was poured onto a Teflon coated metal tray to cure at 100°C for a period of about 10 hours. The HDI-based polyurethanes were white and tough, the LDI-based polyurethanes were slightly yellowed, clear and rubbery.

The quantities of ingredients used in the four polyurethanes are given in Table 4. All contained 0.01% dibutyl tin dilaurate as catalyst, an isocyanate index of 1.03, 50% hard segment, and were 100g total mass.
<table>
<thead>
<tr>
<th>Code</th>
<th>% Hard Segment</th>
<th>Diisocyanate mass (g)</th>
<th>Diisocyanate moles</th>
<th>PCL macrodiol</th>
<th>Macrodiol moles</th>
<th>EG (g)</th>
<th>EG moles</th>
<th>Gel time</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM1-1</td>
<td>50</td>
<td>43.78, mLDI</td>
<td>0.2063</td>
<td>50g 500Mw</td>
<td>0.0000</td>
<td>6.22</td>
<td>0.1002</td>
<td>4:00 min</td>
</tr>
<tr>
<td>TM1-2</td>
<td>50</td>
<td>41.36, mLDI</td>
<td>0.1949</td>
<td>50g 1000Mw</td>
<td>0.0500</td>
<td>8.64</td>
<td>0.1391</td>
<td>3:30 min</td>
</tr>
<tr>
<td>TM1-3</td>
<td>50</td>
<td>41.38, HDI</td>
<td>0.2460</td>
<td>50g 500Mw</td>
<td>0.1000</td>
<td>8.62</td>
<td>0.1388</td>
<td>3:30 min</td>
</tr>
<tr>
<td>TM1-4</td>
<td>50</td>
<td>39.10, HDI</td>
<td>0.2325</td>
<td>50g 1000Mw</td>
<td>0.0500</td>
<td>10.90</td>
<td>0.1755</td>
<td>6:00 min</td>
</tr>
</tbody>
</table>

Table 4 – Composition of polyurethanes from Series 1

### 2.1.2. Synthesis of Polyurethanes from Series 2

In order to investigate the effect of hard segment percentage a series of polymers were made using HDI, PCL macrodiol and EG with varying percentage of hard segment:

Materials: Poly(ε-caprolactone) macrodiol soft segment (molecular weight 402.1 from ERA Polymer Pty Ltd) was dried at 90°C for 4 hours under vacuum (0.1 torr). Ethylene glycol (Aldrich) was degassed at 90°C under vacuum (0.1 torr) for 4 hours and the diisocyanate HDI (Aldrich) was distilled before use (colourless). Stannous octoate (Aldrich) was kept moisture-free and used as received.

Method: A mixture of soft segment macrodiol, EG and stannous octoate were weighed into a 100 ml predried polypropylene beaker, covered with aluminium foil and heated to 70°C under nitrogen in a laboratory oven. The diisocyanate was weighed in a separate wet-tared predried polypropylene beaker and also heated to 70°C. The diisocyanate was then added to the macrodiol/EG/stannous octoate beaker and stirred manually until gelation occurred (~1 min to 5 min), at which time the hot viscous mixture was poured onto a Teflon coated metal tray to cure at 100°C for a period of about 12 hours. The resulting polymers were generally clear and colourless.

The compositions of the ten polyurethanes from Series 2 are shown in Table 5.
<table>
<thead>
<tr>
<th>Code</th>
<th>% Hard Segment</th>
<th>HDI mass (g)</th>
<th>HDI moles</th>
<th>PCL402.1 macrodiol (g)</th>
<th>PCL402.1 moles</th>
<th>EG (g)</th>
<th>EG moles</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM2-1</td>
<td>29.5</td>
<td>8.3661</td>
<td>0.0497</td>
<td>20.0000</td>
<td>0.0497</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>TM2-2</td>
<td>35</td>
<td>10.1215</td>
<td>0.0602</td>
<td>20.0000</td>
<td>0.0497</td>
<td>0.6477</td>
<td>0.0104</td>
</tr>
<tr>
<td>TM2-3</td>
<td>45</td>
<td>12.0767</td>
<td>0.0718</td>
<td>17.0000</td>
<td>0.0423</td>
<td>1.8324</td>
<td>0.0295</td>
</tr>
<tr>
<td>TM2-4</td>
<td>50</td>
<td>42.1601</td>
<td>0.2507</td>
<td>50.0000</td>
<td>0.1243</td>
<td>7.8399</td>
<td>0.1263</td>
</tr>
<tr>
<td>TM2-5</td>
<td>55</td>
<td>14.0773</td>
<td>0.0837</td>
<td>14.0000</td>
<td>0.0348</td>
<td>3.0338</td>
<td>0.0489</td>
</tr>
<tr>
<td>TM2-6</td>
<td>60</td>
<td>48.3370</td>
<td>0.2874</td>
<td>40.0000</td>
<td>0.0995</td>
<td>11.6630</td>
<td>0.1879</td>
</tr>
<tr>
<td>TM2-7</td>
<td>65</td>
<td>36.7325</td>
<td>0.2184</td>
<td>25.0000</td>
<td>0.0622</td>
<td>9.6961</td>
<td>0.1562</td>
</tr>
<tr>
<td>TM2-8</td>
<td>70</td>
<td>38.1598</td>
<td>0.2269</td>
<td>21.0000</td>
<td>0.0522</td>
<td>10.8402</td>
<td>0.1746</td>
</tr>
<tr>
<td>TM2-9</td>
<td>75</td>
<td>23.0410</td>
<td>0.1370</td>
<td>10.0000</td>
<td>0.0249</td>
<td>6.9590</td>
<td>0.1121</td>
</tr>
<tr>
<td>TM2-10</td>
<td>80</td>
<td>24.2763</td>
<td>0.1443</td>
<td>8.0000</td>
<td>0.0199</td>
<td>7.7237</td>
<td>0.1244</td>
</tr>
</tbody>
</table>

Table 5 – Composition of polyurethanes from Series 2

### 2.1.3. Compression Moulding

Compression moulding was performed on the newly synthesised polyurethanes on a hydraulic press with a thermostat and water-cooling capability. The polyurethane was chopped up into small pieces using clean tin snips and pressed into a 1mm thick plaque at a temperature above the melting point of the polymer (typically ~175°C). The temperature was held constant for a period of 5 minutes before cooling in a standard manner under the flow of cold water. A standard mould was used which consisted of a rectangular cavity 100mm x 60mm x 1mm deep cut into a metal plate. Teflon fabric sheet was used on both sides of the mould to prevent adhesion of the polymer to the metal. Frekote® 700-NC releasing interface, (The Dexter Corporation, New Hampshire, USA) was used to prevent the overflow from the mould sticking the metal plates together. The press used was a model 12-10-1T, Wabash hydraulic press.

The polyurethanes were not subjected to annealing at increased temperature in order to avoid unnecessary degradation of the material.

### 2.1.4. Instron Tensile Tests

The compression-moulded plaques were cut into dumbbell shaped specimens with a straight section of 4mm x 12.6mm x 1mm (see Figure 21) and tensile testing was performed on an Instron model 4468 universal testing machine.
The non-standard size of the specimens was due to two reasons, firstly the very high elongation of some of these materials (often over 1000%) and secondly to decrease the amount of polymer required for testing. At least five replicate measurements were taken and averaged and the samples were run after at least one week following compression moulded. A 1kN load cell was used with a crosshead speed of 50mm/min. Strong pneumatic grips were used to prevent slippage. Ambient room temperature was 22°C.

2.1.5. Differential Scanning Calorimetry (DSC)

DSC was conducted on the polymers synthesised for this study typically from -60°C to 210°C with a heating rate of 10°C per minute on a Mettler DSC30. DSC samples were cut from compression moulded sheet and tested at least a week after being moulded. DSC was also conducted on the macrodiols and dimers synthesised for this study from 213K (-60°C) to an appropriate upper temperature for the diol in order to ensure inclusion of the melting endotherm (typically from -60°C to 100°C).

2.1.6. Gel Permeation Chromatography

Two GPC’s were used in the course of this work – one a Waters 150C instrument with tetrahydrofuran (THF) solvent with refractive index detector, and the other a Waters 590 equipped with a refractive index detector (a Waters 410 Differential Refractometer), which ran with dimethyl formamide (DMF) solvent (containing LiBr, 0.05M). Calibration was routinely performed with polystyrene standards for both GPC’s.
The Waters 590 was used for determining the molecular weight of all of the polyurethanes since they dissolve in DMF and not appreciably in THF, and the Waters 150C was used for all other analyses (the synthesised macrodiols and the degradable chain extenders).

### 2.1.7. Shore Hardness Indentor

Shore hardness was measured with calibrated Shore A and Shore D Hardness Indentors on a stack of compression moulded sheet at least 10mm thick on a flat surface and taking the average of five measurements. Ambient temperature was 22ºC. The Shore A hardness scale is used to measure softer materials and Shore D is suitable for harder materials.
2.2. Results and Discussion

The properties of a segmented polyurethane can be tailored simply by altering the percentage of hard segment. Properties such as strength are largely dictated by the strength of the hard segment and this in turn relies principally on the diisocyanate. For this reason a series of four polyurethanes was made with variation of only two factors, namely the diisocyanate and the molecular weight of the macrodiol soft segment (See Table 6). For convenience this series shall hereafter be named ‘Series 1’. Note that the polymers have been named so that the first number designates which series it is from, for example TM1-2 is from series 1, and TM3-1 is from series 3.

<table>
<thead>
<tr>
<th>Code</th>
<th>Hard Segment %</th>
<th>MW of PCL macrodiol</th>
<th>Diisocyanate</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM1-1</td>
<td>50</td>
<td>500</td>
<td>m-LDI</td>
</tr>
<tr>
<td>TM1-2</td>
<td>50</td>
<td>1000</td>
<td>m-LDI</td>
</tr>
<tr>
<td>TM1-3</td>
<td>50</td>
<td>500</td>
<td>HDI</td>
</tr>
<tr>
<td>TM1-4</td>
<td>50</td>
<td>1000</td>
<td>HDI</td>
</tr>
</tbody>
</table>

Table 6 – Series 1: Composition of polyurethanes varying diisocyanate and soft segment length

The four materials were formulated to all have 50% hard segment so that the effect of the different diisocyanate and the molecular weight of the macrodiol could be compared.

<table>
<thead>
<tr>
<th>Code</th>
<th>Elongation at Break %</th>
<th>Young’s Modulus (MPa)</th>
<th>Upper Tensile Strength (MPa)</th>
<th>Hardness (Shore D)</th>
<th>Hardness (Shore A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM1-1</td>
<td>1850 ± 128</td>
<td>1.9 ± 0.3</td>
<td>4.1 ± 0.6</td>
<td>14</td>
<td>53</td>
</tr>
<tr>
<td>TM1-2</td>
<td>1135 ± 191</td>
<td>2.8 ± 0.2</td>
<td>11 ± 1</td>
<td>16</td>
<td>55</td>
</tr>
<tr>
<td>TM1-3</td>
<td>708 ± 160</td>
<td>58 ± 6</td>
<td>12 ± 2</td>
<td>41</td>
<td>96</td>
</tr>
<tr>
<td>TM1-4</td>
<td>960 ± 207</td>
<td>50 ± 5</td>
<td>25 ± 7</td>
<td>45</td>
<td>96</td>
</tr>
</tbody>
</table>

Table 7 – Series 1: Physical properties of polyurethanes varying diisocyanate and soft segment length

An isocyanate index (ratio of NCO to OH) of 1.03 was used for the polyurethanes in Series 1 in order to ensure a more complete reaction and to ensure that a high enough molecular weight was achieved so that the properties of the materials could be evaluated without molecular weight limiting the properties. An isocyanate index of greater than 1.00 results in allophonate crosslinking due to the excess isocyanate reacting with the
urethane linkage. This may render materials less suitable for thermal processing due to excessive gels. Despite allophonate linkages being thermally reversible, the high temperature required to achieve this may cause thermal degradation of the polymer. For the purpose of comparing the effect of diisocyanates and diols on the physical properties it is better to have some gels than to attain insufficient molecular weight.

<table>
<thead>
<tr>
<th>Name</th>
<th>Mn</th>
<th>Mw</th>
<th>MP</th>
<th>Polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM1-1</td>
<td>131,507</td>
<td>234,667</td>
<td>182,242</td>
<td>1.78</td>
</tr>
<tr>
<td>TM1-2</td>
<td>234,314</td>
<td>592,767</td>
<td>-</td>
<td>2.53</td>
</tr>
<tr>
<td>TM1-3</td>
<td>28,994</td>
<td>46,562</td>
<td>49,705</td>
<td>1.61</td>
</tr>
<tr>
<td>TM1-4</td>
<td>132,597</td>
<td>374,591</td>
<td>379,906</td>
<td>2.83</td>
</tr>
</tbody>
</table>

*Table 8 – Series 1: Molecular weights of polymers varying diisocyanate and soft segment length*

It was found that TM1-1 and TM1-2 (the two polyurethanes made using m-LDI) were both yellow after they were synthesised but gradually became colourless over the period of a few months. The polymers were stored in air-tight sealed plastic bags hence it is assumed to be a chemical change. It was noted that the yellow colour occurred when the m-LDI urethanes experienced an excessive reaction exotherm upon curing (the exotherm can be great enough to melt polypropylene beakers). The two HDI-based polyurethanes from series 1 (TM1-3 and TM1-4) were both opaque and white in colour when they were synthesised and became clear and colourless when melt-pressed. Both of the LDI-based polyurethanes were very soft and elastic (see Figure 22) compared with the harder HDI-based materials which had only a very short elastic region (see Figure 24).
Figure 22 – Stress-strain curve for TM1-2

The stress-strain tensile curve for TM1-2 shows a typical elastomer and upon failure at 1400% the material contracted back to its original length and there was no drawing of the material. On the other hand the HDI-based polyurethanes TM1-3 and TM1-4 (see Figure 24) exhibited plastic deformation by way of cold-drawing. Before the point labelled “A” in Figure 24 the tensile specimen appears unchanged and stretches only a little. At point “A” the sample ‘fails’ and cold-drawing begins (the sample is ‘necking’ which refers to the regular narrowing of the specimen) and continues to approximately point “B” where the sample has finished drawing. After point “B” the fully drawn sample becomes increasingly difficult to elongate (since necking is complete) until final failure at point “C” where breakage occurs. The points “A”, “B” and “C” are labelled on Figure 23, which shows a schematic representation of the cold-drawing effect, as well as on Figure 24 which shows a typical tensile stress-strain curve for TM1-3. Note that in Figure 23 that point “A” actually occurs between the first and second sample whereas “B” occurs at the point when the sample is fully drawn and “C” when it breaks.
For certain tissue-engineering applications it is important to have high tensile values at point “A” since inelastic deformation may cause catastrophic failure in a device, for example in the leaflets of a heart valve.

Figure 23 – Diagram of tensile failure showing characteristic cold-drawing of TM1-3

Figure 24 – Stress-strain curve for TM1-3
A comparison of TM1-1 and TM1-2 in Figure 25 shows the materials to be quite similar except that TM1-1 has considerably lower tensile strength. Both curves show soft elastic materials and near-identical elongation at break values.
TM1-3 and TM1-4 display very similar yield points to each other (~11MPa), however TM1-4 displays superior physical properties such as upper tensile strength, elongation at break and toughness. The difference in physical properties could be due in part to the difference in molecular weight between the two materials (see Table 8).

From the properties of the four polyurethanes in series 1, three general observations were made:

- m-LDI based urethanes are very much softer and elastomeric than HDI based urethanes at the same hard segment ratio. This is due to the less ordered hard segment domains seen in m-LDI-based polyurethanes which results in a relatively more amorphous material than HDI-based polyurethanes.
- The higher the molecular weight of the soft segment chain, the stronger the material was (with the same ratio of hard segment to soft segment).
- There was no obvious correlation between Young’s modulus and the molecular weight of the soft segment.

Since one of the objectives of this study was to formulate a range of polyurethanes that display a range of physical properties it was decided to formulate a series based on HDI and a lower molecular weight PCL soft segment macrodiol and to vary the ratio of hard to soft segment in order to determine the effect since HDI-based polyurethanes were shown to be stronger: 4.1MPa and 11MPa tensile strength for m-LDI polyurethanes compared directly with 12MPa and 25Mpa for equivalent HDI polyurethanes (see Table 7, page 46). The reason for choosing a short soft segment was for degradation reasons i.e. to ensure that the length of the hard segment was kept to a minimum. For example, in a 50% hard segment polyurethane the average length of the hard segment is equal to the average length of the soft segment, hence using PCL-1000 will result in an average hard segment length of 1000Mw and PCL-400 will result in an average hard segment length of 400Mw. Hence, the soft segment macrodiol chosen for series 2 was PCL-402 (PCL macrodiol of 402 molecular weight).
<table>
<thead>
<tr>
<th>Name</th>
<th>Hard Segment %</th>
<th>Elongation at Break %</th>
<th>Young’s Modulus (MPa)</th>
<th>Upper Tensile Strength (MPa)</th>
<th>Hardness (Shore D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM2-1</td>
<td>29.5</td>
<td>1530 ± 166</td>
<td>94 ± 7</td>
<td>34 ± 3</td>
<td>45</td>
</tr>
<tr>
<td>TM2-2</td>
<td>35</td>
<td>34 ± 5</td>
<td>77 ± 4</td>
<td>12 ± 0.5</td>
<td>39</td>
</tr>
<tr>
<td>TM2-3</td>
<td>45</td>
<td>1738 ± 108</td>
<td>85 ± 4</td>
<td>36 ± 2</td>
<td>37</td>
</tr>
<tr>
<td>TM2-4</td>
<td>50</td>
<td>1253 ± 142</td>
<td>133 ± 6</td>
<td>50 ± 4</td>
<td>49</td>
</tr>
<tr>
<td>TM2-5</td>
<td>55</td>
<td>1465 ± 202</td>
<td>84 ± 4</td>
<td>37 ± 3</td>
<td>47</td>
</tr>
<tr>
<td>TM2-6</td>
<td>60</td>
<td>899 ± 189</td>
<td>103 ± 5</td>
<td>41 ± 1</td>
<td>44</td>
</tr>
<tr>
<td>TM2-7</td>
<td>65</td>
<td>1300 ± 42</td>
<td>112 ± 3</td>
<td>54 ± 5</td>
<td>52</td>
</tr>
<tr>
<td>TM2-8</td>
<td>70</td>
<td>1537 ± 141</td>
<td>142 ± 7</td>
<td>56 ± 6</td>
<td>57</td>
</tr>
<tr>
<td>TM2-9</td>
<td>75</td>
<td>1191 ± 57</td>
<td>288 ± 10</td>
<td>72 ± 1</td>
<td>60</td>
</tr>
<tr>
<td>TM2-10</td>
<td>80</td>
<td>621 ± 117</td>
<td>245 ± 11</td>
<td>30 ± 4</td>
<td>62</td>
</tr>
</tbody>
</table>

Table 9 – Series 2: Varying the ratio of HDI hard segment to soft segment – mechanical properties

It can be seen in series 2, (Table 9), that by increasing the percentage hard segment, there is a corresponding general increase in the hardness and Young’s modulus. There is also a general increase in the value of the upper tensile strength and although high strength is generally desirable, weaker materials are not necessarily unsuitable. It can be seen that in Table 9 that TM2-2 does not display properties that one would expect and does not appear to fit the trend. This is because of it having a significantly lower molecular weight than the other polymers in the series (see Table 11, page 55). It is believed that this was due to the prepolymer cooling significantly during stirring and phase separation occurring during the curing since the polymer was opaque and white.

The polyurethanes with the lower hard segment content took a longer period of time to react and displayed a less intense exotherm.

The polyurethane TM2-9 (in Table 9) had a very high tensile strength and elongation at break, which shows it to be an exceptionally tough material (toughness is the area under the stress-strain curve) with properties comparable to unoriented nylon (see Figure 27). The highest tensile strength of any HDI-based polyurethane found in the literature was 63MPa\textsuperscript{106}, which shows TM2-9 to have extraordinary strength in combination with high elongation which makes it a remarkably tough polymer.
Once the samples had been stretched to failure on the Instron, the oriented (longitudinally aligned polymer chains) samples were remeasured in their elongated form and retested to determine the strength of the oriented polymer (see Figure 28 and
Table 10). As was expected, there was a large increase in both the Young’s modulus and the upper tensile strength of the polymer in its oriented form.

<table>
<thead>
<tr>
<th>Name</th>
<th>Hard Segment %</th>
<th>Elongation at Break %</th>
<th>Young’s Modulus (MPa)</th>
<th>Upper Tensile Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM2-9-drawn (average)</td>
<td>75</td>
<td>169 ± 47</td>
<td>738 ± 246</td>
<td>251 ± 56</td>
</tr>
<tr>
<td>TM2-9-drawn (maximum)</td>
<td>75</td>
<td>198</td>
<td>1,024</td>
<td>331</td>
</tr>
</tbody>
</table>

**Table 10** – Mechanical properties of cold-drawn TM2-9 (75% hard segment)

Sample preparation greatly affects the tensile properties since a sample that contains defects such as air bubbles; dust; gels; surface scratches or cracks will break prematurely, resulting in lower elongation at break and tensile strength values. When TM2-9 was cold-drawn by the Instron, each sample snapped at different percentage elongations, hence resulting in different draw ratios which varied the properties in Table 10. The usual method of drawing is to stretch the polymer by a predetermined percentage elongation (for example 400%), often directly after melt-extrusion in order to ensure uniform sample treatment.

![Stress-strain curves for TM2-9 showing effect of premature failure](image)

**Figure 29** – Stress-strain curves for TM2-9 showing effect of premature failure

Figure 29 shows the common effect of premature failure. The stress-strain curves of the two samples have approximately the same shape right up until the first breaks at ~920%
elongation. This shows that the failure was due (in this instance) to a defect in the sample as previously mentioned rather than an inherent property of the material. The two specimens were cut from the same sheet of melt-pressed polyurethane and yet there is about 20MPa difference between the tensile strength of the two specimens.

<table>
<thead>
<tr>
<th>Name</th>
<th>Mn</th>
<th>Mw</th>
<th>MP</th>
<th>Polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM2-1</td>
<td>57,497</td>
<td>121,497</td>
<td>106,690</td>
<td>2.11</td>
</tr>
<tr>
<td>TM2-2</td>
<td>18,386</td>
<td>29,231</td>
<td>28,957</td>
<td>1.59</td>
</tr>
<tr>
<td>TM2-3</td>
<td>51,557</td>
<td>95,549</td>
<td>77,048</td>
<td>1.85</td>
</tr>
<tr>
<td>TM2-4</td>
<td>52,272</td>
<td>123,891</td>
<td>115,119</td>
<td>2.37</td>
</tr>
<tr>
<td>TM2-5</td>
<td>52,076</td>
<td>98,294</td>
<td>87,690</td>
<td>1.89</td>
</tr>
<tr>
<td>TM2-6</td>
<td>43,451</td>
<td>186,038</td>
<td>71,708</td>
<td>4.28</td>
</tr>
<tr>
<td>TM2-7</td>
<td>94,849</td>
<td>314,629</td>
<td>174,741</td>
<td>3.32</td>
</tr>
<tr>
<td>TM2-8</td>
<td>63,625</td>
<td>196,431</td>
<td>114,363</td>
<td>3.09</td>
</tr>
<tr>
<td>TM2-9</td>
<td>51,478</td>
<td>172,012</td>
<td>105,154</td>
<td>3.34</td>
</tr>
<tr>
<td>TM2-10</td>
<td>53,650</td>
<td>151,751</td>
<td>69,018</td>
<td>2.83</td>
</tr>
</tbody>
</table>

Table 11 – Series 2: Molecular weights of polymers varying hard segment percentage

Table 11 shows the molecular weight of polymers from series 2. The relatively poor physical properties of TM2-2 as seen in Table 9 are not surprising and are explained by the proportionally low molecular weight achieved. ‘MP’ in Table 11 is the ‘peak molecular weight’ and is the maximum intensity of the peak measured by GPC.

Figure 30 shows that the melting temperature of selected polyurethanes from series 2 (the larger endotherm that occurs between about 100ºC to 170ºC) increases with increasing percentage of hard segment. The smaller endotherm that is apparent at 40ºC to 60ºC is due to the melting of -PCL-HDI-PCL- (where two PCL-macrediols are linked with one HDI).163
The glass transition of the poly(ε-caprolactone) soft segment is expected at around -60°C and is too low to be measured on this apparatus. However, it is not important to determine the exact value of the glass transition since it is known to be substantially below 0°C, hence the material will be relatively soft at room temperature. Significant degradation of the materials can be seen at high temperatures above ~230°C, hence thermal processing at these temperatures is not practical.

2.3. Summary and Conclusion

It was found in this chapter that polyurethanes made using HDI were considerably harder and stronger than those made using m-LDI. It was also found that there was a general increase in hardness, stiffness, tensile strength and melting temperature with increasing hard segment percentage.

It was also shown in this chapter that the HDI-based polyurethanes exhibited a tendency to undergo cold-drawing as a result of tensile stress. The cold-drawn polyurethanes were measured to have very high Young’s modulus and tensile strength (up to just over
1GPa and 300MPa respectively) at the expense of decreased elongation at break when compared to the unoriented specimens. These properties are suitable for spinning strong fibres.

Since the aim of the experimental work was to create a range of polyurethanes that exhibit different properties (physical, thermal and degradative), it is logical to investigate the effect of changing the chemical composition of the soft segment, which has been kept constant thus far (PCL). The synthesis of soft segments and the corresponding synthesis of the polyurethanes using the macrodiols will be investigated in the following chapter.
3. Synthesis and use of Macrodilols

While the poly(ε-caprolactone) macrodiol-based polyurethanes based on HDI were shown in chapter 2 to be excellent materials in terms of physical properties, they are expected to be slow to degrade (this is shown later in Chapter 5). Altering the composition of the soft segment is an alternate way of manipulating the properties of polyester-urethanes.

This chapter describes the design and synthesis of suitable macrodilols in order to investigate the effect of the soft segment chemical structure on the mechanical and thermal properties of poly(ester-urethane)s and their in-vitro degradation rate.

3.1. Methods

3.1.1. Method - General Synthesis of PGA Macrodilols

Glycolic acid (Aldrich) was heated to 200°C with magnetic stirring and nitrogen outgassing in a round-bottomed flask fitted with a side-arm condenser for a period of 5 hours and water was removed (polycondensation). The glycolic acid initially melted into a clear and colourless liquid of low viscosity which eventually solidified into a hard white solid with a melting point >200°C after 4-5 hours as it formed poly(glycolic acid), (PGA).

An amount of ethylene glycol was added to transesterify the PGA to form a macrodiol of the desired molecular weight (transesterification). The ethylene glycol was added to the PGA and heated with magnetic stirring in the same round-bottomed flask, but this time fitted with a vertical condenser topped with a drying tube to prevent moisture from entering the reaction. Within approximately 30-60 minutes at 200°C the white solid all dissolved back into a clear and colourless liquid as the molecular weight decreased. The higher molecular weight PGA solids did not dissolve appreciably in THF or DMF and therefore the molecular weight of the solids could not be accurately measured using the available equipment. The transesterification was monitored by GPC (in THF) since the transesterified PGA macrodil liquid was miscible and the decrease in molecular weight evident.
3.1.2. General Synthesis of PLA, PLGA Macrodiols

PLA and PLGA macrodiols were both made using a polycondensation step followed by an azeotropic distillation in xylene, solvent removal and lastly transesterification using ethylene glycol.

Polycondensation was carried out as for the synthesis of PGA macrodiols. The solid formed was clear and colourless and dissolved in THF hence the molecular weight was shown to be typically between 1000 and 3000 Mn.

Azeotropic distillation – This was based on a published method. Tin metal powder (80-200 mesh) was added as catalyst (0.5% by mass) and xylene was added (2:1, xylene:PLA) and the round-bottomed flask heated to 160°C with magnetic stirring. The round-bottomed flask was fitted with a vertical Dean & Stark apparatus filled with dry molecular sieves in order to dry the solvent before it returned to the reaction vessel. The time taken for the distillation was up to 96 hours. GPC were taken regularly in THF and the molecular weights achieved were up to Mn 33,500, Mw 54,000, MP 56,000. Upon cooling the PLA/PLGA phase separated as a bottom layer with the xylene as an upper layer. The bulk of the xylene was removed on the rotary evaporator and the remainder removed on a Kugelrohr (a bulb-to-bulb vacuum distillation apparatus), and tracked by $^1$HNMR showing the disappearance of the aromatic peaks.

Transesterification was rapid in comparison. Calculated amounts of ethylene glycol were added to the PLA/PLGA solid and heated to 180-200°C with magnetic stirring in a round-bottomed flask fitted with vertical condenser topped with a drying tube. Typical GPC results over time are shown for PLA-352 in Table 17, page 71.

3.1.3. Synthesis of Poly(γ-butyrolactone) Macrodiol

A catalyst (Sn-montmorillonite) (Sn-mont) was made according to a published method. Briefly, Na-cloisite (a montmorillonite clay) was ion-exchanged with Sn$^{2+}$ using SnCl$_4$, washed and filtered numerous times with water then with methanol, dried, ground by mortar and pestle, and sieved to <250µm. The clay was heated at 120°C overnight to dry before use.

The Sn-mont catalyst was used according to a hybrid method arising in part from two papers, H.Miura et al., and J.Kadokawa et al. Sn-mont (13g) was added to a round-
bottomed flask equipped with a mechanical stirrer, ethylene glycol (EG) (180g), γ-butyrolactone (γBL) (250g) and xylene (170g) were added to the flask which was then refluxed at 160°C for 23 hours over which time GPC samples were taken. After 23 hours the reaction mixture was cooled, vacuum filtered and the clay washed with 100ml acetone. The filtrate was then distilled at 60°C under vacuum to remove the EG, xylene, γBL and acetone. The PyBL macrodiol was washed with dichloromethane and saturated NaHCO₃(aq) but the colour remained a lemon yellow colour and yield was 87.2g, (14.3%). The macrodiol was then heated to 80°C on a Kugelrohr to remove low molecular weight diols and 36.02g was removed and characterised by ¹HNMR and GPC to be low molecular weight oligomers.

Acid and hydroxyl number was determined for the remaining macrodiol. The acid number was less than 0.1g(KOH)/g_{macrodiol} and the hydroxyl number was 385.94g(KOH)/g_{macrodiol}, which equates to a molecular weight of 290.7gmol⁻¹.

The final mass after transferring to a 200ml round-bottomed flask was 76g (12.5%).

### 3.1.4. Acid Number Titration

The acid number is the number of mg of KOH required to react with the carboxylic end groups in 1 gram of oligomer. Literature methods were found and used from two sources.¹⁶⁷,¹⁶⁸

The materials used for the titration were chloroform, ethanol and methanol (AR grade). 1% phenolphthalein in methanol solution. 0.5M aqueous KOH. Potassium hydrogen phthalate (KHC₈H₄O₄). 0.5M aqueous KOH was prepared by diluting 27.2ml of 50% KOH solution to 1litre with carbon dioxide-free water. The KOH was standardised with 0.74g KHC₈H₄O₄ in 100ml of carbon dioxide-free water and three drops of phenolphthalein by titrated with the 0.5M KOH.

The method used for the determination of the acid number was as follows: 5g of sample macrodiol was weighed into a 250ml flask and 75 to 100ml of denatured alcohol added. The solution was heated and stirred until the polymer had completely dissolved. 0.5ml of phenolphthalein solution was added and the solution titrated with 0.5M KOH to a pink endpoint.

The acid number is given by the following equation:
Acid Number = (VN x 56.1)/S

Where V = volume of KOH titre, N = normality of the KOH solution, W = weight of the sample used in grams.

3.1.5. Hydroxyl Number

The hydroxyl number is the number of mg of KOH required to react with the hydroxyl end groups in 1 gram of oligomer. Literature methods were found and used to make a modified method as follows.169,170,171,172

The reagents used for the titration were acetic anhydride (AR grade), 1,2-dichloroethane (distilled and colourless), N-methylimidazole (NMIM) (dried over KOH and distilled), chloroform and methanol, thymol blue indicator (10% in methanol), and 0.5M methanolic KOH (standardised using benzoic acid). A stock solution of acetic anhydride in 1,2-dichloroethane (1:6 ratio by volume) was made up fresh each day.

The method used for the hydroxyl number determination was (in triplicate): 3-4 milliequivalents of oligomer was weighed into a 250ml round-bottomed flask and 20ml of 1,2-dichloroethane was added to dissolve the oligomer followed by the addition of 4ml of acetic anhydride stock solution and 4ml of NMIM. The sample was then acetylated at 100°C for 15 minutes under reflux using a condenser and drying tube. The flask was then cooled using ice water, then 3ml deionised water added to the sample to hydrolyse excess acetic anhydride. The sample was then refluxed a second time at 100°C for a further 5 minutes followed by cooling with ice water. The condenser was rinsed carefully with 200ml chloroform followed by 35ml methanol. 5ml of thymol blue indicator solution was added and the flask was titrated with standardised 0.5M KOH to a thymol blue end point. The colour turns from deep yellow/orange to blue.

A blank was also run and this was performed using all the above with the simple omission of the sample.

Hydroxyl Number = (VN x 56.1)/W
Where \( V \) = volume of KOH titre, \( N \) = normality of the KOH solution, 56.1 = molecular weight of KOH, \( W \) = weight of the sample used in grams.

The following formula was used to calculate the molecular weight of the oligomer based on the hydroxyl number (OH\#) and acid number (Acid\#):

\[
\text{Molecular Weight} = \frac{56.1 \times 2 \times 1000}{\text{OH}\# + \text{Acid}\#}
\]

### 3.1.6. Synthesis of Polyurethanes from Series 3 and 4

Polyurethanes from series 3 and series 4 were made using the following general method: The macrodiol soft segments (see sections 3.1.1 to 3.1.3) were dried at 90°C for 4 hours under vacuum (0.1 torr). Ethylene glycol (Aldrich) was degassed at 90°C under vacuum (0.1 torr) for 4 hours and HDI (Aldrich) was used as received (colourless). Stannous octoate (Aldrich) was kept moisture-free and used as received. A mixture of soft segment macrodiol, EG and stannous octoate were weighed into a 100 ml predried polypropylene beaker, covered with aluminium foil and heated to 70°C under nitrogen in a laboratory oven. HDI was weighed in a separate wet-tared predried polypropylene beaker and also heated to 70°C. The HDI was then added to the macrodiol/EG/stannous octoate beaker and stirred manually until gelation occurred (~1min to 5min), at which time the hot viscous mixture was poured onto a Teflon coated metal tray to cure at 100°C for a period of about 12 hours. The resulting polymers were clear but slightly yellow in some cases.

### 3.1.7. Compression Moulding

(See Section 2.1.3, page 43)

### 3.1.8. Instron Tensile Tests

(See Section 2.1.4, page 43)
3.1.9. Differential Scanning Calorimetry (DSC)

(See Section 2.1.5, page 44)

3.1.10. Gel Permeation Chromatography

(See Section 2.1.6, page 44)

3.1.11. Shore Hardness Indentor

(See Section 2.1.7, page 45)

3.1.12. Nuclear Magnetic Resonance

$^1$HNMR and $^{13}$CNMR readings were taken on a Bruker Avance 400 spectrometer. The Bruker Avance 400 spectrometer operated with a Spectrospin 9.4 T magnet (400.13 MHz $^1$H frequency) and was equipped either with a 5 mm $^1$H-$^19$F-$^{13}$C-$^{31}$P QNP autoswitchable probe with z-gradient or a 5 mm inverse $^1$H-X BBI autotuning broadband probe.

All spectra were recorded at 298K (unless otherwise noted) in deuterated DMSO (dimethyl sulfoxide). The spectra were referenced to the residual solvent signal in $^1$H and the deuterated solvent signal in $^{13}$C.

The samples for $^1$HNMR were made up as a dilute solution of ~2 drops of sample in ~0.7ml deuterated DMSO in a NMR tube. The samples for $^{13}$CNMR were made up as a solution of ~ 0.4ml sample in ~ 0.4ml deuterated DMSO in a NMR tube.
3.2. Results and Discussion

3.2.1. Soft Segment Synthesis

Polyester soft segments were typically made by sequential polycondensation (or polyesterification) and transesterification steps. Typically, polycondensation of a hydroxy-acid such as glycolic acid formed poly(glycolic acid) oligomers which were transesterified using a diol (ethylene glycol) to form dihydroxy-functionalised prepolymers.

Equation 1 shows that the concentration of water has a large effect on the extent of polymerisation during a polyesterification.

\[ [H_2O] = \frac{K[M]_0}{\bar{X}_n(X_n - 1)} \]

Equation 1 – Effect of water on polyesterification (from G. Odian, “Principles of Polymerization”)\textsuperscript{173}

Where,

\( \bar{X}_n \) = Degree of polymerisation

\( K \) = Rate constant for the polyesterification

\( [M]_0 \) = Concentration of hydroxy-acid at time 0

\( [H_2O] \) = Concentration of water (g/litre)

The values of \( K \) are generally between 0.1 and 1.0 for polyesterifications.\textsuperscript{173}

In this study the aim was to achieve as high an “Effective Xn” as possible since the added diol will limit the molecular weight. “Effective Xn” can be defined as the ratio of the average number of repeating esterified hydroxy-acids to the number of carboxylic acid groups still present, rather than being simply the degree of polymerisation. This means that the “Effective Xn” can be many times the “actual Xn” since the diols limit the degree of polymerisation. For example, the actual Xn for a 1000Mn PLA chain is \(~13\), however, if every second chain in the mixture is a macrondiol (no acid) then every other chain contains one carboxylic acid and hence the Effective Xn doubles to 26, \(~2000Mn\).
When the Effective Xn is greater than the actual Xn, it means that there has been some success in removing acid by way of reacting with the diol.

<table>
<thead>
<tr>
<th>Effective Mn</th>
<th>Effective Xn</th>
<th>Acid Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>13.03</td>
<td>56.10</td>
</tr>
<tr>
<td>2000</td>
<td>26.92</td>
<td>28.05</td>
</tr>
<tr>
<td>3000</td>
<td>40.81</td>
<td>18.70</td>
</tr>
<tr>
<td>4000</td>
<td>54.69</td>
<td>14.03</td>
</tr>
<tr>
<td>5000</td>
<td>68.58</td>
<td>11.22</td>
</tr>
<tr>
<td>6000</td>
<td>82.47</td>
<td>9.35</td>
</tr>
<tr>
<td>7000</td>
<td>96.36</td>
<td>8.01</td>
</tr>
<tr>
<td>8000</td>
<td>110.25</td>
<td>7.01</td>
</tr>
<tr>
<td>9000</td>
<td>124.14</td>
<td>6.23</td>
</tr>
<tr>
<td>10000</td>
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<td>5.61</td>
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<tr>
<td>15000</td>
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<td>3.74</td>
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</tr>
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<td>30000</td>
<td>415.81</td>
<td>1.87</td>
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<tr>
<td>40000</td>
<td>554.69</td>
<td>1.40</td>
</tr>
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<td>50000</td>
<td>693.58</td>
<td>1.12</td>
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<tr>
<td>100000</td>
<td>1388.03</td>
<td>0.56</td>
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<tr>
<td>200000</td>
<td>2776.92</td>
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</tr>
<tr>
<td>400000</td>
<td>5554.69</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 12 – Relationship between Effective Mn, Effective Xn and Acid number in the absence of diol

It can be seen in Table 12 that the greater the effective Mn, the lower the acid number, of course this is to be expected since the acid number is expressed in grams of KOH to neutralise one gram of sample, hence is inversely proportional to the molecular weight. The Effective Mn is defined as the mass average molecular weight corresponding to the Effective Xn.

Given that K for polyesterification reactions (see Equation 1) is typically in the order of 0.1 to 1.0, the expected acid number can be calculated for this range and the data is presented in Table 13 and Table 14:

<table>
<thead>
<tr>
<th>Xn LA</th>
<th>Effective Mn</th>
<th>Water (ppm)</th>
<th>Acid Number</th>
<th>EoC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1502</td>
<td>23.76</td>
<td>37.35</td>
<td>33.42</td>
</tr>
<tr>
<td>50</td>
<td>3662</td>
<td>3.672</td>
<td>15.32</td>
<td>72.69</td>
</tr>
<tr>
<td>100</td>
<td>7262</td>
<td>0.909</td>
<td>7.73</td>
<td>86.23</td>
</tr>
<tr>
<td>200</td>
<td>14462</td>
<td>0.227</td>
<td>3.88</td>
<td>93.09</td>
</tr>
<tr>
<td>500</td>
<td>36062</td>
<td>0.036</td>
<td>1.56</td>
<td>97.23</td>
</tr>
</tbody>
</table>

Table 13 – Polyesterification data, assuming K = 0.1
Table 13 and Table 14 are modified from (G.Odian, “Principles of Polymerization”)\textsuperscript{174} to include theoretical Acid Numbers and efficiency of conversion (EoC\%). The EoC\% is calculated to be 100\% minus the percentage of carboxyl-terminated chains in the reaction mixture.

<table>
<thead>
<tr>
<th>K=1.0, Water (g)</th>
<th>Xn LA</th>
<th>Effective Mn</th>
<th>Water (ppm)</th>
<th>Acid Number</th>
<th>EoC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1502</td>
<td>237.600</td>
<td>37.35</td>
<td>33.42</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>3662</td>
<td>36.720</td>
<td>15.32</td>
<td>72.69</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>7262</td>
<td>9.090</td>
<td>7.73</td>
<td>86.23</td>
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<tr>
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<td>14462</td>
<td>2.268</td>
<td>3.88</td>
<td>93.09</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>36062</td>
<td>0.362</td>
<td>1.56</td>
<td>97.23</td>
<td></td>
</tr>
</tbody>
</table>

\textit{Table 14 – Polyesterification data, assuming K=1.0}

It could be expected that the real values would fall somewhere between these two sets of figures shown in Table 13 and Table 14 depending on a number of factors (catalyst, reflux rate, stirring rate, temperature, solvent, viscosity, etc.). If this were the case then in order to get an acid value of 1 or less one must reduce water content to about 0.3-0.03ppm water in the reaction mixture by the end of the polycondensation reaction. However having said this, the syntheses in this study are slightly different to the one described by G.Odian\textsuperscript{173} in that there is a molecular weight-limiting diol present. Since there is a diol present we do not experience some of the effects that are specific to high molecular weight syntheses, such as greatly increasing mixture viscosity, increased diffusion limitations, or as substantial a change in the ratio of esters to endgroups. Hence our experimental data may not conform to that shown in Table 13 or Table 14 but one would expect it to be in the same order of magnitude.

The equation of the limiting equilibrium is shown in Equation 2:

\[ K_1 \]

\[ \text{R-COOR’} + \text{H}_2\text{O} \rightleftharpoons \text{R-COOH} + \text{HOR’} \]

\textit{Equation 2 – Equation of the limiting equilibrium in esterification}
If water is removed then the equilibrium is pushed to the left (esterification). This is an equilibrium between free water that is present in the reaction mixture and water bound as –COOH. At the very start of a condensation reaction involving glycolic acid, every glycolic acid has one carboxylic acid group and one hydroxyl group. As the molecular weight increases and oligomers are formed, the ratio of carboxylic acid groups to glycolic repeat units decreases. Water that was formed during the condensation must be removed from the reaction to prevent hydrolysis of the chains back to carboxylic acid.

In the case where solvents are used to efficiently remove water by azeotropic distillation\textsuperscript{156} there is another equilibrium:

\[
\text{H}_2\text{O}_{(\text{liq})} + \text{Solvent}_{(\text{liq})} \rightleftharpoons \text{H}_2\text{O}_{(\text{g})} + \text{Solvent}_{(\text{g})}
\]

All solvents are soluble in all other solvents to varying extents and this becomes very important when ppm amounts of water are problematic. Azeotropic distillation removes most of the water however unless the solvent is replenished with totally dry solvent some amount of water remains. Some references have commented on this problem and have shown that refluxing through molecular sieves can reduce the water content in the solvent to 3ppm or less.\textsuperscript{156} Some groups have also replaced the wet solvent with dry solvent rather than drying it and allowing it to return to the reaction mixture but this is less desirable since this could take a very large amount of dry solvent to lower the water concentration to acceptable levels.

Azeotropic distillation was used in the synthesis of many of the macrodiols in this study in order to minimise the amount of water remaining in the reaction mixture. As a result, very low acid numbers were achieved in some cases.

For example: PLA-352 was a linear macrodiol of 352 molecular weight (by acid and hydroxyl number determination) and had an acid number of only 1.45mg KOH/gram of PLA which equates to 0.45% acid groups in the PLA and an EoC% of 99.1%.

This compares more than favourably with literature values of acid numbers for similar macrodiols (see Table 15).
<table>
<thead>
<tr>
<th>Macrodiol Mw</th>
<th>Acid Number (mgKOH per gram of polymer)</th>
<th>EoC% (calc)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>7200</td>
<td>1</td>
<td>87</td>
<td>175</td>
</tr>
<tr>
<td>5600</td>
<td>2</td>
<td>80</td>
<td>175</td>
</tr>
<tr>
<td>7200</td>
<td>2</td>
<td>74</td>
<td>175</td>
</tr>
<tr>
<td>7300</td>
<td>2</td>
<td>74</td>
<td>175</td>
</tr>
<tr>
<td>5100</td>
<td>3</td>
<td>73</td>
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</tr>
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<td>3</td>
<td>71</td>
<td>175</td>
</tr>
<tr>
<td>8200</td>
<td>2</td>
<td>71</td>
<td>175</td>
</tr>
<tr>
<td>6200</td>
<td>3</td>
<td>67</td>
<td>175</td>
</tr>
<tr>
<td>6300</td>
<td>3</td>
<td>66</td>
<td>175</td>
</tr>
<tr>
<td>6700</td>
<td>3</td>
<td>64</td>
<td>175</td>
</tr>
<tr>
<td>7100</td>
<td>3</td>
<td>62</td>
<td>175</td>
</tr>
<tr>
<td>7700</td>
<td>3</td>
<td>59</td>
<td>175</td>
</tr>
<tr>
<td>7100</td>
<td>4</td>
<td>49</td>
<td>175</td>
</tr>
<tr>
<td>4900</td>
<td>6</td>
<td>48</td>
<td>175</td>
</tr>
<tr>
<td>5900</td>
<td>5</td>
<td>47</td>
<td>175</td>
</tr>
<tr>
<td>8000</td>
<td>4</td>
<td>43</td>
<td>175</td>
</tr>
<tr>
<td>5900</td>
<td>6</td>
<td>37</td>
<td>175</td>
</tr>
<tr>
<td>8400</td>
<td>5</td>
<td>25</td>
<td>175</td>
</tr>
<tr>
<td>1600</td>
<td>1</td>
<td>97</td>
<td>129</td>
</tr>
<tr>
<td>1200</td>
<td>1.5</td>
<td>97</td>
<td>129</td>
</tr>
<tr>
<td>3200</td>
<td>1.6</td>
<td>91</td>
<td>129</td>
</tr>
<tr>
<td>6600</td>
<td>1.4</td>
<td>84</td>
<td>129</td>
</tr>
<tr>
<td>37000</td>
<td>1</td>
<td>34</td>
<td>129</td>
</tr>
<tr>
<td>4700</td>
<td>12</td>
<td>-1</td>
<td>129</td>
</tr>
<tr>
<td>4900</td>
<td>10</td>
<td>13</td>
<td>176</td>
</tr>
<tr>
<td>17100</td>
<td>2.1</td>
<td>36</td>
<td>177</td>
</tr>
</tbody>
</table>

Table 15 – Literature values of acid number for degradable macrodiols

It is generally thought that a lower acid number is better with some authors expressing the opinion that an acid number of less than 1mg KOH per gram of prepolymer is “good”. However the acid number only tells part of the story. The acid number is inversely proportional to the molecular weight of the macrodiol (containing some acid functionality) since it is expressed in units of mg KOH per gram of prepolymer rather than per mole. It can be seen in Table 15 that a low acid number does not always mean a good EoC%.

One of the EoC% values in Table 15 is negative. What this means is that the acid number is higher than would be expected if all chains contained one hydroxyl-terminated end and one carboxylic acid-terminated end. This could possibly be due to residual acid catalyst or acid contamination which both may interfere with the titration. Etherification could also theoretically cause this as this would result in a dicarboxylic acid, however it is not the likely cause since high temperatures and quite specific catalysts must be employed to catalyse the reaction, hence it is highly improbable.
3.2.2. Macrodials Synthesised For This Study

![Structures of synthesised macrodials](image)

The initiator for each of the synthesised macrodials was ethylene glycol giving the predicted structures in Figure 31. At room temperature all of the macrodials were liquid with the exception of PGA macrodiol which was a white slurry. However, the PGA slurry was fully melted at 70°C, the temperature at which it was used.

Table 16 shows the characterisation of the various macrodials that were used in the synthesis of Series 4. Note that the “Mw (calc)” is generally considered more accurate than the GPC values since it is based on the hydroxyl number and acid number titration values which should have an error of less than one percent, whereas the GPC values are by comparison to polystyrene standards. While comparing molecular weight to polystyrene standards does not give a true value, it does give the polydispersity and allows weight comparisons between the oligomers.

<table>
<thead>
<tr>
<th>Soft Segment macrodiol</th>
<th>Hydroxyl #</th>
<th>Acid #</th>
<th>Mw (calc)</th>
<th>GPC Mn</th>
<th>GPC Mw</th>
<th>GPC PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL</td>
<td>263.3</td>
<td>0.2</td>
<td>426</td>
<td>720</td>
<td>852</td>
<td>1.18</td>
</tr>
<tr>
<td>PEG</td>
<td>284.2</td>
<td>0.1</td>
<td>395</td>
<td>567</td>
<td>612</td>
<td>1.08</td>
</tr>
<tr>
<td>PLA</td>
<td>317</td>
<td>1.45</td>
<td>352</td>
<td>719</td>
<td>966</td>
<td>1.34</td>
</tr>
<tr>
<td>PGA</td>
<td>231</td>
<td>141</td>
<td>300</td>
<td>528</td>
<td>575</td>
<td>1.09</td>
</tr>
<tr>
<td>PLGA</td>
<td>280</td>
<td>18.2</td>
<td>376</td>
<td>680</td>
<td>839</td>
<td>1.23</td>
</tr>
<tr>
<td>PγBL</td>
<td>387.9</td>
<td>0.2</td>
<td>291</td>
<td>418</td>
<td>462</td>
<td>1.10</td>
</tr>
</tbody>
</table>

Table 16 – Characterisation of soft segment macrodials used in Series 3 and 4
Each of the macrodiols consists of a mixture of different molecular weight oligomers. The polydispersity (PD) of the oligomers is shown in Table 16 and shows a reasonably narrow distribution, where 1.0 is monodisperse. The molecular weight of the macrodiols varied from 290 to 426 suggesting that the amount of ethylene glycol required for transesterification was overestimated in all cases resulting in lower than the desired 400 molecular weight. The reasons for this are firstly that the calculation was based on the assumption of complete dehydration in the polycondensation step, and secondly some of the hydroxy acid was lost during the polycondensation step (carried over with the water), which means less ethylene glycol is required. If an exact molecular weight were required then one would need to compensate to take these into account. In this case it is not critical since a small variation in the length of the soft segment is not expected to significantly affect the properties and comparisons between materials can still be made.

Figure 32 shows $^1$HNMR of a number of different batches of PGA that have different effective ratios of ethylene glycol to glycolic acid. The GPC values are not exact values as has been mentioned before as the dimer is Mw 120, not the indicated Mw 167 (both theoretically and by $^1$HNMR characterisation). The results of the $^1$HNMR of this series suggests that with some optimisation it might be possible to obtain the molecular weight of the macrodiols by $^1$HNMR through comparison of the peaks between $\sim 3.6 - 5.0$ which would be much quicker and easier than by titration. This could be done by comparison of the integrals of selected peaks (as a percentage of the total spectrum), such as the peak at 4.9 ppm (due to the methylene protons from the glycolic residues) which becomes more pronounced in the higher molecular weight oligomers.
The transesterification step (see Table 17) is a relatively rapid reaction compared with polycondensation. The molecular weight of the polymer changes quickly upon addition of the ethylene glycol and heat, approximately halving each 30 minutes until equilibrium is reached somewhere between 150 minutes and 24 hours.

The polydispersity increases during the transesterification as the chains are glycolysed (transesterified with a glycol) and decreases again over time as the reaction equilibrates. This is important since low polydispersities are preferred and despite the ethylene glycol

<table>
<thead>
<tr>
<th>Time</th>
<th>Mn</th>
<th>Mw</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>33,562</td>
<td>54,230</td>
<td>1.62</td>
</tr>
<tr>
<td>40 minutes</td>
<td>10,741</td>
<td>16,152</td>
<td>1.50</td>
</tr>
<tr>
<td>90 minutes</td>
<td>3,226</td>
<td>7,544</td>
<td>2.34</td>
</tr>
<tr>
<td>150 minutes</td>
<td>1,866</td>
<td>4,126</td>
<td>2.21</td>
</tr>
<tr>
<td>24 hours</td>
<td>719</td>
<td>966</td>
<td>1.34</td>
</tr>
</tbody>
</table>

Table 17 – Molecular weight change during transesterification for PLA-352
being fully reacted after typically less than 4 hours, the polydispersity continues to decrease. If there are many short chains and some long chains, the hydroxyls of the short ones will react with the long chains over time to transesterify and shorten the long chains and add to themselves which causes a narrower distribution.

The ring-opening polymerisation of γ-butyrolactone is shown in Table 18, (see page 59 for the method). Higher molecular weights can be achieved by decreasing the amount of ethylene glycol. The reaction was not monitored between 6 hours and 21 hours and based on the Mn and Mw, the reaction appears to have reached equilibrium between these two time points.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Mn (GPC)</th>
<th>Mw (GPC)</th>
<th>PD (Mn/Mw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>265</td>
<td>336</td>
<td>1.27</td>
</tr>
<tr>
<td>4</td>
<td>281</td>
<td>367</td>
<td>1.31</td>
</tr>
<tr>
<td>5</td>
<td>290</td>
<td>389</td>
<td>1.34</td>
</tr>
<tr>
<td>6</td>
<td>299</td>
<td>455</td>
<td>1.52</td>
</tr>
<tr>
<td>21</td>
<td>344</td>
<td>624</td>
<td>1.82</td>
</tr>
<tr>
<td>24</td>
<td>338</td>
<td>511</td>
<td>1.51</td>
</tr>
<tr>
<td>26</td>
<td>341</td>
<td>520</td>
<td>1.53</td>
</tr>
<tr>
<td>30</td>
<td>406</td>
<td>547</td>
<td>1.35</td>
</tr>
<tr>
<td>After solvent removal</td>
<td>354</td>
<td>520</td>
<td>1.47</td>
</tr>
</tbody>
</table>

Table 18 – Molecular weight of oligo-γ-butyrolactone over time

The \(^1\text{HNMR}\) of oligo-γ-butyrolactone is shown in Figure 34 and a comparison with spectra (dimer, 400Mw and 1000Mw) \(^{165}\) (H.Miura, 1999) confirms that this is γ-butyrolactone macrodiol of less than Mn 400. The γ-butyrolactone macrodiol was liquid at room temperature and had a slight yellow tint to it – most likely due to trace thermal degradation impurities.
Figure 33 – Structure of poly(γ-butyrolactone) initiated using ethylene glycol

Figure 34 – 400MHz $^1$HNMR of poly(γ-butyrolactone) of 291 molecular weight
3.2.3. Synthesis of Polyurethanes with Varying Soft Segments

It would be an advantage to be able to control the rate of degradation since some tissue-engineering uses require a faster-degrading polymer, hence if polymers were formulated to degrade in a range of time periods, they could conceivably be used in a broader range of applications. One of the simplest ways to achieve a change in degradation time is to alter the composition of the soft segment to incorporate chemical groups that degrade more quickly than the urethane hard segment. The effect of altering the soft segment is investigated in this section.

The linear polyester family has a degradation rate inversely proportional to the length of the repeating monomer, i.e. PGA>P3PA>P4HB>P5VL>PCL, note that P3PA is poly(3-hydroxypropionic acid) and P5VL is poly(5-valerolactone). The reason can be in part attributed to the increasing hydrophobicity of the polymers with increasing methylene units. Hence, a PCL-based series of polyurethanes would be expected to degrade at a slower rate than a corresponding series based on PGA. In order to be suitable for polyurethane synthesis it is necessary to synthesise the polyester macrodiols. Macrodiols used in polyurethanes have often been compared in the literature on the basis of molecular weight alone. This can be misleading since the relative length of the macrodiol has an influence on the properties (See Figure 35).

![Figure 35 – Chain length varying with macrodiol composition (similar molecular weights)](image-url)
When comparing the relative rate of hydrolysis of polyurethanes made from these macrodiols one must take into account the number of ester groups present. For example the macrodiols shown in Figure 35 are all within 10 atomic mass units of 413Mw and yet have very different lengths and number of esters (see Table 19).

<table>
<thead>
<tr>
<th>Macrodiol</th>
<th>Mw</th>
<th>Chain length (atoms)</th>
<th>Functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG</td>
<td>414.49</td>
<td>28</td>
<td>8 ethers</td>
</tr>
<tr>
<td>PCL</td>
<td>404.50</td>
<td>25</td>
<td>3 esters</td>
</tr>
<tr>
<td>PGA</td>
<td>410.28</td>
<td>22</td>
<td>6 esters</td>
</tr>
<tr>
<td>PLA</td>
<td>422.38</td>
<td>19</td>
<td>5 esters</td>
</tr>
</tbody>
</table>

Table 19 – Comparison of ~400 Mw macrodiols

It can be seen in Table 19 that PCL macrodiol has only half the number of ester groups that the PGA macrodiol has, despite being approximately the same molecular weight. Assuming the same percentage hard segment in two polyurethanes made from PLA and PCL, the PLA-based polyurethane will have twice the number of esters per gram than the PCL-based polyurethane and would therefore be expected to degrade more quickly. Even without taking into account the relative lability of the \( \alpha \)-hydroxy acid compared with the \( \epsilon \)-hydroxy acid, one would expect the PLA-based polyurethane to degrade more quickly.

A series of polyurethanes was made while keeping the hard to soft segment ratio constant (65%) and as far as practicable the same molecular weight macrodiol (~400MW as used in series 2) (See Table 20).

It should be noted that the physical properties of the polyurethanes are expected to vary markedly with different soft segments, for example PCL has a melting point of 57ºC and PGA has a melting point of 225ºC. \(^{83}\)

<table>
<thead>
<tr>
<th>Polyurethane Code</th>
<th>Soft Segment Macrodiol</th>
<th>Post processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM3-1</td>
<td>PCL-402</td>
<td>Extrusion and FDM</td>
</tr>
<tr>
<td>TM3-2</td>
<td>PEG-395</td>
<td>Extrusion and FDM</td>
</tr>
<tr>
<td>TM3-3</td>
<td>PLGA-425</td>
<td>Extrusion and FDM</td>
</tr>
<tr>
<td>TM3-4</td>
<td>PGA-491</td>
<td>Not enough material</td>
</tr>
</tbody>
</table>

Table 20 – Series 3: Changing the Soft Segment - Composition
It was found that the four polymers varied in physical properties considerably. TM3-1, TM3-2 and TM3-4 were all reasonably similar in properties (relatively hard, flexible and strong), whereas TM3-3 was very brittle at room temperature and snapped rather than bent which is indicative of a high glass transition temperature. TM3-1 and TM3-2 were both colourless and had very good mechanical properties, whereas TM3-3 and TM3-4 were somewhat opaque, slightly yellowed, and not as tough.

<table>
<thead>
<tr>
<th>Polyurethane</th>
<th>Soft Segment</th>
<th>Hard Segment</th>
<th>Elongation at Break %</th>
<th>Young’s Modulus (MPa)</th>
<th>Upper Tensile Strength (MPa)</th>
<th>Hardness (Shore D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM3-1</td>
<td>PCL-402</td>
<td>65</td>
<td>1300 ± 42</td>
<td>112 ± 3</td>
<td>54 ± 5</td>
<td>52</td>
</tr>
<tr>
<td>TM3-2</td>
<td>PEG-395</td>
<td>65</td>
<td>1482 ± 270</td>
<td>253 ± 31</td>
<td>55 ± 5</td>
<td>47</td>
</tr>
<tr>
<td>TM3-3</td>
<td>PLGA-376</td>
<td>65</td>
<td>47 ± 64</td>
<td>265 ± 205</td>
<td>13 ± 2</td>
<td>44</td>
</tr>
<tr>
<td>TM3-4</td>
<td>PGA-491</td>
<td>65</td>
<td>Too brittle to cut specimens but similar to TM3-3</td>
<td></td>
<td></td>
<td>41</td>
</tr>
</tbody>
</table>

Table 21 – Series 3: Effect on physical properties of changing the soft segment

Series 3 and series 4 have been kept as separate series despite both having the same variable, namely the soft segment. The reason for this is that series 3 was made earlier than series 4 for use in an accelerated degradation study however the same PEG-based polyurethane was reused in series 4, that is TM3-2 was reused as TM4-2.

<table>
<thead>
<tr>
<th>Polyurethane</th>
<th>Soft Segment</th>
<th>Hard Segment</th>
<th>Elongation at Break %</th>
<th>Young’s Modulus (MPa)</th>
<th>Upper Tensile Strength (MPa)</th>
<th>Hardness (Shore D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM4-1</td>
<td>PCL-402</td>
<td>65</td>
<td>947 ± 128</td>
<td>210 ± 16</td>
<td>32 ± 3</td>
<td>54</td>
</tr>
<tr>
<td>TM4-2</td>
<td>PEG-395</td>
<td>65</td>
<td>1482 ± 270</td>
<td>253 ± 31</td>
<td>55 ± 5</td>
<td>47</td>
</tr>
<tr>
<td>TM4-3</td>
<td>PLA-352</td>
<td>65</td>
<td>Too brittle to cut specimens but similar to TM3-3</td>
<td></td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>TM4-4</td>
<td>PLGA-376</td>
<td>65</td>
<td>Too brittle to cut specimens but similar to TM3-3</td>
<td></td>
<td></td>
<td>72</td>
</tr>
<tr>
<td>TM4-5</td>
<td>PGA-300</td>
<td>65</td>
<td>Too brittle to cut specimens but similar to TM3-3</td>
<td></td>
<td></td>
<td>67</td>
</tr>
<tr>
<td>TM4-6</td>
<td>P4HB-291</td>
<td>65</td>
<td>151 ± 80</td>
<td>248 ± 10</td>
<td>16 ± 1</td>
<td>49</td>
</tr>
</tbody>
</table>

Table 22 – Series 4: Mechanical properties of polymers with different soft segments
Figure 36 – Series 4: Thermal properties (Differential Scanning Calorimetry)

Figure 36 is consistent with TM4-3, TM4-4 and TM4-5 (the top three) all being brittle while the other polymers were not. The reason is that the glass transition occurs for these three polymers at around room temperature whereas the other three display glass transition temperatures significantly lower (-30°C or below). The glass transition in Figure 36 is labelled “Tg” and is seen as a slight ‘dip’ in the graph, the melting endotherm of the polymer is “B” and the melting of the “-soft segment-HDI-soft segment-” phase is “A”, (as previously discussed in section 2.2, page 56).

The stress-strain curve for TM4-2 (see Figure 37) was similar to those for the polymers in series 1, characterised by three regions: firstly a relatively short elastic region, secondly a plastic region of cold-drawing where the stress to draw the polymer is relatively low and constant, and thirdly a region of increasing stress until failure. The ‘bumps’ in the curve between ~400-1000% elongation are believed to be due to slippage of the sample in the grips as it stretched.
The main reason for the significant difference in tensile strength between the high-strength TM4-1 and TM4-2 and the other polymers is due to molecular weight as can be seen in Table 23.

**Figure 37** – Stress-strain curve for TM4-2

**Figure 38** – Stress-strain curve for TM4-6
The stress-strain curve for TM4-6 (PγBL-based polyurethane) is shown in Figure 38 and illustrates that the polymer displays the same-shaped curve as the stronger two polymers up until failure, i.e. it shows the elastic region and the plastic cold-drawing region but it then fails rather than continuing into the third region.

The reason for failure in this case was twofold, firstly the molecular weight was quite low and secondly the sample was difficult to prepare (some materials melt-press more easily than others for a variety of reasons) and contained some defects in the form of small air bubbles.

<table>
<thead>
<tr>
<th>Polyurethane</th>
<th>Macrodial</th>
<th>Mw</th>
<th>Mn</th>
<th>MP</th>
<th>Polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM4-1</td>
<td>PCL-402</td>
<td>52,313</td>
<td>35,869</td>
<td>52,885</td>
<td>1.46</td>
</tr>
<tr>
<td>TM4-2</td>
<td>PEG-394</td>
<td>111,352</td>
<td>46,316</td>
<td>96,330</td>
<td>2.40</td>
</tr>
<tr>
<td>TM4-3</td>
<td>PLA-352</td>
<td>15,197</td>
<td>9,107</td>
<td>14,481</td>
<td>1.67</td>
</tr>
<tr>
<td>TM4-4</td>
<td>PLGA-376</td>
<td>16,274</td>
<td>10,847</td>
<td>17,415</td>
<td>1.50</td>
</tr>
<tr>
<td>TM4-5</td>
<td>PGA-300</td>
<td>15,272</td>
<td>9,281</td>
<td>16,752</td>
<td>1.65</td>
</tr>
<tr>
<td>TM4-6</td>
<td>Py-HB-291</td>
<td>33,769</td>
<td>22,856</td>
<td>31,373</td>
<td>1.48</td>
</tr>
</tbody>
</table>

**Table 23** – Series 4: Molecular weights of polymers with different soft segments

Table 23 explains the poor physical properties of the weaker polyurethanes as being due to insufficient molecular weight.

Some papers\textsuperscript{179,180,106} have reported molecular weights (Mw) lower than 15,000 for similar polyurethanes (lower than in Table 23), however this does not mean that these molecular weights are acceptable since they appear to result in very poor physical properties. Synthesis conditions could be optimised to increase the molecular weight by varying the reaction temperature, catalyst, and other factors. By keeping the conditions of synthesis constant it appears that one-step polymerisation appears to be less suitable for the shorter hydroxy-esters than for the longer ones since PCL and PEG-based soft segments consistently gave higher molecular weights. This may also be due in part to the glycolic and lactic chains hydrolysing more easily or solidifying at a lower molecular weight rather than remaining in the melt like ε-caprolactone-based polyurethanes. It is suspected that the higher hydrolysability of the glycolic and lactic chains might have caused inaccurate hydroxyl number determinations which could also have resulted in low molecular weight polymers.
3.3. Summary and Conclusion

In this chapter it has been shown that the soft segment has a large effect on the thermal and mechanical properties of the polyurethanes. HDI-based polyurethanes were made using ethylene glycol as chain extender and 65% hard segment while varying the chemistry of the soft segment.

Biodegradable aliphatic polyurethanes containing poly(γ-butyrolactone) were made for the first time and were shown to display some similar physical properties (low Tg, similar Young’s modulus, tendency to cold-draw) to poly(ε-caprolactone)-based polyurethanes. The poly(γ-butyrolactone) was synthesised by ring-opening polymerisation using a clay catalyst in xylene to form low molecular weight telechelic diols.

The molecular weights of the PLA, PGA and PLGA-based polyurethanes were substantially lower than those of the PCL, PyBL and PEG-based polyurethanes. The low molecular weights were a result of keeping synthesis conditions constant for comparison purposes. PLA, PGA and PLGA-based polyurethanes also showed higher glass transition temperatures than the other polyurethanes which is useful for tailoring properties of the materials. The high glass transition results in a high Young’s modulus which can be used to control the properties of the polyurethanes which was an original objective of this study.
4. Polyurethanes with Degradable Chain Extenders

The effect of different diisocyanates, hard segment percentages and soft segments were investigated in chapters 3 and 4. The other major component of segmented polyurethanes that is almost always overlooked in the literature is the effect of different chain extenders. Details are given in this chapter of a new series of degradable chain extenders which have been designed and synthesised in order to make a novel range of polyurethanes. The rationale is that the polyurethanes will hydrolyse in the hard segment at a higher rate than conventional polyurethane hard segments through the incorporation of hydrolysable linkages. The chain extenders in this study will be named based on the constituent monomers, for example GA-EG represents the glycolic acid – ethylene glycol dimer which has a lengthy systematic name ‘hydroxy-acetic acid 2-hydroxy-ethyl ester’ despite being the simplest of all of the degradable chain extenders synthesised for this work.

4.1. Methods

4.1.1. Synthesis of Novel Chain Extenders

4.1.1.1. GA-EG Degradable Chain Extender

GA-EG1 (Glycolic Acid – Ethylene Glycol)

150g of glycolic acid (GA) (Sigma) was heated at 220°C under nitrogen outgassing in a round-bottomed flask equipped with a magnetic stirrer bead, a still-head sidearm and condenser to collect the water runoff. After 4 hours the clear liquid was cooled to 200°C where it solidified into a white opaque brittle solid (PGA). 650g of dry ethylene glycol (Aldrich) was added to the PGA in a mole ratio of 5:1 (EG:GA) in order to transesterify the polymer and obtain reasonable yield of dimer. This was heated to 200°C for a period of 5 hours in total. The EG was removed under vacuum (~0.01 torr) at 120°C and trapped in liquid nitrogen trap as an opaque white solid. The liquid nitrogen trap was cleaned out and the temperature was then increased to 160°C where the dimer distilled over and trapped as a clear colourless solid. Yield of dimer was 121g, (51% yield -
some was lost in other fractions as well as in tests since purity was considered more important than yield). The Refractive index of the dimer was 1.45604 at 22.4°C.

GA-EG2, a scale-up reaction of GA-EG1 in order to maximise yield
434g of 70% glycolic acid in water was heated at 150°C to remove water for 16 hours under nitrogen outgassing in a large round-bottomed flask equipped with a magnetic stirrer bead, a still-head sidearm and condenser to collect the water runoff. To the approximately 232g of white solid PGA was added 1240g of ethylene glycol (five to one ratio) and the temperature increased to 210°C at which temperature the solid dissolved over one hour and the temperature was decreased to 180°C and cooled to room temperature after a total of 8 hours and 45 minutes of transesterification. Interestingly the liquid was perfectly colourless and clear at this point (thermal degradation can often cause yellowing or browning at high temperatures).

The dimer-containing liquid was then heated on the Kugelrohr at 50°C under vacuum (0.01torr) to remove unreacted ethylene glycol and then the temperature was increased to 75°C to distil the dimer. The dimer fraction was collected and then distilled a second time to remove any ethylene glycol present. In total there was 1031.58g of ethylene glycol, 314.79g GA-EG dimer (79% yield), 102.95g trimer and higher oligomers. The ethylene glycol and dimer were both clear and colourless liquids at room temperature however the trimer and higher oligomers had a hint of yellow to it (concentrated from the larger volume).

### 4.1.1.2. LA-EG Degradable Chain Extender

LA-EG (Lactic acid – Ethylene Glycol)

200g of 90% lactic acid in water was heated to 160°C for 6 hours under nitrogen outgassing in a round-bottomed flask equipped with a magnetic stirrer bead, a still-head sidearm and condenser to collect the water runoff. A five to one ratio of ethylene glycol (650g) was added to the PLA and heated to 180°C for 21 hours. The ethylene glycol was then distilled from the round-bottomed flask at 70°C and caught in a liquid nitrogen trap. The trap was cleaned and then the temperature was raised to 130°C and the LA-EG dimer was distilled. After four hours some solid crystallised in the condenser showing
that most likely trimer was distilling and that dimer had finished. Yield was 130g (48.5% yield).

### 4.1.1.3. GA-1,3-PD Degradable Chain Extender

GA-1,3-PD (Glycolic Acid-1,3-Propane Diol)

56.7g of glycolic acid was heated to 220°C for 5 hours under nitrogen outgassing in a round-bottomed flask equipped with a magnetic stirrer bead, a still-head sidearm and condenser to collect the water runoff. A five to one ratio of 1,3-propane diol (283.6g) was added to the PGA and heated to 200°C for 17:30 hours. The 1,3-propane diol was then removed on the Kugelrohr under vacuum at 70°C. The GA-1,3-PD dimer was distilled on the Kugelrohr and was found to be an opaque white solid at room temperature whereas the 1,3-propane diol was a clear and colourless liquid. The dimer was rinsed out with ethyl acetate that was subsequently removed by Kugelrohr leaving some undistilled yellow-orange residue. Yield was 53g (53% yield).

### 4.1.1.4. EG-Suc-EG Degradable Chain Extender

EG-Suc-EG (Ethylene Glycol – Succinic Acid – Ethylene Glycol)

23.6g of succinic acid was heated with 248g of ethylene glycol (1:10 mole ratio) to 170°C for 20 hours in a round-bottomed flask under nitrogen outgassing. This was then heated under vacuum (0.01torr) on the Kugelrohr to remove ethylene glycol at 40-50°C and then increased to 120°C to distil the EG-Suc-EG trimer which came over as a colourless liquid. Yield was 22.7g, (55.1% yield).

### 4.1.1.5. EG-Seb-EG Degradable Chain Extender

EG-Seb-EG (Ethylene Glycol – Sebacic Acid – Ethylene Glycol)

50.5g of sebacic acid was heated with 155g of ethylene glycol (1:10 ratio) to 175°C for 6 hours in a round-bottomed flask under nitrogen outgassing with a side-arm condenser to remove water. This was then heated under vacuum (0.01torr) on the Kugelrohr to
remove ethylene glycol at 80°C. EG-Seb-EG was left in the flask as a colourless liquid which solidified to an opaque solid at room temperature. The sebacic acid-bis(ethylene glycol) could not be distilled due to the high boiling point and the apparent high yield (calculated by mass of residue) is due to the presence of higher oligomers which were not separated. Yield (impure) was 69.61g, (96.0% yield). Characterisation included IR, GPC, $^1$HNMR, $^{13}$CNMR, $^1$H-$^1$H-COSY, DSC.

4.1.1.6. EG-Fum-EG Degradable Chain Extender

EG-Fum-EG, (Ethylene Glycol – Fumaric Acid – Ethylene Glycol)

116g of fumaric acid was heated with 620g of ethylene glycol (1:10 ratio) to 180°C for 4 hours in a round-bottomed flask under nitrogen outgassing. This was then heated under vacuum (0.01torr) on the Kugelrohr to remove ethylene glycol at 40-50°C and then increased to 130-140°C to distil the EG-Fum-EG trimer which came over as a colourless liquid. The liquid crystallised into a white solid with large spherulites. Yield was 117.8g, (57.7% yield)

4.1.2. Synthesis of Polyurethanes from Series 5 and 6

Materials: The macrodiacol soft segments (PCL-macrodiool of 426 molecular weight from ERA Polymers Pty Ltd, PEG-macrodiool of 396 molecular weight from Aldrich, and see section 3.1.1 for synthesis of PGA-macrodiool) were dried at 90°C for 4 hours under vacuum (0.1 torr). The degradable chain-extenders were degassed at 60°C under vacuum (0.1 torr) for 4 hours and HDI (Aldrich) was used as received (colourless). Stannous octoate (Aldrich) was kept moisture-free and used as received.

Method: A mixture of soft segment macrodiacol, degradable chain extender and stannous octoate were weighed into a 100 ml predried polypropylene beaker, covered with aluminium foil and heated to 70°C under nitrogen in a laboratory oven. HDI was weighed in a separate wet-tared predried polypropylene beaker and also heated to 70°C. The HDI was then added to the macrodiacol/degradable chain extender/stannous octoate beaker and stirred manually until gelation occurred (~1min to 5min), at which time the
hot viscous mixture was poured onto a Teflon coated metal tray to cure at 100°C for a period of about 12 hours. The resulting polymers were clear and colourless.

### 4.1.3. Crosslinking of the EG-Fum-EG Polyurethane

This was a ‘proof-of-concept’ experiment designed to prove that crosslinking can occur through the fumaric acid residues in polyurethanes containing EG-Fum-EG. 0.142g of TM5-5 (polyurethane made using HDI, EG-Fum-EG and PCL macrodiol with 65% hard segment) was dissolved in 2.846g dimethylformamide (DMF). This was then filtered using a syringe filter (0.4 micron pores). 0.008g of camphoroquinone and 0.012g of 2-(methylamino)ethylmethacrylate were added (photoinitiator and sensitiser respectively). The liquid was yellow (from the camphoroquinone) and of low viscosity – similar to water. The liquid was quite stable at room temperature with ambient lighting. Upon exposure to intense blue light (3M ESPE Elipar™ Freelight 2, 1000mW/cm², 440-480nm) for 2 minutes, it gelled to a solid that was firm enough to keep its shape when removed from the flask.

### 4.1.4. NMR – GA-EG Transesterification Kinetics

The determination of GA-EG transesterification kinetics was carried out in deuterated DMSO in a NMR tube on a Bruker DRX500 spectrometer (see Section 4.1.6, page 86) for further details. The tube was heated to the reaction temperature and a $^1$HNMR spectrum was taken periodically (each 15min for higher temperatures and each 30min for lower temperatures) typically overnight. 0.4g sample and 0.4g of deuterated DMSO were used for each sample.

### 4.1.5. Melt Flow Index

ASTM D 1238 was used for melt flow index (MFI) determination. Briefly, a 2.16kg weight was used to push the polymer out of a standard diameter nozzle at the specified temperature. The mass of polymer extruded per unit time was measured and converted
to grams of polymer extruded per 10 minutes. The polymer was held at temperature for six minutes before extruding to ensure adequate melting. The instrument used was a MFI-B-289 from I.D.M. (Instrument Design & Manufacture, Dandenong, Australia).

4.1.6. Nuclear Magnetic Resonance

Readings were taken on two different machines: a Bruker Avance 400 spectrometer and a Bruker DRX500 spectrometer. The 400MHz machine was used for routine samples and the 500MHz machine was used for the transesterification kinetics experiments. The Bruker Avance 400 spectrometer operated with a Spectrospin 9.4 T magnet (400.13 MHz ¹H frequency) and is equipped either with a 5 mm ¹H,¹⁹F,¹³C,³¹P QNP autoswitchable probe with z-gradient or a 5 mm inverse ¹H-X BBI autotuning broadband probe. The Bruker DRX500 spectrometer operates with a Spectrospin 11.74 T magnet (500.13 MHz ¹H frequency) and a 5 mm inverse-detection ¹H,¹³C,¹⁵N TXI probe equipped with z-gradients.

All spectra were recorded at 298K (unless otherwise noted) in deuterated DMSO (dimethyl sulfoxide). The spectra are referenced to the residual solvent signal in ¹H and the deuterated solvent signal in ¹³C. The samples for ¹HNMR were made up as a dilute solution of ~2 drops of sample in ~0.7ml deuterated DMSO in a NMR tube. The samples for ¹³CNMR were made up as a solution of ~ 0.4ml sample in ~ 0.4ml deuterated DMSO in a NMR tube.

4.1.7. Compression Moulding

(See Section 2.1.3, page 43)

4.1.8. Instron Tensile Tests

(See Section 2.1.4, page 43)

4.1.9. Differential Scanning Calorimetry (DSC)

(See Section 2.1.5, page 44)
4.1.10. Gel Permeation Chromatography
(See Section 2.1.6, page 44)

4.1.11. Shore Hardness Indentor
(See Section 2.1.7, page 45)
4.2. Results and Discussion

4.2.1. Degradable Chain Extenders

The slowest degrading portion of the polyurethane is usually the hard segment since it is composed entirely of urethane bonds (see Figure 39).

![Figure 39 – Polyurethane hard segment composed of hexamethylene diisocyanate and ethylene glycol](image)

The polyurethanes that were synthesised for series 4 consisted of only 35% soft segment and 65% hard segment by mass, hence one would expect that increasing the degradation rate of the slower degrading hard segment would have a greater impact than altering the soft segment. This leads to a novel concept – it is proposed for the first time that an ester-containing diol can be incorporated into the hard segment of the polyurethane allowing increased degradation. Previously there had been some work in this area where amino acids have been incorporated into chain extenders with a view to enhance enzymatic degradation of the hard segment. These chain extenders were actually diamines rather than diols and form urethane-urea linkages rather than urethane linkages.

The properties of an ideal degradable chain extender were reasoned to be the following:

- Must contain an easily hydrolysable α-hydroxy-ester linkage.
- The chain extender must be a diol (to form urethane bonds with diisocyanate rather than other bonds such as urethane-urea which take longer to degrade)
- Must be as short as possible in order to avoid disruption of the hard segment ordering. If it were too bulky then it would not separate as a hard segment phase since it would no longer have the high regularity and hydrogen bonding which are characteristic of the hard segment.
- The chain extender must degrade to non-toxic products.

The simplest ester that could be proposed for this application that fits all the these criteria is as follows (see Figure 40):
As can be seen in Figure 40, this molecule cannot be designed as a stable diol to be any shorter and still contain an ester. Upon hydrolysis, the hydroxyacetic acid-2-hydroxyethylester (GA-EG, so-named from its expected degradation products) would be expected to give glycolic acid and ethylene glycol as degradation products which are relatively non-toxic (see Figure 41).

If this chain extender were used to make a polyurethane by the reaction of a diisocyanate with the hydroxyl groups on the chain extender then an ester linkage would be incorporated into the hard segment (see Figure 42).

Through incorporation of an ester chain extender into the hard segment, the maximum number of consecutive urethane linkages in the hard segment is only two. For example, when the degradable dimer GA-EG is used as chain extender, every urethane linkage is next to one degradable ester (see Figure 43), i.e. -E-U-U-E-U-U-E-U-U-E-
This compares favourably with the conventional diisocyanate and diol hard segment in which case the entire hard segment is composed of urethane linkages causing considerably slower degradation.

Assuming the esters were to completely degrade before the urethane bonds began to degrade in a polyurethane composed of HDI, GA-EG chain extender, and a poly(glycolic acid) soft segment, only three species would remain - the longest of which can contain only two urethane linkages. This is a reasonable approximation since the ester linkage does hydrolyse much faster than the urethane linkage and one would expect that these compounds would make up a significant proportion of the degradation products. The possible molecular masses are 292, 306 or 320 (see Figure 44).

---

**Figure 43** – Incorporation of the GA-EG chain extender into the hard segment

**Figure 44** – Some expected degradation products of GA-EG chain extender and HDI hard segment
In comparison, if ethylene glycol were used as the chain extender in a polyurethane composed of HDI, EG and PCL400 with 70% hard segment, the average number of consecutive urethane linkages in the hard segment would be just over eight with a molecular weight of more than 1,120 (see Figure 45). This highlights the usefulness of the degradable chain extender in polyurethane hard segments and shows how the degradation may be enhanced without having to reduce the hard segment percentage.

![Figure 45 – Approximate length of a 70% hard segment of HDI and EG with PCL400 as soft segment](image)

Polyesters and polyurethanes of over 1000Mw are usually insoluble in water and are unlikely to be excreted quickly from the body until some urethane linkages have been broken.

<table>
<thead>
<tr>
<th>PCL400/EG/HDI Polyurethane</th>
<th>PCL1000/EG/HDI Polyurethane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hard Segment %</td>
<td>Average consecutive urethane linkages</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>29.6</td>
<td>2.00</td>
</tr>
<tr>
<td>30</td>
<td>2.02</td>
</tr>
<tr>
<td>40</td>
<td>2.84</td>
</tr>
<tr>
<td>50</td>
<td>4.02</td>
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<tr>
<td>60</td>
<td>5.74</td>
</tr>
<tr>
<td>70</td>
<td>8.64</td>
</tr>
<tr>
<td>80</td>
<td>14.44</td>
</tr>
<tr>
<td>90</td>
<td>31.80</td>
</tr>
<tr>
<td>100</td>
<td>∞</td>
</tr>
</tbody>
</table>

Table 24 – Effect of hard segment % and chain length on the number of consecutive urethane linkages

Note in Table 24 that 29.6% is the lowest hard segment percentage possible an HDI – PCL400 polyurethane since this equates to a 1 to 1 ratio of HDI:PCL400.
The ‘average consecutive urethane linkages’ in Table 24 show that the number of consecutive ethylene glycol moieties (and hence urethane linkages) increases with the percentage hard segment and that it also depends upon the length of the soft segment. This is evident in the same table where the PCL1000 polyurethane has more than double the number of consecutive urethane linkages than the PCL400 polyurethane at the same hard segment compositions. In comparison, by using a degradable chain extender there cannot be more than two consecutive urethane linkages no matter what the hard segment percentage is.

Unfortunately however, this proposed degradable chain extender is not commercially available, neither is there a published method for complete synthesis and purification. Therefore an optimised method was devised and it was synthesised specifically for this study. Two literature methods were found which claimed to have synthesised the dimer, however the dimers were not pure:

The first involved synthesis from ethylene oxide and hydrogen peroxide via irradiation with ultra violet light with side-products (acetaldehyde and acetic acid). The reported yield of the glycolic acid-ethylene glycol dimer was good, 90.8%, and the authors also reported the following characterisation of the product: “(b.p. 133°C (10mm), \(d_4^{20}\) 1.0330, \(n_D^{20}\) 1.4162, MR\(_D\) 22.31; calc. 22.36).” The authors also found that saponification of this ester with caustic solution gave ethylene glycol and glycolic acid. There is doubt as to whether GA-EG was indeed the major product formed as claimed or whether this was actually a mixture of oligomers. The refractive index the authors measured did not match the experimental value obtained from the pure dimer that was characterised in this work (1.45604 at 22.4°C).

The second literature method involved condensation of ethylene glycol and glycolic acid, was published in a patent of 1940 and included the following (albeit abbreviated) method:

5 moles of ethylene glycol and 5 moles of powdered polyglycolide (prepared by dehydrating glycolic acid at 100-220°C) were heated to 100-200°C for five hours. Yield was substantially quantitative of a pale amber liquid containing 98-99% of glycol-monoxyglcolate. Characterisation was performed by specific gravity (1.283 at 60°F/60°F) and the saponification number was 460.
The authors (Loder & Teeters, 1940) were incorrect in their assumption that they had synthesised 98-99% glycol-monoglycolate (GA-EG dimer). They obviously based their assumption on the saponification number (460) which equates to an average molecular mass of 122 (theoretical mass of GA-EG is 120). The problem with this assumption is that it neglects the fact that the saponification number would not change during the transesterification since the average molecular weight and the number of ester linkages both remain constant. This is exactly what was found in this study when repeating this experimental method on a smaller scale – (GA:EG, 1:1), see Figure 46. The authors did not have the benefit of GPC to confirm whether or not their product was monodisperse, hence it is understandable that they assumed complete conversion of the reactants to GA-EG.

![Figure 46 – GPC after esterification of EG and GA (1:1) for 12 hours](image)

It was obvious from GPC characterisation of the product that as well as dimer there was a substantial amount of ethylene glycol, trimer, tetramer and even higher oligomers present. In fact, the desired dimer comprises much less than one half of the reaction mixture with the remainder being EG and higher oligomers. The authors (Loder & Teeters, 1940) did not purify their product nor did they characterise it fully. In the transesterification reaction of poly(glycolic acid) with ethylene glycol there is an equilibrium that affects the final composition of the product. In the reaction mixture any hydroxyl end-group can initiate transesterification with any ester on an adjacent chain at a sufficiently high temperature (see Figure 47).
When EG (Mn 62) reacts with the PGA oligomer (Mn 398) (see Figure 47), the EG could react with any of the ester groups, in this case it has transesterified to form two identical oligomers (Mn 230 each). One of the oligomers or monomers increases in size and the other decreases in size in all of the possible transesterification reactions except in the case of auto-transesterification where a hydroxyl could conceivably react with an ester from its own chain without altering its length. It is possible to form cyclic oligomers by transesterification as well as by condensation (see Figure 48).
It is not expected that there would be a significant amount of cyclic material present since at low molecular weights there are a high number of hydroxyl groups present to transesterify and open the cyclic structure. There are reports in the literature where cyclic oligomers of poly(lactic acid) formed during polyesterification and have been isolated and characterised.\textsuperscript{156}

4.2.2. Kinetics and Effect of Transesterification in Dimer Syntheses

No species in the reaction mixture, including the desired dimer, remain inert at high temperature. For example, if a pure sample of dimer (GA-EG) were heated to 180°C, (a temperature at which transesterification is known to rapidly occur), at equilibrium the mixture would no longer contain only dimer but rather it would contain a mixture of oligomers and ethylene glycol: EG, GA-EG, GA-GA-EG and GA-EG-GA, GA-GA-EG and GA-GA-EG-GA etc. Figure 49 gives the first step of the transesterification of the GA-EG dimer where 2GA-EG react to form EG and GA-EG-GA. Of course the asymmetrical trimer (GA-GA-EG) is approximately equally likely to occur.

\[
\text{Figure 49} \quad \text{ Transesterification of GA-EG to give EG and GA-EG-GA}
\]

The GA-EG-GA trimer that is formed in Figure 49 can react with another dimer to form a tetramer or could react with an EG to revert to two dimers.

In reality the reaction mixture is often complicated by the presence of some water (even trace amounts) which reacts to form carboxylic acid functional groups that allow the presence of the GA monomer, which is totally absent in this idealised case.
This transesterification of the GA-EG dimer was shown to occur by heating a sample of pure dimer in deuterated DMSO for extended periods of time while measuring the formation of ethylene glycol through $^1$HNMR to track the change in the sample. Figure 50 shows the change over time in the $^1$HNMR spectra of GA-EG at 150°C, the peak due to the methylene protons of ethylene glycol at 3.48ppm can be seen to increase over time. There is a broad peak at 4.16ppm at 900 seconds and this moves to 4.45ppm by 69840 seconds (19 hours and 24 minutes). The broad peak is due to hydroxyl protons of the dimer, and the shape and position of the peak is well known to be temperature dependant. Ethylene glycol is used to calibrate temperature for $^1$HNMR because of this inherent property.

![Figure 50](image)

**Figure 50** – $^1$HNMR spectra of GA-EG over time at 150°C in deuterated DMSO

The method chosen to measure the formation of ethylene glycol was to integrate a peak that was known to be due to ethylene glycol and to compare it with one known to be due to the dimer. The singlet at 3.48ppm was chosen to represent the amount of ethylene glycol (from the methylene group) and the triplet at 3.63ppm was chosen to represent the dimer (see Figure 51 where the corresponding protons are labelled ‘a’ and ‘b’
respectively). The reason for monitoring these specific protons is due to the fact that they are relatively free from overlapping peaks at all temperatures unlike most of the other protons which overlap with hydroxyl protons at some temperatures.

![Figure 51 – GA-EG dimer and ethylene glycol – choice of protons for monitoring transesterification](image)

Figure 51 – GA-EG dimer and ethylene glycol – choice of protons for monitoring transesterification

![Figure 52 – Formation of EG during transesterification of GA-EG dimer at various temperatures](image)

Figure 52 – Formation of EG during transesterification of GA-EG dimer at various temperatures

It can be seen in Figure 52 that the error in the graph from 0-5000 seconds is high, possibly due either to the sample warming to temperature or maybe to the high error involved in integrating the very small EG peak (see Figure 50). Each of the data points in Figure 52 correspond to one ¹HNMR plot after a certain time and at a given temperature. As was expected, each of the series appear to approach the same value but at different rates. The initial straight part of each graph (after the initial ‘roughness’) was replotted to determine the gradient at each temperature (see Figure 53) and hence the rate constants, ‘k’ and these k values were then plotted as an Eyring plot (see Figure 54).
Figure 53 – First-order rate plots of EG formation during transesterification of the GA-EG dimer

Figure 54 – Eyring plot for the transesterification reaction of GA-EG

The Eyring plot should give a straight line that can be used to interpolate the rate of reaction (sans catalyst) over these temperatures.
4.2.3. Theoretical Modelling of the Transesterification Reaction

Allowing three assumptions and approximations, we can statistically predict the amount of each product that will be present at equilibrium based on the starting ratio and correlate this with the observed values. The first assumption is that each hydroxyl group in the reaction is as reactive towards transesterification as each other hydroxyl despite the chain length. The second assumption is that each ester is as reactive as each other ester whether on a short or long chain. The third assumption is that there is no water or carboxylic acid present in the reaction mixture.

Given a 1:1 starting ratio of EG:GA, for each solitary EG present there must be an extra GA to be found in a trimer or higher oligomer (See Table 25), therefore the molar fraction of EG can be expressed as being equivalent to the sum of the product of the oligomers higher than dimer multiplied by a factor determined by the amount of GA:

\[ n_{EG} = \sum n_3 + 2n_4 + 3n_5 + \ldots + (y-2)n_y \]

Equation 3 – Amount of EG present in a 1:1 EG:GA esterification

Where, \( n_{EG} = \) moles of ethylene glycol in the reaction mixture
- \( n_3 = \) moles of trimer
- \( n_4 = \) moles of tetramer
- \( n_5 = \) moles of pentamer
- \( n_y = \) moles of oligomer \( y \) units long

The reason that the dimer itself is not included in the sum is because it does not have an extra GA above the average 1:1 ratio, hence it does not contribute to the number of solitary EG present.
### Table 25 – Composition of glycolic acid / ethylene glycol oligomeric diols

<table>
<thead>
<tr>
<th>Oligomer</th>
<th>Statistically possible compositions</th>
<th>GA</th>
<th>Esters</th>
<th>OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomer</td>
<td>EG, (note that GA is not possible in the absence of H₂O)</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Dimer</td>
<td>EG-GA, GA-EG</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Trimer</td>
<td>EG-GA-GA, GA-EG-GA, GA-GA-EG</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>n-mer</td>
<td>n combinations</td>
<td>n-1</td>
<td>n-1</td>
<td>2</td>
</tr>
</tbody>
</table>

The above series of rules regarding the transesterification were devised by the author of this thesis and Alf Uhlherr (Mol. Sci. CSIRO) wrote a computer program (see Appendices) to simulate the transesterification reaction of poly(glycolic acid) (sans water) and ethylene glycol at different feed ratios. This program gave the data of Table 26 and Figure 55.

### Table 26 – Computer generated data for predicted products of EG / GA esterification

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Predicted Products</th>
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</thead>
<tbody>
<tr>
<td>GA:EG</td>
<td>Fraction GA</td>
</tr>
<tr>
<td>1:9</td>
<td>0.1000</td>
</tr>
<tr>
<td>1:8</td>
<td>0.1111</td>
</tr>
<tr>
<td>1:7</td>
<td>0.1250</td>
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<tr>
<td>1:6</td>
<td>0.1429</td>
</tr>
<tr>
<td>1:5</td>
<td>0.1667</td>
</tr>
<tr>
<td>1:4</td>
<td>0.2000</td>
</tr>
<tr>
<td>1:3</td>
<td>0.2500</td>
</tr>
<tr>
<td>1:2</td>
<td>0.3333</td>
</tr>
<tr>
<td>1:1</td>
<td>0.5000</td>
</tr>
<tr>
<td>2:1</td>
<td>0.6667</td>
</tr>
<tr>
<td>3:1</td>
<td>0.7500</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fraction GA</th>
<th>EG 2mer</th>
<th>3mer</th>
<th>4mer</th>
<th>5mer</th>
<th>6mer</th>
<th>7mer</th>
<th>8mer</th>
<th>%dimer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.902</td>
<td>0.087</td>
<td>0.010</td>
<td>0.001</td>
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<td>88.8</td>
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<td>0.891</td>
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<td>0.877</td>
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<td>0.015</td>
<td>0.002</td>
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<td>0.002</td>
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<td>0.047</td>
<td>0.013</td>
<td>0.003</td>
<td>0.001</td>
<td></td>
<td></td>
<td>73.6</td>
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<td>0.027</td>
<td>0.009</td>
<td>0.003</td>
<td>0.001</td>
<td></td>
<td>64.6</td>
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<td>0.521</td>
<td>0.233</td>
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<td>0.060</td>
<td>0.033</td>
<td>0.017</td>
<td>0.009</td>
<td>0.005</td>
<td>49.2</td>
</tr>
<tr>
<td>0.364</td>
<td>0.207</td>
<td>0.136</td>
<td>0.091</td>
<td>0.063</td>
<td>0.044</td>
<td>0.030</td>
<td>0.020</td>
<td>35.0</td>
</tr>
<tr>
<td>0.281</td>
<td>0.178</td>
<td>0.128</td>
<td>0.097</td>
<td>0.075</td>
<td>0.058</td>
<td>0.043</td>
<td>0.033</td>
<td>29.1</td>
</tr>
</tbody>
</table>

Graphing the fraction of GA in the reaction mixture against the predicted percentage of dimer in the product (from Table 26) it can be seen in Figure 55 that there is an approximately linear relationship between expected yield and fraction of GA.
Predicted yield of dimer

\[ y = -92.504x + 96.826 \]

\[ R^2 = 0.9977 \]

Figure 55 – Graph of expected yield of dimer vs fraction of glycolic acid in feed

While it appears in Figure 55 to be a simple matter to obtain a high yield of dimer simply by adding a higher proportion of ethylene glycol, it is not so simple. A practical limit exists since very large excesses of ethylene glycol are required to achieve high yields and the excess glycol must be removed by vacuum distillation, which is a time-consuming process.

4.2.4. Refinement of the Degradable Chain Extender

The length of the chain extender is expected to have a marked effect on the properties of a polyurethane. It has been shown that the inter-chain hydrogen bonding in the hard segment between the amine and carbonyl of the urethane bonds provides polyurethanes with a high degree of strength and crystallinity. The length of the chain extender can be expected to have an effect on the amount of inter-chain hydrogen bonding in the hard segment (see Figure 56 and Figure 57), hence it could be an important control of the properties of the polyurethane.

Figure 56 shows the structure of a hard segment composed of the GA-EG degradable chain extender and hexamethylene diisocyanate. Because of the length of the chain extender, the polyurethane contains approximately half the hydrogen bonding as a chain
extender that is an even number of atoms long (see Figure 57). This is expected to affect the properties since more hydrogen bonding should result in a stronger and more crystalline hard domain. This is a simplified representation of the hard segment but it should also be kept in mind that hydrogen bonding could also occur between the carbonyl of the chain extender and the urethane linkage however it would result in a lower number of hydrogen bonds, which makes it energetically less likely to occur.

In Figure 56 there are two hydrogen bonds per seventeen atoms in length and this can be compared with Figure 57 where there are two hydrogen bonds per nine atoms in length. One would certainly expect the physical, thermal and degradative properties to be affected by doubling the amount of hydrogen bonding in the polyurethane. Having said that, it is not necessarily better to have a stronger material with a greater hydrogen bonding density for degradable applications since the penetration of water would be hindered and hence degradation due to hydrolysis also retarded. It is useful to compare the two materials in order to gain a better understanding of how the properties may be tailored using this effect. A stronger polyurethane means that scaffolds made from it can
be thinner and retain the same strength as a weaker material, leading to higher surface area per volume and hence faster degradation (assuming all else is equal).

The structures of some of the degradable chain extenders designed and synthesised for this study are shown in Figure 58 and these will be discussed in some detail in the following sections.

![Figure 58 – Structure of the Degradable Chain Extender diols](image-url)
4.2.5. GA-EG Degradable Chain Extender

![Structure of glycolic acid-ethylene glycol degradable chain extender (GA-EG)](image)

**Figure 59** – Structure of glycolic acid-ethylene glycol degradable chain extender (GA-EG)

In order to produce a higher yield and a pure GA-EG dimer it was necessary to improve on literature methods. Better yields were expected by changing from a 1:1 ratio of GA:EG to a higher proportion of EG as the excess EG should transesterify oligomers to smaller units, hence increasing the yield of dimer. This would leave unreacted EG which could be removed by vacuum distillation.

After optimising various conditions such as reaction times and temperatures, the following method was used to conveniently obtain a high-purity product:

- Condensation polymerisation of glycolic acid with removal of water
- Transesterification using excess ethylene glycol
- Fractional distillation under vacuum

Fortunately, ethylene glycol can be effectively distilled under high vacuum (0.01-0.001 torr) at only ~40°C, whereas the dimer was distilled at ~70-80°C under the same reduced pressure which fortunately avoids high temperatures where further transesterification would be a major problem (as shown in section 4.2.2). It was found that the fractional distillation step had the added advantage of separating any coloured impurities from the product leaving a clear and colourless liquid dimer that was characterised by $^1$HNMR, $^{13}$CNMR, IR and GPC to be of high purity. The coloured impurities are pyrolysis products caused by degradation of the hydroxy acid due to excess heat and have been shown to contain a complex mixture of products by GPC, $^1$HNMR and $^{13}$CNMR (see Figure 60 and Figure 64).
It can be seen in Figure 61 that there are many more different carbon environments than in the pure dimer shown later in Figure 66. Interestingly after distillation of an originally yellowish solution on a scale of ~1 litre, the residue darkens as it concentrates through brown to a black coloured liquid with only a couple of grams remaining, even
so there is a large proportion of dimer still present (compare with Figure 66) which shows the impurities that contribute to the colour are very intense.

Figure 62 shows the quantities used and the masses recovered for GA-EG dimer synthesis (GA-EG2, see section 4.1.1.1 page 81). Once the dimer has been distilled, it is possible to reuse the oligomer residues and the distilled ethylene glycol to create more dimer, they need not be wasted. It is therefore possible to devise a continuous process to synthesise the dimer involving multiple streams where the distilled ethylene glycol and higher molecular weight residues could be reinjected into the starting feed to transesterify to form more dimer. Since the residual oligomer is already dehydrated, it would be returned at the second stage of the process (transesterification) rather than the first stage (dehydration).

![Figure 62 – Schematic diagram of GA-EG dimer synthesis (GA-EG2)](image)

It would be possible to reuse the ethylene glycol in order to minimise waste and costs if ever the process was scaled up beyond bench scale. However, in a continuous process
there would be some additional complications which are not seen in this single batch process. One of these complications is that the glycolic acid is not entirely dehydrated and hence still contains some water (as well as uncondensed carboxylic acid end-groups which react with ethylene glycol to form water). Therefore the water would be distilled along with ethylene glycol during the fractional distillation and wet ethylene glycol would be returned unless there is an additional drying stage. Simply drying the unreacted ethylene glycol before returning it to the reaction would suffice. The drying and return of ethylene glycol is shown in Figure 63 with a heavy dotted line (along with the return of oligomer residue).

A second complication is that coloured impurities gradually build up in the oligomer residue, and it would have to be cleaned to prevent contamination that might occur if the concentration of pyrolysis products built up too high. The cleaning could be achieved by using a secondary bleed circuit that transesterifies the residue with ethylene glycol rather than returning it to the primary circuit, distils the dimer, returns the ethylene glycol and disposes of the impurities which have been shown to be of negligible mass. This secondary circuit is shown in Figure 63 with light dotted lines. The heavy dotted lines in Figure 63 details the primary circuit and show how the process could be improved to avoid wasting ethylene glycol and glycolic acid residues.
Figure 63 also shows the relative number of moles of glycolic acid and ethylene glycol during the reaction and where they end up. The percentages are based on the reaction GA-EG2 (see page 81). The bold text boxes represent product being removed or reactants being added to the system. The percentages are in mole percent rather than mass since water is lost during the reaction which affects the mass. Overall there was a 1.5% discrepancy in the overall mass balance and was most likely due to entrained glycolic acid lost during the dehydration since ~99 mol% of ethylene glycol was accounted for but only ~96 mol% of glycolic acid. The water collected during the dehydration was strongly acidic according to universal indicator test strips showing the likely presence of glycolic acid. It is certain that some material was also lost on glassware since solvents were not used to rinse. Solvents were avoided in order to avoid
contamination as well as to minimise the number of steps (solvent removal can be time-consuming, difficult to completely remove, and expensive), hence it is advantageous if the process can do without the use of solvents. The loss of material could be minimised with further optimisation of conditions, especially with a view to avoiding loss of glycolic acid during the condensation stage. The dotted lines in Figure 63 represent how the process could be made continuous by returning the EG and glycolic acid oligomer to the reaction. This has been shown possible in a batch reaction by reacting the residual oligomer with EG to create more dimer which was then purified.

The purified dimer (GA-EG1) was characterised and shown to be of high purity by IR, $^1$HNMR and $^{13}$CNMR.

The IR of the GA-EG chain extender is very similar to that of the lactic acid-ethylene glycol dimer, LA-EG (Figure 68), with the major exception being LA-EG contains a methyl substituent which shows C-H and CH$_3$ bending vibrations as marked on the spectrum.

**Figure 64** – Infrared Spectrum of GA-EG degradable chain extender (GA-EG1)
It is interesting that the protons labelled “a” and “e” in Figure 65 display splitting into triplets due to the proximity to the glycolic acid methylene group.

![Figure 65 – ^1H NMR Spectrum of GA-EG degradable chain extender (GA-EG1)](image)

**Figure 65 – ^1H NMR Spectrum of GA-EG degradable chain extender (GA-EG1)**

Additional notes or explanations can be added here if necessary.
The GA-EG dimer was a clear and colourless liquid of low viscosity at room temperature. It is stable at room temperature but is not stable at elevated temperatures as was shown previously in Figure 52.

### 4.2.6. LA-EG Degradable Chain Extender

![Structure of lactic acid-ethylene glycol degradable chain extender (LA-EG)](image)

**Figure 67 – Structure of lactic acid-ethylene glycol degradable chain extender (LA-EG)**

LA-EG was synthesised by a similar method to GA-EG: condensation polymerisation of LA, transesterification using excess EG followed by fractional distillation. The only variations were the temperatures and times for each step (see method, section 4.1.1.2 page 82).

![Infrared Spectrum of LA-EG degradable chain extender](image)

**Figure 68 – Infrared Spectrum of LA-EG degradable chain extender**
In Figure 68, only the peaks that show differences from the GA-EG IR spectrum (Figure 64) are labelled. The peak occurring at about 2890 (the right-hand peak of the triplet) is due to the tertiary C-H stretch and comparison with Figure 64 shows it is absent in the GA-EG dimer. The CH\textsubscript{3} bending vibrations are marked on Figure 68 and do not occur in GA-EG, the unlabelled peaks are similar to GA-EG in Figure 64 with the exception of the C-O stretch at ~1100. The C-O stretches no longer overlap since the methyl group causes a shift to longer wavelengths of one of the C-O stretches.

![Figure 68 - IR Spectrum](image)

**Figure 68** – IR Spectrum of GA-EG dimer

The 1H NMR spectrum of the LA-EG dimer (Figure 69) also shows splitting of the hydroxyl protons, this time into a doublet, “a”, and triplet, “f”, as would be expected. This is confirmation that the coupling is occurring between the hydroxyl proton and the protons on the adjacent carbon.

![Figure 69 - 1H NMR Spectrum](image)

**Figure 69** – 1H NMR Spectrum of LA-EG degradable chain extender
The LA-EG dimer was a clear and colourless liquid of low viscosity at room temperature very similar to the GA-EG dimer.

### 4.2.7. GA-1,3-PD Degradable Chain Extender

A dimer was synthesised using 1,3-propane diol and glycolic acid for comparison with the GA-EG dimer as previously mentioned.

The only difference between GA-EG and GA-1,3-PD is an extra methylene unit. The methylene C-H stretch in Figure 72 (GA-1,3-PD) is considerably larger than the same peak in Figure 64 (GA-EG) which is expected since there is one extra methylene unit.
**Figure 72** – Infrared Spectrum of GA-1,3-PD degradable chain extender

**Figure 73** – $^1$H NMR Spectrum of GA-1,3-PD degradable chain extender
The GA-1,3-PD degradable chain extender dimer was a white and somewhat waxy solid at room temperature unlike the slightly shorter GA-EG which was liquid.

### 4.2.8. EG-Suc-EG Degradable Chain Extender

There are a number of different ways to cause the hard segment to degrade more quickly. One of these is to increase the proportion of esters in the hard segment. One would expect that there would be a limiting number of consecutive esters that can be incorporated into a hard segment before the two-phase morphology was lost, hence any degradable ester to be used for such an application would have to be reasonably short. Chain extenders are typically less than 300 molecular weight and soft segments which show phase separation are generally over 400 molecular weight. The previous three degradable chain extenders all insert one ester into the hard segment for every two urethane linkages. It was therefore proposed to design a chain extender capable of inserting exactly two ester linkages for every two urethane linkages to enhance the rate of degradation.

It is not possible to synthesise high yields of trimer from a diol and hydroxy-acid due to the nature of the reaction (see section 4.2.3) therefore an alternative method was
devised. A diacid and excess diol was employed rather than using a hydroxy-acid (see Figure 76).

The R group in Figure 76 could be chosen from any of the diacids shown in Table 27 however one would expect that there might be a limiting size of the trimer if it needs to be distilled. In order to obtain a high purity trimer by this method it is essential that the trimer be distilled since there will always be some tetramer and higher oligomers present at equilibrium (see Figure 77).
<table>
<thead>
<tr>
<th>Name</th>
<th>Common Name</th>
<th>Molecular Formula</th>
<th>Melting Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethane dioic acid</td>
<td>Oxalic acid</td>
<td>HOOC(\text{COOH})</td>
<td>189.5</td>
</tr>
<tr>
<td>Propane dioic acid</td>
<td>Malonic acid</td>
<td>HOOC((\text{CH}_2))(\text{COOH})</td>
<td>135</td>
</tr>
<tr>
<td>Butane dioic acid</td>
<td>Succinic acid</td>
<td>HOOC((\text{CH}_2))(\text{COOH})</td>
<td>185-187</td>
</tr>
<tr>
<td>Pentane dioic acid</td>
<td>Glutaric acid</td>
<td>HOOC((\text{CH}_2))(\text{COOH})</td>
<td>97.5-98</td>
</tr>
<tr>
<td>Hexane dioic acid</td>
<td>Adipic acid</td>
<td>HOOC((\text{CH}_2))(\text{COOH})</td>
<td>152</td>
</tr>
<tr>
<td>Heptane dioic acid</td>
<td>Pimelic acid</td>
<td>HOOC((\text{CH}_2))(\text{COOH})</td>
<td>105.7-105.8</td>
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<td>Suberic acid</td>
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<td>140-144</td>
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<td>Azelaic acid</td>
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<td>Decane dioic acid</td>
<td>Sebacic acid</td>
<td>HOOC((\text{CH}_2))(\text{COOH})</td>
<td>134.5</td>
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</table>

Table 27 – Formula and melting point of common diacid monomers

The melting points shown in Table 27 are included to show the effect of chain length on the properties. Diacids that have an even number of carbons in the chain exhibit higher melting points than diacids that have an odd number along with a general decrease in melting point as the chain length increases. Obviously the melting point is proportional to the intermolecular forces, which are higher for even-numbered chain lengths. One might reasonably expect to see a difference in polyurethanes made from chain extenders containing these diacids depending on this odd / even trend due to the effect that would have on hydrogen bonding in the hard segment.

Succinic acid was chosen for use as a precursor from amongst the other diacids due to the following reasons:

- It is a natural metabolite and known to be non-toxic *in vivo*.
- It is reasonably short and hence should be less compatible with the soft segment than the longer diacids
- Oxalic acid while shorter is relatively toxic
- Malonic acid has an odd number of atoms in the chain, and although the smallest diacid apart from oxalic acid, it would be expected to be less crystalline in the hard segment than an even-numbered diacid.
- Succinic acid has an even number of units in the chain and could be expected to exhibit greater crystallinity.
Succinic acid was heated in the presence of excess ethylene glycol with the removal of condensed water. Once the condensation was complete and the water formation ceased, the EG-Suc-EG trimer was separated by fractional distillation under vacuum. EG-Seb-EG which contains sebacic acid was also synthesised and is shown in the methods at the beginning of this chapter but it was found to not distil even at high temperature (180°C) and high vacuum (0.001 torr) due to its relatively high molecular weight. For this reason it could not be obtained as pure as EG-Suc-EG and was not used except to show the limitation of distillation for this application.

Figure 78 – Comparison of EG-Seb-EG and EG-Suc-EG
The trimer EG-Suc-EG was a clear and colourless liquid of low viscosity at room temperature similar to the GA-EG and LA-EG dimers.
4.2.9. EG-Fum-EG Degradable Chain Extender

The fumaric acid trimer is almost identical in structure to the succinic acid trimer from the previous section with the exception of the unsaturated bond. The fumaric acid trimer with ethylene glycol (EG-Fum-EG) adds the attractive ability to the polymer of being able to crosslink through the double bond under certain conditions.\textsuperscript{183} Hence, the linear polymer chains could conceivably be thermally processed and then crosslinked upon demand.

**Figure 81** – Structure of fumaric acid/ethylene glycol diol degradable chain extender (EG-Fum-EG)

**Figure 82** – \textsuperscript{1}HNMR Spectrum of EG-Fum-EG degradable Chain Extender (EG-Fum-EG)
Figure 83 – $^{13}$CNMR Spectrum of EG-Fum-EG degradable Chain Extender (EG-Fum-EG)

The trimer EG-Fum-EG was beautifully crystalline, white, hard solid with large spherulites at room temperature and melted to a clear and colourless low-viscosity liquid upon gentle warming (melting point 39-41°C).
4.3. Polyurethanes Synthesised Using Degradable Chain Extenders

A series of polyurethanes (series 5) was made using HDI that contained the novel degradable chain extenders in place of ethylene glycol. In the cases where a soft segment was employed, poly(ε-caprolactone) macrodiol of molecular weight 426 was used and it constituted 35% by weight of the polymer.

<table>
<thead>
<tr>
<th>Name</th>
<th>Chain extender</th>
<th>Hard Segment %</th>
<th>Elongation at Break %</th>
<th>Young’s Modulus (MPa)</th>
<th>Upper Tensile Strength (MPa)</th>
<th>Hardness (Shore D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM5-1</td>
<td>GA-EG</td>
<td>65</td>
<td>605 ± 126</td>
<td>205 ± 14</td>
<td>14 ± 4</td>
<td>49</td>
</tr>
<tr>
<td>TM5-2</td>
<td>LA-EG</td>
<td>65</td>
<td>935 ± 39</td>
<td>58 ± 2</td>
<td>4.8 ± 0.3</td>
<td>32</td>
</tr>
<tr>
<td>TM5-3</td>
<td>GA-1,3-PD</td>
<td>65</td>
<td>700 ± 350</td>
<td>181 ± 32</td>
<td>12 ± 1</td>
<td>54</td>
</tr>
<tr>
<td>TM5-4</td>
<td>EG-Suc-EG</td>
<td>65</td>
<td>18 ± 4</td>
<td>225 ± 35</td>
<td>14 ± 1</td>
<td>47</td>
</tr>
<tr>
<td>TM5-5</td>
<td>EG-Fum-EG</td>
<td>65</td>
<td>657 ± 156</td>
<td>135 ± 13</td>
<td>22 ± 5</td>
<td>46</td>
</tr>
<tr>
<td>TM5-6</td>
<td>GA-EG</td>
<td>100</td>
<td>712 ± 64</td>
<td>12 ± 4</td>
<td>2.7 ± 0.7</td>
<td>38</td>
</tr>
<tr>
<td>TM5-7</td>
<td>LA-EG</td>
<td>100</td>
<td>187 ± 89</td>
<td>406 ± 178</td>
<td>12 ± 4</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 28 – Series 5: Physical properties of polyurethanes containing degradable chain extenders

<table>
<thead>
<tr>
<th>Name</th>
<th>Mn</th>
<th>Mw</th>
<th>MP</th>
<th>Polydispersity</th>
</tr>
</thead>
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<tr>
<td>TM5-1</td>
<td>20,208</td>
<td>34,568</td>
<td>36,452</td>
<td>1.71</td>
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<tr>
<td>TM5-2</td>
<td>25,540</td>
<td>45,701</td>
<td>51,540</td>
<td>1.79</td>
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<tr>
<td>TM5-3</td>
<td>22,872</td>
<td>32,878</td>
<td>34,326</td>
<td>1.38</td>
</tr>
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<td>TM5-4</td>
<td>21,482</td>
<td>29,060</td>
<td>29,862</td>
<td>1.35</td>
</tr>
<tr>
<td>TM5-5</td>
<td>32,199</td>
<td>80,476</td>
<td>51,670</td>
<td>2.50</td>
</tr>
<tr>
<td>TM5-6</td>
<td>18,029</td>
<td>25,838</td>
<td>24,181</td>
<td>1.43</td>
</tr>
<tr>
<td>TM5-7</td>
<td>39,079</td>
<td>89,140</td>
<td>62,553</td>
<td>2.28</td>
</tr>
</tbody>
</table>

Table 29 – Series 5: Molecular weight of polyurethanes containing degradable chain extenders

In each case an isocyanate index of 1.00 was used in order to prevent excessive crosslinking. Higher molecular weight polymers would have been achieved by increasing the isocyanate index however this was not desirable for thermal processing applications which are one of the objectives of this study. While the physical properties of the polymers improve substantially with molecular weight and thus could be improved by optimising synthesis conditions, however synthesis conditions were kept the same as for series 2 for consistency.
While the properties of the materials shown in Table 28 are not particularly surprising and might be considered predictable, it is a good confirmation of the expected theoretical trends. For example in comparing the effect of the GA-EG chain extender to the LA-EG chain extender on the 65% hard segment polyurethane (TM5-1 and TM5-2 respectively), one can see that LA-EG results in a more amorphous material due to the methyl side group, displaying characteristic relative softness and lower Young’s modulus than TM5-1.

**Figure 84** – Stress-strain tensile curve for TM5-1


**Figure 85** – Stress-strain tensile curve for TM5-2

**Figure 86** – Stress-strain tensile curve for TM5-3

Despite TM5-3 being reasonably weak when compared with polymers from previous chapters and of quite low molecular weight (Mn 20,208), the oriented fibre gave four to five-fold better tensile strength (see Figure 87).
The tensile stress-strain curve of TM5-4 showed only the elastic portion of the three regions (see Figure 24, page 49) with early fracture and none of the sample displayed cold-drawing to any extent.

Figure 87 – Stress-strain tensile curve for an oriented fibre of TM5-3

Figure 88 – Stress-strain tensile curve for TM5-4
It is not coincidence that the strongest and weakest materials were also the highest and lowest molecular weights. The fumaric acid double bond is thought to have crosslinked to some degree after polymerisation since the melt-pressed specimen contracted in length and width and thickened which is often evident in material that has crosslinked after synthesis. The stress-strain curve of TM5-5 resembled those of Series one with the characteristic three regions – elastic, cold-drawing and plastic.

In order to show that the fumaric acid-containing polyurethane (TM5-5) was indeed crosslinkable through the fumarate alkene, an experiment was devised to show whether or not this can occur (see method at start of chapter for full details). In short, TM5-5 was dissolved in DMF (1 part polymer to 20 parts DMF) and filtered to remove any undissolved or crosslinked matter. A sensitis er and photoinitiator were added and the resulting liquid was stable and of low viscosity. Upon exposure to intense blue light the thin solution turned to a firm, jellylike solid which was strong enough to support its shape when removed from the container despite being about 95% DMF by weight. This shows that the polyurethane can be crosslinked on demand and further investigation would be appropriate to determine the extent of this highly desirable property.
TM5-6 and TM5-7 are different to other polymers in this series in that they are composed only of chain extender and diisocyanate and do not contain 35% PCL macromdiol. The properties are expected to differ from the others and differences can be seen in the stress-strain curves (see Figure 90 and Figure 91).

![Stress-strain tensile curve for TM5-6](image)

**Figure 90** – Stress-strain tensile curve for TM5-6

TM5-6 displays a very low Young’s modulus of only 12.5 MPa and was a very soft material at room temperature. TM5-7 on the other hand was stronger but didn’t have as good elongation at break which led both materials to have similar toughness (area under the curve which corresponds to the energy required to break the sample).
For thermal processing applications it is important for a material to have a high melt flow index. For a material to be suitable for FDM it must have a melt flow index of $>$ ~10g/10min. Representative polymers from series 5 were tested at 175°C to determine suitability:

<table>
<thead>
<tr>
<th>Material</th>
<th>Temperature (°C)</th>
<th>MFI (g/10min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM5-1</td>
<td>175</td>
<td>139</td>
</tr>
<tr>
<td>TM5-2</td>
<td>175</td>
<td>51</td>
</tr>
<tr>
<td>TM5-3</td>
<td>175</td>
<td>306</td>
</tr>
</tbody>
</table>

*Table 30 – Melt Flow Index of selected polymers from series 5*

It can be seen that all three of the polymers tested showed MFI’s greater than 10g/10minutes. This is good since the polymers could therefore be used at lower temperatures than 175°C, and thus avoid thermal degradation of the polymer.
4.4. Summary and Conclusion

A new range of degradable chain extenders were made by polycondensation, transesterification followed by purification by fractional distillation. These chain extenders were used in the synthesis of polyurethanes. The degradable chain extenders reported in this study included both dimer and trimer diols which contained hydrolysable ester linkages: GA-EG, LA-EG, GA-1,3PD, EG-Suc-EG, and EG-Fum-EG. The monomers used in these degradable chain extenders included hydroxy-acids such as glycolic acid, diacids such as succinic acid, and diols such as ethylene glycol. Polyurethanes were made using the degradable chain extenders and reasonable molecular weights were attained (Mw from 25,838 to 89,140).

It was shown that EG-Seb-EG (based on sebacic acid) was impossible to distil, even at temperatures up to 180°C and 0.001 torr. This showed a limit to the range of possible degradable chain extenders that can be purified by this particular method.

A polyurethane containing a fumaric acid-based chain extender (EG-Fum-EG) was shown to crosslink on demand under certain conditions. This is an interesting property which may have potential applications in modifying the properties of the polymer after thermal processing or for curing in situ.
5. Degradation and Cell Growth Studies

All of the polymers synthesised in this study have been tested in this chapter for *in vitro* biocompatibility and biodegradability, which was an original objective of this study.

5.1. Materials and Methods

5.1.1. Cell Culture Biocompatibility Testing

Polymer samples were melt-pressed between two metal plates to form a thin sheet between 100-400µm in thickness. In triplicate, these sheets were cut into 10mm x 10mm squares and glued onto glass coverslips using cyanoacrylate (Wanbang, ASTM D-4236) leaving an exposed upper polymer surface. The coverslips were used to prevent the polymer sheets floating in the culture medium. The coverslips bearing the polymer were sterilised by washing sequentially in 70% ethanol, PBS buffer and distilled water before being placed in six-well cell-culture plates.

Primary ovine fibroblasts were grown from explants of aortic valves from 1-year old animals to confluence in a 75cm² flask (3x10⁶ cells). They were passaged up to 10 times before use. The cells were harvested by trypsinising and mixed with DMEM (Dulbecco’s Modified Eagle’s Medium) to provide a suspension of fibroblast cells (120ml). 2ml of the cell suspension was added to each of the samples (5,000 cells cm⁻²) as well as a control glass coverslip with no polymer.

The plates were incubated in a 5% CO₂ atmosphere at 37°C and the medium was changed in all samples on a weekly basis.

Digital photographs of each sample were taken at 50x microscope magnification daily for comparison purposes. The camera used was an Olympus C-5050 Zoom, 5.0 megapixels with an adapter (DCA-TCP (ISSCO, Sydney Australia)) fitted to an inverted microscope (Olympus CK2).
5.1.2. Accelerated Degradation study

A degradation study was performed on a number of selected samples varying the soft segment composition and keeping the hard segment percentage and composition constant. The polymers used were series 3. Melt-pressed sheets of material (~1mm thick) were cut to approximately 1cm x 2cm, weighed, and the molecular weight of each polymer was measured by GPC. The weighed sheets were placed in a Teflon® cage which allowed buffer freely in and out through ~3mm holes, yet trapped the sample inside to prevent flotation. A 1 litre Schott bottle filled with buffer (0.01M phosphate buffered saline (PBS), pH 7.4) and containing the Teflon-encased samples was placed stationary in an oven at 70°C.

5.1.3. Detailed Degradation study

A detailed degradation study was performed according to ASTM F-1635-04 and included all of the polyurethanes used in this thesis for comparison. Briefly, GPC molecular weight of melt pressed specimens (~1mm thick and between 0.1g and 0.25g) was taken before samples were accurately weighed in triplicate and placed into individual 20ml glass vials with screw-capped lids. 16g of PBS buffer (pH7.4, 0.1M) was poured into each vial then the vials were capped and placed into a 37°C oven. The pH was checked regularly using a calibrated electronic pH meter and maintained within the range pH 7.2-7.6 using 0.1M NaOH. The samples all sank to the bottom of the vials and did not float, and they were not agitated or stirred during the experiment.

5.1.4. Scanning Electron Microscopy

An XL30 Field Emission Scanning Electron Microscope (FESEM) was used for all the SEM pictures. Samples were first carbon coated using a Dynavac CS300 carbon coater that coated the samples with a layer of carbon approximately 250 angstroms thick.
5.1.5. Light Microscopy

Three optical light microscopes were used in the course of this work – a Nikon Labophot-2 stereomicroscope and a Kyowa Tokyo stereomicroscope each coupled with a Sony CCD-Iris DXC-107AP camera. The third was an Olympus CK2 stereomicroscope which was used for the cell growth study pictures.
5.2. Results and Discussion

Unfortunately in the literature there are hundreds of different methods of doing degradation and toxicity studies so it is very difficult to compare one to another. These can be generally categorised as either in vivo or in vitro tests. For this thesis it was decided to carry out tests in vitro which is ethically responsible – to gain as much information about the degradation and cytotoxicity of the materials as possible before testing in vivo.

In vitro tests generally involve polymer samples being characterised then immersed in a buffer solution at a given temperature for a specified period, and either agitated or static, then dried and recharacterised to determine any change in the samples. In order to accelerate the test (some materials may take a number of years to degrade) there are a number of different methods employed which include combinations of the following: high or low pH, high temperatures, agitation, mechanical stress on the sample and addition of enzymes such as urethanases or esterases. The sample morphology also greatly affects the time taken to degrade, eg. a sheet of 5mm thickness will take longer to degrade than a 5µm thick sheet since less surface area per volume is exposed to the degradation medium. For this reason the sample thickness of 1mm was kept constant in both studies. Polyurethanes are relatively impermeable to water compared with some polymers so it would be expected that degradation would start at the surface.

5.2.1. Accelerated Degradation study

An accelerated degradation study was carried out on the polymers from series 3 in phosphate buffered saline (PBS) pH 7.4, static for seven weeks at 70°C and the mass loss, molecular weight change and physical appearance recorded. All the initial samples were melt pressed into a 1mm thick non-porous sheet. The difference in formulation between the four materials was the composition of the soft segment. The reason for carrying out this accelerated study was to determine a suitable timeframe for the more comprehensive study. If none of the materials degraded appreciably in 7 weeks at 70°C then one would expect that milder conditions would have little effect over even a much
longer period of time. Some of the materials did degrade considerably as can be seen in Figure 92.

![Images of polyurethanes with different soft segments after 7 weeks at 70°C in PBS buffer](image)

**Figure 92** – Four polyurethanes with different soft segments after 7 weeks at 70°C in PBS buffer

In Figure 92 it can be seen that TM3-1 appeared to barely degrade at all and while there was a change in molecular weight (see Table 32), there was negligible mass loss (Table 31) and it remained a clear and colourless material. TM3-2 yellowed and became slightly cracked, losing some of its initial flexibility. TM3-3 and TM3-4 both degraded significantly, becoming relatively brittle and porous with a mass loss of around 40% (see Table 31). It is noteworthy that this corresponds to more than just the 35% of mass found in the soft segment. All four of the samples were still in one piece at the end of the degradation study however TM3-3 and TM3-4 were both brittle and broke when they were forced into vials for storage, as can be seen in Figure 92.

<table>
<thead>
<tr>
<th>Code</th>
<th>TM3-1</th>
<th>TM3-2</th>
<th>TM3-3</th>
<th>TM3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diisocyanate</td>
<td>HDI</td>
<td>HDI</td>
<td>HDI</td>
<td>HDI</td>
</tr>
<tr>
<td>Hard Segment %</td>
<td>65</td>
<td>65</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>Soft Segment</td>
<td>PCL</td>
<td>PEG</td>
<td>PLGA</td>
<td>PGA</td>
</tr>
<tr>
<td>Macrodil MW</td>
<td>402.1</td>
<td>394.8</td>
<td>425.4</td>
<td>491.3</td>
</tr>
</tbody>
</table>

**Table 31** – Composition of polyurethanes used in preliminary degradation study

In the following tables “% Mass Loss” has been calculated by taking the average of three samples both before and after degradation and expressing the mass difference as a
percentage. “%ΔMn” was calculated as the percentage difference between the initial Mn and the final Mn by GPC expressed as a percentage.

<table>
<thead>
<tr>
<th>Code</th>
<th>% Mass Loss</th>
<th>Pre Degradation</th>
<th>Post Degradation</th>
<th>%ΔMn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mn (10^3)</td>
<td>Mw (10^3)</td>
<td>PD</td>
</tr>
<tr>
<td>TM3-1</td>
<td>0.7</td>
<td>94.849</td>
<td>314.629</td>
<td>3.32</td>
</tr>
<tr>
<td>TM3-2</td>
<td>4.2</td>
<td>47.072</td>
<td>100.331</td>
<td>2.13</td>
</tr>
<tr>
<td>TM3-3</td>
<td>38.9</td>
<td>11,100</td>
<td>17,842</td>
<td>1.61</td>
</tr>
<tr>
<td>TM3-4</td>
<td>41.3</td>
<td>8,336</td>
<td>12,661</td>
<td>1.52</td>
</tr>
</tbody>
</table>

Table 32 – Series 3: Mass loss and GPC molecular weights before and after degradation

The mass loss for TM3-3 and TM3-4 would be expected to be higher simply because the starting molecular weight was lower than TM3-1 and TM3-2. Mass loss only occurs when the polymer is hydrolysed into oligomers or monomers that are small enough to be water-soluble, which depending upon the chemical composition occurs at less than about 1,000Mn. For this reason TM3-2 displays only 4.2% mass loss compared with losing over half of its initial Mn. The final Mn was 22,730 which is still a long way from being soluble but this is of course an average, and while the materials may degrade mostly by random hydrolysis there may possibly be increased scission of the terminal monomers.

Since an in vitro study is unlikely to accurately represent what happens in vivo (a very complex and dynamic environment), the results obtained in the preliminary degradation study must be viewed with caution, bearing in mind that they may not reflect actual degradation times in the body. It does however show promising results that proves the materials are degradable, and that even the polyurethanes that might be predicted to be the slowest degrading, such as the polyurethanes containing poly(ε-caprolactone) and poly(ethylene glycol), were shown to degrade by hydrolysis, measurable by change in molecular weight by GPC. In vivo degradation would be expected to include enzymatic and oxidative degradation in addition to simple hydrolysis.
5.2.2. Detailed Degradation Study

The accelerated high temperature degradation study may not be a good guide to degradability as there may not be a linear relationship between temperature and degradation. This is because the glass transition temperature of some polymers is between physiological temperature and 70°C which means that they will degrade quite differently at 70°C compared with at 37°C since they would be plastic and likely to absorb water more easily than when they are glassy. Ideally they should be measured as closely as possible under the same conditions they would experience in their intended application. The second study was designed bearing this in mind and the temperature was kept to only 37°C and the relevant ASTM method was followed (ASTM F-1635-04).

The degradation rate for this study would be expected to be slower than would be experienced in the body. This is because pH was kept neutral (pH 7.2-7.6), temperature kept to 37°C, and samples were not agitated or stressed in any way. Samples were non-porous (1mm thick sheet). No enzymes or cells were present so enzymatic and oxidative degradation could not occur – only hydrolysis.

<table>
<thead>
<tr>
<th>Series 1</th>
<th>Pre Degradation</th>
<th>Post Degradation</th>
<th>%ΔMn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code</td>
<td>% Mass Loss</td>
<td>Mn</td>
<td>Mw</td>
</tr>
<tr>
<td>TM1-1</td>
<td>1.88 ± 0.3</td>
<td>99,842</td>
<td>228,040</td>
</tr>
<tr>
<td>TM1-2</td>
<td>1.77 ± 0.2</td>
<td>158,729</td>
<td>324,817</td>
</tr>
<tr>
<td>TM1-3</td>
<td>1.61 ± 0.3</td>
<td>33,362</td>
<td>53,124</td>
</tr>
<tr>
<td>TM1-4</td>
<td>1.04 ± 0.3</td>
<td>39,696</td>
<td>66,659</td>
</tr>
</tbody>
</table>

Table 33 – Series 1: Degradation results (PBS buffer pH 7.4, 37°C, 3 months)

%ΔMn is the percentage change in number average molecular weight.

Degradation of series 1 resulted in greater degradation of the LDI-based urethanes than the HDI-based urethanes (49.2 and 58.5 %ΔMn compared with 11.7 and 0.937%ΔMn).

The fact that LDI-based urethanes degrade more rapidly than HDI-based urethanes can be attributed to the relatively greater amorphous character of the LDI-based polyurethanes which permits greater water penetration, and hence, exposure to hydrolysis.
Degradation of series 2 (HDI-EG-PCL400, varying hard segment %) showed that the materials in this series are relatively slow to degrade – the average $\% \Delta M_n$ was 4.0 ± 4.8 %. The polymers from this series are the slowest degrading of all the series. This would appear to limit the polymers from series 2 to longer-term degradable implants rather than short-term implants. By ‘longer-term’, one might estimate a period of greater than one year until it fully degrades and in some cases it may be much longer. *In vivo* studies might show a different degradation profile and a highly porous sample might also enhance degradation so this is by no means conclusive.

GPC is not a highly accurate method and evidence of this can be seen in TM2-4 where the $M_n$ was measured to increase by 3.66%, however the $M_w$ was measured to decrease.

Some polymers from series 4 (65% hard segment HDI-EG, varying soft segments) shown in Table 35 had degraded to a greater extent than those of series 2:
The polyurethanes containing PGA and PLGA (TM4-4 and TM4-5) degraded much more than the polyurethane containing PLA (TM4-3) despite initially being of similar molecular weight. Predictably, the PCL, PEG and PyBL polyurethanes (TM4-1, TM4-2 and TM4-6) were the slower degrading in this series. There was only slight mass loss of these three when compared with the PLGA-based polyurethane, which lost 57%.

<table>
<thead>
<tr>
<th>Series 5</th>
<th>Pre Degradation</th>
<th>Post Degradation</th>
<th>%ΔMn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code</td>
<td>% Mass Loss</td>
<td>Mn</td>
<td>Mw</td>
</tr>
<tr>
<td>TM5-1</td>
<td>1.08 ± 0.1</td>
<td>31,401</td>
<td>58,487</td>
</tr>
<tr>
<td>TM5-2</td>
<td>1.83 ± 0.2</td>
<td>39,462</td>
<td>71,927</td>
</tr>
<tr>
<td>TM5-3</td>
<td>0.88 ± 0.1</td>
<td>28,096</td>
<td>46,838</td>
</tr>
<tr>
<td>TM5-4</td>
<td>2.82 ± 0.2</td>
<td>15,802</td>
<td>26,973</td>
</tr>
<tr>
<td>TM5-5</td>
<td>0.51 ± 0.2</td>
<td>34,123</td>
<td>91,025</td>
</tr>
<tr>
<td>TM5-6</td>
<td>10.25 ± 0.8</td>
<td>15,308</td>
<td>24,111</td>
</tr>
<tr>
<td>TM5-7</td>
<td>6.66 ± 0.6</td>
<td>27,891</td>
<td>58,098</td>
</tr>
</tbody>
</table>

Table 36 – Series 5: Degradation results (PBS buffer pH 7.4, 37°C, 3 months)

Series 5 (65% hard segment HDI-varying degradable chain extenders, PCL400) is the most interesting of the series in this degradation study as it shows that the degradable chain extenders enhance degradation considerably. A direct comparison of the first five in series 5 can be made with series 2 (none of which degraded) since many of the polyurethanes from the two series are within a similar molecular weight range and differ only in chain extender and hard segment percentage, since both series share a PCL400 soft segment. TM5-6 and TM5-7 are composed of 100% hard segment and cannot be directly compared with series 2. The average %ΔMn for the first five polymers in series 5 is 28.5 ± 10.7 % which compares favourably with series 2 which was 4.0 ± 4.8 %. The least degradable in series 5 (17.3 %ΔMn) was more degradable than the most degradable of series 2 (11.0 %ΔMn). This clearly shows an increase in the rate of degradation due to the degradable chain extenders.

One would expect that a polyurethane composed of a faster-degrading soft segment such as PGA-300 and a faster degrading chain extender such as GA-EG should degrade even faster than any of the previous series. This was tried and included in the degradation study (for uniformity it has been included as series 6 despite only consisting of one polymer):
The soft segment used was PGA-300 and has been previously described and also used in TM4-5. The chain extender was GA-EG and 65% hard segment.

<table>
<thead>
<tr>
<th>Code</th>
<th>% Mass Loss</th>
<th>Mn</th>
<th>Mw</th>
<th>PD</th>
<th>Mn</th>
<th>Mw</th>
<th>PD</th>
<th>%ΔMn</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM6-1</td>
<td>87.9 ± 0.6</td>
<td>13,283</td>
<td>25,053</td>
<td>1.89</td>
<td>1,331</td>
<td>1,416</td>
<td>1.06</td>
<td>90.0</td>
</tr>
</tbody>
</table>

Table 37 - Series 6: Degradation results (PBS buffer pH 7.4, 37°C, 3 months)

TM6-1 lost 87.9 ± 0.6% of its original mass and the final Mn was only one tenth that of the initial Mn. This clearly shows that it degraded much more than any of the other samples from any of the series and further validates the methods used to increase the rate of degradation.

Some of the poly(ester-urethanes) showed severe cracking by the end of the degradation study (see Figure 93) whereas most polymers showed no obvious change in appearance.

Figure 93 – Optical microscope pictures of representative polymers post-degradation
TM2-6 in Figure 93 is typical of most of the polyurethanes and shows no visible degradation. TM5-7 shows surface whitening (likely to be surface degradation) whereas TM5-6 and TM4-3 each show cracking. It is interesting that TM4-3 and TM5-6 each shows different cracking patterns – TM5-6 appears to be more mosaic-like whereas TM4-3 is quite different and appears to have a different network of cracks on each side of the specimen which do not extend from one side of the sheet to the other (see close-up in Figure 94).

![Figure 94 – Optical microscope picture of TM5-6 and TM4-3 post-degradation (close up)](image)

It is evident from the pictures of these partially-degraded polyurethanes that different polyurethanes from the various series display different ways of degrading depending upon their individual composition. This is a factor which must be taken into account when considering a material for a specific application; not only the time taken to degrade but also the mode of degradation. The mode of degradation also has an effect on the time taken until the strength of the polymer is lost. Since the polyurethanes do not all degrade in exactly the same manner or rate, it may be possible to use this to tailor them for different applications. In this chapter it has been shown that the rate of degradation can be controlled through the chemistry, however further work could be done to shed light on the mode of degradation which has not been thoroughly investigated in this work.
5.2.3. Cytocompatibility

A cell growth study was conducted on selected polymers in order to adjudge biocompatibility. Each of these polymers were seeded with primary ovine fibroblasts (as outlined in methods, section 5.1.1 page 130) in triplicate and photographed daily for ten days then left for a further ten days and photographed again. The photographs were given a subjective ranking between 0 and 5, where 0 indicated that no cells were visible and 5 indicated a confluent layer of cells. Quantitative cell counts were not practical since the density of cells varied widely in some cases across the field of view and buckling of the polymers in some cases made it impossible to attain adequate focus of the entire field of view.

Figure 95 shows representative images taken on a polymer (TM4-5) on five consecutive days after seeding. On the first day it can be seen that the fibroblasts attached to the surface of the polymer and they spread out with their typical fibroblast morphology. By the fifth day the cells were confluent which showed that the number had greatly increased and that the cells could multiply on the polymer.

Cells survived and grew on the bottom of all the wells for the full twenty days and none of the polymers appeared to give off any toxic or inhibitory leachate since cells grew to confluence on the bottom of all the wells.
There were a few difficulties with the method used for the cell growth study. Firstly, within a day of the polymers being placed in the cell-growth media the polymer squares detached from the glass slide. Surprisingly, the cyanoacrylate (super-glue) that was used to glue the polymers down was not suitable for the application despite the slides being glued down a week prior to being used in order to ensure adequate bonding time. As a result, some of the polymers buckled which made photography difficult since the

Figure 95 – Optical microscope photograph of fibroblasts over time on 18TM4-5
surface was not always flat and some of the polymers floated to the surface of the growth media causing the attached cells to die. A dash was used in the table to represent cases where it was uncertain whether there were cells present or not. For example, Poly(ε-caprolactone), (PCL80000) which was used as a positive control was opaque, hence the cells could not be seen on the surface and the result was given as dashes to represent the uncertainty, whereas it appeared at times as though there were no cells present on TM2-3 so the result was given as a 0.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>PCL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TM2-3</td>
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<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td></td>
</tr>
<tr>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>TM3-3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
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</tr>
<tr>
<td>TM4-1</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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</tr>
<tr>
<td>TM4-5</td>
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<td>3</td>
<td>3</td>
<td>4</td>
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<td>5</td>
<td>5</td>
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<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>TM4-6</td>
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<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
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<td>1</td>
<td>2</td>
<td>4</td>
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<tr>
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<td>2</td>
<td>1</td>
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<td>2</td>
<td>2</td>
<td>1</td>
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</tr>
<tr>
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<td>1.7</td>
<td>1.9</td>
<td>2.1</td>
<td>2.1</td>
<td>2.2</td>
<td>2.3</td>
<td>2.6</td>
<td>3.2</td>
<td>3.3</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Table 38 – Cell study results (relative number of cells each day from 0-5)

There is clearly an increase in the average cell numbers (average of all the materials excluding the blank) over time shown in Table 38. There were only two materials in which cells could be seen and were not half-covered after the twenty days and these were TM2-3 and TM5-3 which each scored a 2. This shows that the materials are generally quite biocompatible since the cells are able to grow and multiply – if cell numbers had decreased over time then it would indicate that the materials are unsuitable for cell-growth in vitro and hence most likely to be unsuitable in vivo.
5.2.3.1. Cell Growth Study – Polymer Degradation

It was noticed that TM4-5 showed visible signs of degradation over the twenty-day cell growth study. The polymer displayed extensive cracking and the pH of the growth medium changed more rapidly than in the other samples (the growth medium changed from a pinky-red through to yellow as the pH decreased due to phenol red indicator). Degradation of TM4-5 began to show on day 6 when circular markings appeared; focusing on the markings showed that they were between the upper and lower polymer surface (see Figure 96).

![Circular cracks forming](image-url)

Figure 96 – Degradation of TM4-5 beginning showing circular cracks forming (6 days)
Figure 97 – Degradation of TM4-5 showing fibroblast growth on cracked surface (10 days)

Figure 98 – Degradation of TM4-5 showing differential degradation inside circular cracks (36 days)

It can be seen in Figure 98 that the areas inside the circular defects are degrading differently to the areas outside the circles. The inner surface has small rounded bead-like particles on the surface possibly slower-degrading and exposed due to surface erosion of the surrounding material.
5.3. **Summary and Conclusion**

The key findings of this chapter were the comparative rates of degradation of the polyurethanes from all the series that were synthesised in the preceding chapters and the fact that these materials are shown to be biocompatible.

It was found that the polyurethanes based on HDI, EG and PCL (from series 1 and 2) were amongst the slowest degrading polymers in this study with negligible mass and molecular weight loss after 3 months at 37°C. Changing the soft segment to PLA, PLGA and PGA rather than PCL caused a greater mass and molecular weight loss due to increased hydrolysis. It was further shown that by incorporating degradable chain extenders the rate of hydrolysis in the hard segment increased (series 5). Bearing this in mind it is possible to control the rate of degradation of these polyurethanes through control of the chemistry of the hard segment and the soft segment which was an original objective of this study.

An *in vitro* cell study showed these materials were biocompatible as primary mammalian cells adhered and multiplied over time.
6. FDM Scaffolds

Selected polymers were tested for performance on the FDM in order to show that the polymers can be made into scaffolds for tissue engineering and the results are shown in this chapter.

6.1. Materials and Methods

6.1.1. Brabender Extruder

The solid polymer sheet was chopped into ~ 1cm$^3$ pieces with clean tin-snips, cooled in liquid nitrogen and ground into powder using a cryogrinder which ground the polymer into a coarse powder. The polymer powder was then dried at 100°C under vacuum overnight. The polymer was extruded on a small single screw Brabender extruder equipped with a 1.7mm die at 175°C and 40rpm. The polymer was taken off by a variable-speed belt conveyor and cooled at ambient temperature in air without water bath to avoid unnecessary degradation. The filament was spooled and kept under nitrogen in a moisture-free environment for at least one week prior to use.

6.1.2. Mini-Extruder

The solid polymer sheet was chopped into ~ 1cm$^3$ pieces with clean tin-snips, cooled in liquid nitrogen and ground into powder using a cryogrinder as above. The polymer powder was then dried at 100°C under vacuum overnight. The polymer was extruded on a small single screw Randcastle extruder – model RC-025-CF equipped with a 1.7mm die at 175°C and 40rpm. The polymer was taken off by a belt conveyor and the filament treated and stored as above.

The mini-extruder was used for initial testing of the polymers since relatively small amounts of polymer could be used compared with the Brabender (~20-30g compared with 100-200g).
6.1.3. FDM

The FDM used was a “FDM Modeller 1600” purchased from Stratasys®, Inc. The polymer filament (1.7mm diameter) was fed through the FDM apparatus and small rectangular blocks of porous scaffold (~15 mm x 20 mm x 4 mm) were made to show that the material was suitable for FDM. The polymer was extruded from the smallest available nozzle, a T10 nozzle (254 micron diameter).

There are three variables on the FDM: the first is the temperature inside the extrusion head; the second is the envelope temperature inside the machine and the third is the percentage speed that the machine runs at – this is set as a percentage of the maximum roller and extrusion head speed. The operating envelope temperature inside the FDM machine was set at 25°C, the heating zone was set at 168°C and the speed of the head and extrusion rate were set at 70%.

6.1.4. Cell Growth on FDM Scaffolds

Rectangular lattice scaffolds ~15 mm x 20 mm x 4 mm, were made using TM3-2 with the FDM by the method described in section 6.1.3, page 148.

The scaffolds were sterilised by washing sequentially in 70% ethanol, PBS buffer and distilled water before being placed in six-well cell-culture plates.

Primary ovine fibroblasts were grown from explants of aortic valves from 1-year old animals to confluence in a 75cm² flask (3x10⁶ cells). They were passaged up to 10 times before use. The cells were harvested by trypsinising and mixed with DMEM to provide a suspension of fibroblast cells. The cell suspension was added to each of the samples and the plates were incubated in a 5% CO₂ atmosphere at 37°C. The medium was changed on a weekly basis.

After 9 weeks the scaffolds were fixed using 3% glutaraldehyde and photographs were taken using an Olympus C-5050 Zoom, 5.0 megapixels with an adapter (DCA-TCP (ISSCO, Sydney Australia)) fitted to an inverted microscope (Olympus CK2). The scaffolds were then dehydrated through ethanol in preparation for SEM and dried.
6.2. Results and Discussion

Suitable candidate polymers were extruded into 1.7mm diameter filaments that can be fed into FDM (see Figure 99). The diameter of the filament must be kept as close as possible to 1.7mm as even slight variations in the thickness affect the dimensions of the finished scaffold.

![Figure 99 – TM2-6 filament after extrusion on the mini-extruder](image)

The extruded filaments were fed through the FDM to create lattice-like porous scaffolds, as shown in Figure 100.

![Figure 100 – SEM - Plane view of an FDM scaffold (TM2-7)](image)
Figure 100 shows a plane view of an FDM’d scaffold using TM2-7, (65% hard segment, PCL 402 macrodiol, HDI, EG). A rectangular block was made with spaces between the strands to allow cell ingrowth. The diameter of the strands was 284µm as estimated by SEM.

![Figure 101 – SEM - Views of a sectioned scaffold (TM2-7)](image)

Sections of the scaffold in different orientations were made using a sharp razor blade in order to show the regular channels running throughout the structure. The cut ends of the fibres in the upper two images in Figure 101 show oval sections. This was due to the angle that they were cut at, the lower two images are more circular. The top strand of the lower right image can be seen to be rough and damaged – this is because it was the bottom strand as the scaffold was being built and suffered damaged as it was removed from the substrate. The same effect can be seen on the top right image, however this time it is the fibres at the bottom of the picture that are damaged, it can also be seen that that this row is somewhat more compressed than the other rows.
Figure 102 – SEM - Views of the scaffold (TM2-7) showing welding and the overlap

Figure 102 shows closer views of the scaffold to highlight the welding points between fibres. In the bottom left image it can be seen that there is no obvious weakness in the join between the two fibres since they melted intimately together. The debris is simply displaced polymer caused during sectioning.

The polymers were clear and colourless. Pictures were taken using an optical microscope with various lighting effects in order to show the polymer clearly, (see Figure 103).
The weld between the top layer and second layer can be seen in the bottom left image of Figure 103 through the upper fibre. The lower right image shows the corners of the scaffold. The pictures in Figure 103 were taken at various magnifications but the fibre thickness is constant ~284µm. Figure 104 on the other hand shows 400µm diameter fibres which results in a smaller gap between fibres.
The polyurethane TM2-7 was used in the FDM to make a scaffold of an aortic heart-valve (see Figure 105). The scaffold was made simply to show that complex three-dimensional scaffolds could be made using the biodegradable polyurethanes. The item was made in one piece from a CAD model of a heart valve model. The settings of the FDM program used resulted in filaments with no spacing, hence there was little if any porosity in this model.

The material was flexible and the leaflets did bend however they were certainly not as flexible as native leaflets, which are extremely thin and pliable. The design of this heart valve whilst crude does show that the poly(ester-urethane)s made in this thesis can be used to create precise 3D architectures and are suitable for thermal processing.

Figure 105 – Trileaflet heart valve scaffold made by FDM using TM2-7
6.3. Cell Colonisation of FDM Scaffolds

Creating FDM scaffolds using the new polyurethanes demonstrated their suitability for thermal processing. In order to demonstrate that the processed polyurethane scaffolds were biocompatible, cells were seeded onto the scaffold and grown for an extended period of time.

![Sectioned scaffold and Cell growth](image)

Figure 106 – Polyurethane scaffold (PEG soft segment, TM3-2) seeded with sheep fibroblasts

Figure 106 shows cells bridging around the corners of the scaffold whereas the right hand side of Figure 107 shows the scaffold filled with the cells which have fully bridged the gaps in the scaffold by providing their own support structure in the form of extracellular matrix which is primarily collagen. Figure 107 was an optical microscope photograph taken at an edge of the scaffold which was exposed to nutrient supply whereas Figure 106 was taken in the middle of the scaffold which had less access to the nutrients.
The peripheral pores of the scaffold are completely filled with cell-growth.

**Figure 107** – Polyurethane scaffold (TM3-2) seeded with sheep fibroblasts

SEM was also taken of the cell-seeded scaffolds and showed the cells confluent over all the exposed surfaces of the scaffold, both internally and externally. Sections were cut so that the inside of the scaffold could be seen (see Figure 108).

**Figure 108** – Polyurethane scaffolds (TM3-2) seeded with sheep fibroblasts (SEM)
Figure 109 – SEM showing a solid fibroblast sheet covering the scaffold surface (TM3-2)

The right hand image in Figure 109 is a close-up of the cell sheet that is shown at the bottom of the left hand image. The fibroblasts span the gaps between the polymer fibres by secreting a fibrous network of extracellular matrix (see Figure 110). The underlying concept of tissue engineering is that this extracellular matrix will provide support for the cells, thus allowing the polymer to degrade.

Figure 110 – Fibrous extracellular matrix produced by fibroblasts on the scaffold

The *in vitro* cell growth on the scaffolds shows that the scaffolds are still biocompatible after extrusion and FDM. Further studies could be carried out to prove that the scaffolds are biocompatible *in vivo*. 
6.4. Summary and Conclusion

It has been shown in this chapter that the polyurethanes can be thermally processed into tissue engineering scaffolds capable of supporting cell growth in vitro. The polymers were extruded as a 1.7mm diameter filament suitable for fused deposition modelling (FDM). They were processed by FDM into lattice-like scaffolds. Excellent control over the layout and spacing of the strands was shown by SEM. A trileaflet heart valve scaffold was constructed by FDM to show that scaffolds with complicated curves, overhangs and architectures could be made using the polyurethanes. High resolution SEM pictures showed that cells had secreted extracellular matrix which is important for structural integrity as the scaffold degrades.
7. Conclusions and Recommendations

The conclusions and recommendations outlined in this chapter refer to the original aims and outline of the study which can be found in Section 1.6, on page 39.

In Chapter 2 it was found that HDI-based polyurethanes were harder and less elastomeric than their LDI-based counterparts. HDI-based polyurethanes containing PCL soft segments were shown to exhibit cold-drawing and the oriented material was very tough (high elongation in combination with high strength). The tensile strength of the polyurethanes was up to $72 \pm 1$ MPa (extremely strong), and up to $251 \pm 56$ MPa as an oriented fibre. The strength and modulus of the oriented fibres suggest the materials are suitable for spinning and weaving. It was also found that the length of the soft segment in the polyurethanes affected the physical properties with the longer soft segment giving rise to higher tensile strength, however it had been suspected that a shorter soft segment might cause an increase in Young’s Modulus but this was shown not to be the case. The hardness, Young’s modulus and strength of the polyurethanes were shown to increase with increasing hard segment percentage.

Chapter 3 showed the effect of changing the chemistry of the soft segment. Soft segment macrodiols were synthesised and used to make polyurethanes which exhibit a range of mechanical and thermal properties. Commercially available PCL and PEG were used and the soft segments synthesised for this study were PLA, PGA, PLGA and $\gamma$-BL. It is concluded that altering the soft segment in the polyurethane can give a wide variety of glass transition temperatures as well as a range of different mechanical properties. A soft segment synthesised from $\gamma$-butyrolactone by ring opening polymerisation was used for the first time to make a biodegradable aliphatic polyurethane.

The mechanical properties of some of the polyurethanes in this chapter were limited by the molecular weight attained since the one-step method of synthesis employed appeared to be unsuitable for the more degradable soft segments (PLA, PGA and PLGA). The reason could possibly also be attributed to less accuracy in the molecular weight determination by titrimetric methods for these more hydrolysable macrodiols.
Chapter 4 investigated a range of degradable chain extenders which were synthesised and used to make biodegradable polyurethanes for the first time. The degradable chain extenders synthesised and in this study included high purity dimers and trimers, namely: LA-EG, GA-EG, GA-1,3-PD, EG-Suc-EG and EG-Fum-EG (see Chapter 4 for details). All of these degradable chain extenders were diols that contained one or two ester linkages and were suitable for making polyurethanes. The synthesis method employed for these dimers and trimers (polycondensation, transesterification and purification by fractional distillation) was demonstrated to give up to 79% yield based on the starting amount of hydroxy-acid and the resulting product was of high purity. One of the trimers (EG-Fum-EG) was designed to contain fumaric acid and a polyurethane made using it was shown to be photocrosslinkable on demand.

Chapter 5 showed the materials to be both biodegradable and biocompatible. A cell study was performed with primary ovine fibroblasts which were shown to proliferate on the polyurethanes and in most cases grow to confluence. An in vitro degradation study showed that the polyurethanes are degradable. A comparison of a series containing the degradable chain extenders (series 5) and the series with a regular chain extender (series 2) showed that the degradable chain extender increases the degradation of the polyurethanes and that this is happening in the hard segment since both series contained the same soft segment (PCL400).

Chapter 6 shows that the polyurethanes can be used to make scaffolds for tissue engineering. Fused deposition modelling (FDM) was used to construct grid-like scaffolds which were demonstrated to support cell-growth in vitro. The FDM was shown suitable for constructing complicated 3D scaffolds by making a tri-leaflet heart-valve scaffold.

There are a number of recommendations for further work which can be made as a result of this study. These will be addressed by chapter:

Chapter 2 showed some materials with exceptional strength which is worth further investigation since high strength allows one to use less material which is often desirable in tissue engineering. Chapter 2 also showed some highly elastic materials based on m-
LDI which was not investigated further since HDI was chosen for more detailed study rather than m-LDI. Elasticity is often very much desired for tissue engineering scaffolds and is less common than plastic polymers and hence these materials warrant further study.

Chapter 3 contains some polyurethanes that were of lower molecular weight than is preferred. It would be worth optimising the conditions of synthesis to attain higher molecular weights and hence better mechanical properties since these materials showed stark differences to those made using PCL and PEG soft segments, namely a higher glass transition temperature and faster degradation. This may be achieved by altering the catalyst type and concentration and hence the reaction time to prevent excessive temperatures due to the reaction exotherm.

Chapter 4 gave details of representative degradable chain extenders and some polyurethanes made using them. Further examples of degradable chain extenders could be made by using the methods described in chapter 4 in order to provide a broader range of ‘building blocks’ which can be used to tailor the thermal, physical and degradative properties. For this reason it would be valuable to have a more complete and systematic study of the compounds of the types: HO-R₁-COO-R₂-OH and HO-R₁-OOC-R₂-COO-R₁-OH. One of the chain extenders, (EG-Fum-EG) which contained fumaric acid was shown to undergo crosslinking by photopolymerisation and further investigation could be undertaken to determine the implications of this property since this has potential cure-on-demand applications.

Chapter 5 consists of the degradation and cell compatibility studies. Further work could be carried out to investigate the effect of these materials on other cell types such as endothelial cells or stem cells, and to take the next logical step to test the polymers in vivo.

The degradation study could be extended to investigate further time points and until complete degradation. Information about the mode of degradation could be garnered through analysis of the degradation products over time.

Chapter 6 showed the scaffolds made using fused deposition modelling and fibroblast cells growing on the scaffolds. This work could be extended to trial 3D scaffolds for
implants and to determine the response to the material \textit{in vivo}. Strength and flexibility testing of the scaffolds during degradation would also provide useful information.

In conclusion, this work has produced polymers in a methodical fashion that have been shown to be biocompatible, biodegradable and suitable for scaffold fabrication. There are a number of areas where this work can be carried on to provide an even broader range of materials. It is intended that this thesis should provide further insight into the methodologies and the important factors that can be systematically varied to produce biodegradable polyurethanes with desired properties.
APPENDIX – A – Transesterification modelling program

program transester
  c Calculates length distribution of ethylene glycol / glycolic acid comonomers subject to random transesterification
  parameter (maxmol=100000, maxlen=1000, maxloop=1000000)
  parameter (interval=1000000)
  integer length(maxmol), glycol(maxmol), lcount(maxlen), gi, gj
  data lc / maxlen*0 /
  
  write(*,'(6.3)') float(lc(k))/float(maxmol), k=1,20

  c Define initial lengths
  do i = 1, maxmol
    glycol(i) = 1
    length(i) = int(avlength*i) - int(avlength*(i-1))
    lcount(length(i)) = lcount(length(i)) + 1
  end do

  write(*,'(20f6.3)') (float(lc(k))/float(maxmol), k=1,20)

  c Main loop
  do loop = 1, maxloop
    continue
    i = int(rand()*maxmol + 1)
    j = int(rand()*maxmol + 1)
    if (i.eq.j) goto 10

    c Select 2 reacting molecules and the target ester site
    idir = int(2*rand() + 1)
    jsite = int(rand()*length(j))
    jdir = 1
    if (jsite.ge.glycol(j)) jdir = 2

    c Calculate new oligomer lengths
if (jdir.eq.1) then
  gi = jsite + glycol(i)
  if (idir.eq.2) gi = jsite + length(i) - glycol(i) + 1
  lj = length(j) - jsite
  gj = glycol(j) - jsite
else
  gi = length(i) - glycol(i) + 1
  if (idir.eq.2) gi = glycol(i)
  lj = jsite
  gj = glycol(j)
end if
li = length(i) + length(j) - lj

write(*,*) length(i),glycol(i),length(j),glycol(j),
*  idir,jdir,jsite,li,gi,lj,gj
lcount(length(i)) = lcount(length(i)) - 1
lcount(length(j)) = lcount(length(j)) - 1
lcount(li) = lcount(li) + 1
lcount(lj) = lcount(lj) + 1
length(i) = li
length(j) = lj
glycol(i) = gi
glycol(j) = gj

if (loop.eq.interval*int(loop/interval)) then
  write(*,'(20f6.3)') (float(lcount(k))/float(maxmol),k=1,20)
end if
end do
end
APPENDIX – B – Toxicity Data

The degradation products of implantable polymers must be non-toxic to avoid potential harm to the patient. This section gives toxicity values from the literature for the expected final degradation products of polymers synthesised in this study. Most of the cited values are acute toxicity data such as LD$_{50}$ which is arguably not the ideal measure for a chronic implant where very small amounts are leaching out over a very long period of time, however it could also be argued that it gives a reasonable approximation of comparative toxicity.

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<thead>
<tr>
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<th>Route</th>
<th>Dose</th>
<th>Dosage (mg/kg)</th>
<th>Reference</th>
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<td>Mouse</td>
<td>IP</td>
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<td>LD$_{50}$</td>
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Table 39 – Acute toxicity data for ethylene glycol from the literature

* 80% solution in H$_2$O

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Table 40 – Acute toxicity data for 1,6-hexanediamine (the degradation product of HDI)

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Table 41 – Acute toxicity data for various degradation products
Chemosynthesis of bioresorbable poly(γ-butyrolactone) by ring-opening polymerisation: a review

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Received 4 August 2004; accepted 2 October 2004
Available online 11 November 2004

Abstract

Recent advances in the synthesis of poly(γ-butyrolactone) have yielded homopolymers of up to 50,000 Mw from the low-cost monomer γ-butyrolactone. This monomer has for the better part of a century been thought impossible to polymerise. Poly(γ-butyrolactone) displays properties that are ideal for tissue-engineering applications and the bacterially derived equivalent, poly(4-hydroxybutyrate) (P4HB), has been evaluated for such uses. The glass transition temperature (–48 to –51 °C), melting point (53–60 °C), tensile strength (50 MPa), Young’s modulus (70 MPa) and elongation at break (1000%) of P4HB make it a very useful biomaterial. Poly(γ-butyrolactone) degrades to give γ-hydroxybutyric acid which is a naturally occurring metabolite in the body and it has been shown to be bioresorbable.

Investigation into the synthesis of poly(γ-butyrolactone) has recently produced homo-oligomeric diols 400–1000 Mw that are suitable for reacting with diisocyanates to form polyurethanes. Biodegradable polyurethanes made from diols of polyglycolide (PGA) and poly(e-caprolactone) (PCL) have the disadvantage of high glass transition and slow degradation, respectively. Poly(γ-butyrolactone) can be thought of as being the missing link in the biodegradable polyester family immediately between PGA and PCL and displaying intermediate properties.

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Keywords: Polyhydroxybutyric acid; Polymerisation; Scaffold; Degradation

Contents

1. Introduction ........................................... 3772
2. Some background nomenclature .......................... 3772
3. Synthesis .............................................. 3773
   3.1. Zeolites and clay as catalyst ..................... 3776
   3.2. Enzymatic .................................... 3776
   3.3. Bacterial/microbial ............................. 3776

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1. Introduction

Degradable polyesters have found a wide range of uses in medical applications such as absorbable sutures and tissue-engineering scaffolds. The ester linkage is susceptible to hydrolysis and has been shown to degrade both hydrolytically and enzymatically. The degradation products of the biodegradable polyesters are typically hydroxy acids, such as glycolic acid and lactic acid that are generally recognized as being non-toxic. For these reasons there has been a large volume of research into the biodegradable polyester family in the past few decades.

Recently, poly(4-hydroxybutyrate) (P4HB) has attracted the interest of researchers for use as a biodegradable polymer. It has been evaluated as scaffolds [1–3] in combination with other degradable polyesters for tissue engineering of cardiovascular tissue. P4HB displays similar physical properties to poly(ε-caprolactone) (PCL), while degrading in a shorter period of time [4] which makes it a valuable addition to the tissue-engineer’s repertoire.

This paper reviews aspects of chemosynthesis by ring-opening polymerisation of γ-butyrolactone (γ-BL) as well as degradation and toxicity of P4HB.

2. Some background nomenclature

There are two BL isomers (Fig. 1): γ-BL, which is the subject of this paper, and β-butyrolactone (β-BL), which is more easily polymerised by ring opening due to increased ring strain.

Many authors in the past have referred to P3HB as simply polyhydroxybutyrate (PHB) since P4HB was not known, and this nomenclature is often still in use. The mechanical and thermal properties of the two materials are very different (Table 1) where P4HB is straight chain and P3HB has a methyl side-group (Table 2). Note that P3HB contains a chiral centre (as does lactic acid) and the properties of the polymer depend upon the tacticity.

In the literature, the nomenclature ‘lactone’ has often been used interchangeably with ‘cyclic ester’ and they have been named based on the number of members in the ring as well as the number of carbons in the monomer (Table 2).

Polymers in general are usually named after the starting monomers. For example, “polyglycolide”
(PGA) is synthesised by ring opening the glycolide cyclic dimer and it can be distinguished from “poly(glycolic acid)” which is polymerised by condensation of glycolic acid. Unfortunately, this has not always been the case with lactones where the polymer has sometimes been named after the repeat unit rather than the starting monomer, as in the example of “poly(3-hydroxybutyrate)” which was made by ring opening of β-BL [5,11]. Another reference named the same polymer as “poly(β-hydroxybutyrate)” [12], while some references name the polymer after the lactone: β-BL was polymerised to give poly-β-BL, “poly(β-butyrolactone)” [13].

β-Propiolactone is a relatively potent carcinogen—it has a TD50 of 1.16 mg/kg body weight/day [14]. Due to the carcinogenicity of β-propiolactone, poly(β-propiolactone) has not usually been considered for use in biomedical applications. Bearing this in mind, one sees an obvious gap in properties of degradable polyesters (Table 3) where P3PL is unsuitable and P4HB has been unpolymerisable, which leaves the very rigid and fast-degrading PGA and the slow-degrading Poly(δ-Valerolactone) (PVL) and PCL (Table 4).

3. Synthesis

Of all the degradable polyesters, P4HB (or poly(γ-butyrolactone)) has attracted the least attention and has arguably been the most misunderstood due to a misconception γ-BL cannot undergo ring-opening polymerisation. Initial research into the polymerisation of γ-BL by Carothers et al. [29] stated in 1932: “Thus, we have heated samples of pure γ-butyrolactone both with and without catalysts (zinc chloride, potassium carbonate) at 80°C for 12 months; none of the samples showed any detectable increase in viscosity.” A conclusion was drawn from this that γ-BL was not able to undergo ring-opening polymerisation at all and this conclusion has since been explained thermodynamically [30–34]. Despite this failure to polymerise, there have been numerous groups [29,35,36] that have published attempts to homopolymerise γ-BL without success and one would assume that many more groups might have been reluctant to publish negative results considering it had already been reported as being unable to polymerise.

In 1951, γ-BL was shown to ring open to form oligomers (a degree of polymerisation of 2–3) with diethoxy terminal groups [37]. This was a side-product of the reaction and was not a desired product; however, it showed for the first time that γ-BL could homopolymerise, or more correctly oligomerise.

Despite this, it was still thought [38] that γ-BL was unable to ring-open polymerise at all and it was not until 1966 that this was for the first time purposely shown to be not correct [39]. In that case, extreme conditions were
used to achieve polymerisation (20,000 atm and 165 °C) and homopolymers of between 1200 and 3350 Mw were formed (the molecular weight was measured using a vapour pressure osmometer).

Kricheldorf et al. [40] stated in 1985: “Concerning copolymerizations of γ-butyrolactone, it is noteworthy that this monomer for thermodynamic reasons cannot be homopolymerised at temperatures above 50 °C.” In 1991, Jedlinski et al. [41] also stated that γ-BL cannot homopolymerise for thermodynamic reasons. As recently as 2003, γ-BL has been referred to in the literature as “nonhomopolymerizable” [42]. While there is some merit to these statements, they neglect to take into account the effect of catalysts and pressure that have facilitated the formation of quite high molecular weight at temperatures above 50 °C. The reason these authors stated that it cannot form a high molecular weight homopolymer is that it has a very small ring strain, so small that ΔGp (Gibbs free energy of polymerisation) is positive [43]. Simply put, this means that the ester in the lactone ring is less likely or as likely to break and join onto the polymer chain (due to its stability) than the ester in the free polymer chain is likely to transesterify or undergo ring closure under normal conditions. The thermodynamic parameters involved in the polymerisation of γ-BL to P4HB at both normal pressures [33] and in the super-cooled state under high pressures [44] have been investigated.

γ-BL does not undergo ring-opening polymerisation as easily as β-BL does because of the aforementioned small ring strain [43]. High molecular weight poly(3-hydroxybutyrate), e.g. Mw 430,000 [45] and 580,000 [6], has been made by ring-opening polymerisation without much difficulty since the four-membered ring has a greater strain than the five-membered ring.

While it has been calculated [46] to be thermodynamically impossible to chemically synthesise a high molecular weight homopolymer under normal conditions, numerous papers have shown that γ-BL can be copolymerised with other lactones and hydroxy acids, to form copolyesters [5,34,46,47,48] (Table 5) and can even form homopolymers of low molecular weight [38,49,50] (Table 6).

The molecular weight of homopolymers of γ-BL achieved by ring opening that have been reported to date are low, e.g. 200 Da (a degree of polymerisation of 2.4 was published [49] based on 1H NMR data).

Mn (by GPC) of 800 Da has been achieved [57] by using a lipase from *Pseudomonas* sp. to catalyse the ring-opening reaction. This reaction was carried out with 0.10 mmol (0.0086 g) of γ-BL and 0.040 g of lipase at 45 °C over a period of 20 days and yet achieved only an 8% yield and Mw/Mn = 2.23 after precipitation. Precipitation tends to reduce Mw/Mn and increase Mw by removing some of the low molecular weight oligomers.
Oligomers of 4HB are useful prepolymers that can undergo chain-linking reactions such as with diisocyanates to form polyurethanes [50] or other polymers. Many of the attempts to polymerise \(\gamma\)-BL have not been carried out with the intention to yield the diols necessary for polyurethane chemistry. The initiators used are typically unsuitable, e.g. ethanol [49], which gives an ethoxy-terminated chain, which prevents further polymerisation (Fig. 2). One obvious way to circumvent this problem is to use a difunctional initiator like a diol rather than the monofunctional alcohol, e.g. ethylene glycol [50] (Fig. 2). This gives a diol that could be used for the synthesis of biodegradable polyurethanes (Table 7).

Bailey et al. [66] in 1976 describe developments in synthesis of alternating poly(ester–ether)s from spiro-ortho-esters, some which include \(\gamma\)-BL as a monomer (Fig. 3). These do not form the P4HB homopolymer but

![Table 5](image)

<table>
<thead>
<tr>
<th>Year</th>
<th>Co-monomer</th>
<th>Temperature ((^\circ)C)</th>
<th>Time (h)</th>
<th>Mn</th>
<th>Max % (\gamma)-BL in product</th>
<th>Yield %</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1964</td>
<td>(\beta)-PL</td>
<td>50</td>
<td>20 days</td>
<td>NA</td>
<td>Feed 98.2, BL:PL</td>
<td>0.2</td>
<td>[51]</td>
</tr>
<tr>
<td>1964</td>
<td>BCMO</td>
<td>0</td>
<td>3–35 h</td>
<td>NA</td>
<td>89.7%</td>
<td>6.0</td>
<td>[52]</td>
</tr>
<tr>
<td>1964</td>
<td>(\beta)-PL</td>
<td>30</td>
<td>21 days</td>
<td>NA</td>
<td>Feed 7.3, BL:PL</td>
<td>43</td>
<td>[52]</td>
</tr>
<tr>
<td>1969</td>
<td>BCMO</td>
<td>20</td>
<td>20 h</td>
<td>NA</td>
<td>50%</td>
<td>17.4</td>
<td>[53]</td>
</tr>
<tr>
<td>1970</td>
<td>BCMO</td>
<td>25</td>
<td>6.4 h</td>
<td>NA</td>
<td>36%</td>
<td>7.9</td>
<td>[54]</td>
</tr>
<tr>
<td>1985</td>
<td>GA</td>
<td>60</td>
<td>44 h</td>
<td>NA</td>
<td>26%</td>
<td>1.4</td>
<td>[40]</td>
</tr>
<tr>
<td>1989</td>
<td>(\tau)-LA</td>
<td>200</td>
<td>20 h</td>
<td>640</td>
<td>19%</td>
<td>NA</td>
<td>[36]</td>
</tr>
<tr>
<td>1990</td>
<td>GA</td>
<td>200</td>
<td>7 h</td>
<td>1500</td>
<td>16%</td>
<td>NA</td>
<td>[48]</td>
</tr>
<tr>
<td>1995</td>
<td>(\beta)-BL</td>
<td>100</td>
<td>4 h</td>
<td>2700</td>
<td>35%</td>
<td>13</td>
<td>[5]</td>
</tr>
<tr>
<td>1996</td>
<td>(\tau)-lactide</td>
<td>140</td>
<td>4 days</td>
<td>14,600</td>
<td>17%</td>
<td>6</td>
<td>[55]</td>
</tr>
<tr>
<td>1997</td>
<td>(\beta)-BL</td>
<td>25</td>
<td>7 days</td>
<td>1800</td>
<td>56%</td>
<td>24</td>
<td>[56]</td>
</tr>
<tr>
<td>1998</td>
<td>(\omega)-CL</td>
<td>140</td>
<td>4 days</td>
<td>29,500</td>
<td>16%</td>
<td>16</td>
<td>[47]</td>
</tr>
<tr>
<td>1998</td>
<td>(\beta)-VL</td>
<td>140</td>
<td>4 days</td>
<td>18,600</td>
<td>15%</td>
<td>12</td>
<td>[47]</td>
</tr>
<tr>
<td>1998</td>
<td>(\beta)-PL</td>
<td>140</td>
<td>4 days</td>
<td>1600</td>
<td>23%</td>
<td>9</td>
<td>[47]</td>
</tr>
<tr>
<td>1998</td>
<td>GA</td>
<td>140</td>
<td>4 days</td>
<td>NA</td>
<td>26%</td>
<td>26</td>
<td>[47]</td>
</tr>
<tr>
<td>1998</td>
<td>(\omega)-CL</td>
<td>45</td>
<td>20 days</td>
<td>2900</td>
<td>5%</td>
<td>45</td>
<td>[57]</td>
</tr>
<tr>
<td>1999</td>
<td>(\omega)-CL</td>
<td>25</td>
<td>2 h</td>
<td>57,000</td>
<td>22%</td>
<td>31</td>
<td>[34]</td>
</tr>
<tr>
<td>2002</td>
<td>(\delta)-VL</td>
<td>25</td>
<td>24 h</td>
<td>DP=4.3</td>
<td>30%</td>
<td>5.7</td>
<td>[49]</td>
</tr>
<tr>
<td>2003</td>
<td>(\omega)-CL</td>
<td>25</td>
<td>48 h</td>
<td>2880</td>
<td>33%</td>
<td>38</td>
<td>[42]</td>
</tr>
</tbody>
</table>

![Table 6](image)

<table>
<thead>
<tr>
<th>Year</th>
<th>Temperature ((^\circ)C)</th>
<th>Time (h)</th>
<th>Length of polymer or oligomer formed</th>
<th>Characterisation of molecular weight</th>
<th>Yield %</th>
<th>Notes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1951</td>
<td>NA</td>
<td>NA</td>
<td>Low DP, 2–3 and more</td>
<td>Mp</td>
<td>NA</td>
<td>Diethoxy-terminated (side-product)</td>
<td>[37]</td>
</tr>
<tr>
<td>1966</td>
<td>160</td>
<td>4</td>
<td>1200–3350 by osmometry</td>
<td>IR (not shown)</td>
<td>20</td>
<td>20,000 atm, recrystallised three times, mp 61–62 °C</td>
<td>[39]</td>
</tr>
<tr>
<td>1996</td>
<td>60</td>
<td>430</td>
<td>888–932Mw</td>
<td>MALDI-TOF MS</td>
<td>25–42</td>
<td>Lipase catalyst in (n)-hexane using methanol as initiator (~2:1 of lipase to (\gamma)-BL by mass)</td>
<td>[58]</td>
</tr>
<tr>
<td>1998</td>
<td>45</td>
<td>480</td>
<td>Mn 800 (by GPC) Mw/Mn = 2.23 400–1000Mn by GPC</td>
<td>HNMR and CNMR (not shown)</td>
<td>8</td>
<td>8.9 mg (\gamma)-BL to 40 mg of lipase</td>
<td>[57]</td>
</tr>
<tr>
<td>1999</td>
<td>144</td>
<td>21</td>
<td></td>
<td>HNMR and GPC shown</td>
<td>56</td>
<td>Clay catalyst, initiated with ethylene glycol and diethylene glycol</td>
<td>[50]</td>
</tr>
<tr>
<td>2000</td>
<td>40–160</td>
<td>10–70</td>
<td>10,000–50,000Mw</td>
<td>GPC</td>
<td>9–74</td>
<td>Lewis acid catalyst at very high pressure. Less than 4 g reactants</td>
<td>[59]</td>
</tr>
<tr>
<td>2002</td>
<td>25</td>
<td>3</td>
<td>DP 2.4 (Mn=200)</td>
<td>HNMR (not shown)</td>
<td>5.5</td>
<td>Ethanol initiated, clay catalyst (ion-exchanged montmorillonite)</td>
<td>[49]</td>
</tr>
<tr>
<td>2003</td>
<td>180</td>
<td>6</td>
<td>NA (oligomers)</td>
<td>None shown</td>
<td>47.2</td>
<td>Not the intended product, zeolite catalyst</td>
<td>[60]</td>
</tr>
<tr>
<td>2003</td>
<td>40–160</td>
<td>5–300</td>
<td>Mn = ‘‘5000 or more’’</td>
<td>GPC</td>
<td>5–23</td>
<td>Metal complex catalyst at very high pressure. Less than 1 g of reactants</td>
<td>[61]</td>
</tr>
</tbody>
</table>
are considered biodegradable and useful for biomedical applications.

3.1. Zeolites and clay as catalyst

Mesoporous zeolites were shown to be effective catalysts for ring-opening polymerisation of δ-valerolactone and ε-caprolactone [67]; however, the authors did not report attempting γ-BL. Another group reported that an unexpected oligomeration of γ-BL occurred during an attempted alkylation over zeolites [60]. They did not show the characterisation of these oligomers, however reported a 47% yield of oligomer; the reaction temperature was 180 °C and reaction time was 6 h. As a result, zeolite shows potential as a catalyst for γ-BL ring-opening polymerisation and has not yet been thoroughly investigated.

3.2. Enzymatic

Uyama and Kobayashi [68] showed for the first time that lactones could be ring-opening polymerised with a lipase to form polymers in 1993 when they polymerised ε-caprolactone and δ-valerolactone to a maximum molecular weight of 7700Mn.

There are not many cases of ring-opening polymerisation of γ-BL using enzymes in the literature to date; however, this may change as lipases have been shown to be effective catalysts for the polymerisation. However, there is a major disadvantage with all the reported oligomerationisations of γ-BL using lipases: they use a large ratio of lipase to γ-BL making it quite expensive, especially since enzymes are known to lose their catalytic effect over time. For example, lipase from Pseudomonas sp. was used to ring-open γ-BL to give 800Mn at 45 °C, 480 h, and with a yield of 8% [57]. Unfortunately, the reaction used 40 mg lipase to 8.9 mg of γ-BL yielding only 0.71 mg poly(γ-butyrolactone) which makes this an expensive exercise.

3.3. Bacterial/microbial

It is worth noting that P4HB of high molecular weight has been made using microbes (Table 8). The feedstock for the micro-organisms has included γ-BL for

![Fig. 2. Ring opening of γ-BL with ethanol or ethylene glycol.](image)

![Fig. 3. Formation of poly(ester-ether) alternating copolymer including γ-BL.](image)

**Table 7** Some reported unsuccessful attempts to polymerise γ-BL

<table>
<thead>
<tr>
<th>Year</th>
<th>Catalyst/initiator</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1932</td>
<td>Zinc chloride, potassium carbonate and none</td>
<td>80</td>
<td>12 months</td>
<td>[29]</td>
</tr>
<tr>
<td>1961</td>
<td>14 different organometallic catalysts</td>
<td>0–78</td>
<td>3–72 h</td>
<td>[62]</td>
</tr>
<tr>
<td>1964</td>
<td>BF3.Et2O</td>
<td>30–80</td>
<td>9–30 days</td>
<td>[52]</td>
</tr>
<tr>
<td>1977</td>
<td>Al2/Zn μ-oxoisopropoxide</td>
<td>40</td>
<td>NA</td>
<td>[35]</td>
</tr>
<tr>
<td>1989</td>
<td>None</td>
<td>200</td>
<td>20 h</td>
<td>[36]</td>
</tr>
<tr>
<td>1997</td>
<td>Cationic zirconocene dimethyl complexes</td>
<td>25–60</td>
<td>2 h</td>
<td>[63]</td>
</tr>
<tr>
<td>1999</td>
<td>Samarium(II) aryloxide complexes</td>
<td>25</td>
<td>1–5 min</td>
<td>[34]</td>
</tr>
<tr>
<td>2003</td>
<td>Zwitterionic titanoxanes</td>
<td>20</td>
<td>NA</td>
<td>[64]</td>
</tr>
<tr>
<td>2003</td>
<td>Amino isopropoxyl strontium</td>
<td>25–80</td>
<td>70 min</td>
<td>[65]</td>
</tr>
<tr>
<td>2003</td>
<td>SmI2/Sm</td>
<td>25</td>
<td>48 h</td>
<td>[42]</td>
</tr>
</tbody>
</table>
P(3HB-co-4HB) synthesis using bacteria (1992) and up to 100% composition of P4HB has been made [7]. Thermal degradation of P4HB shows γ-BL as a major degradation product (1994) [69]. Properties of P4HB (bacterial) have been characterised and show it to be a strong flexible polymer (Table 1) [8].

Poly-4-hydroxybutyrate has recently been made by a fermentation process using genetically engineered Escherichia coli that is capable of producing up to 50 g of poly-4-hydroxybutyrate per litre of fermentation broth in 48 h [4].

4. Mechanism of ring-opening polymerisation of γ-butyrolactone

Ring-opening polymerisation has been carried out using either cationic catalysts or anionic catalysts. The generally accepted mechanism for the cationic ring-opening polymerisation of γ-BL involves coordination and alignment of the carbonyl oxygen with the metal centre (e.g. Al) followed by insertion.

γ-BL has been used to determine the mechanism of initiation of ring-opening polymerisation using a strontium-based initiator system [65]. The authors chose γ-BL because it would react with the initiator but not further polymerise under normal conditions. The results showed the ring-opening polymerisation followed a coordination–insertion mechanism. The general mechanism for P3HB has also been proposed and is considered to follow a similar mechanism [107].

Data have been published detailing the relative catalytic copolymerisation parameters of some common lactones including γ-BL and as one would expect it is unlikely to homopolymerise [108]. Formation of an alternating copolymer between γ-BL and 3,3-bis(chloromethyl)oxacyclobutane at high BL feed content was explained by the authors as due to the inability of BL to homopolymerise [53].

The mechanism can be explained in part by the fact that γ-BL is shown to form a one-to-one adduct with certain initiators including for example yttrium methoxycarbonyl with the yttrium metal centre.

5. Degradation of poly(4-hydroxybutyrate)

Nakayama et al. [47] showed that introduction of γ-BL units into polyesters resulted in both enhanced biodegradability and flexibility. Polyesters copolymerised were: PLLA; PGA; poly-β-propiolactone; poly-δ-valerolactone and poly-ε-caprolactone.

It has been published that the body absorbs P4HB in a period of 8–52 weeks [4]. It is also stated in the same reference that due to cyclisation of the degradation product from hydroxy acid to lactone, the degradation products are somewhat less acidic than those of PGA and PLA which both have lower pKₐ values.

P4HB has been tested for degradability [8] in river water (containing micro-organisms) at 25°C and compared with other polyesters. P4HB was the polyester that degraded at the highest rate (by biological oxygen demand) in the presence of these micro-organisms.

Thermal degradation of P4HB has shown that the pyrolysate contains mainly γ-BL and higher oligomers both cyclic and linear to pentamer [69].

6. Toxicity

When considering toxicity one must take into account the biocompatibility of the initial polymer as well as that of the degradation products. There has been some work published regarding the biocompatibility of bacterially derived P4HB for tissue engineering [70–75].

The main use of P4HB has been to provide strength to biodegradable non-woven PGA and PLLA scaffolds by dip coating into a 1% solution of P4HB in tetrahydrofuran [70,71]. These scaffolds have been reported in a number of papers to have good biocompatibility and fast degradation. A scaffold for a human pulmonary conduit was shown to be replaced by living cells in vitro [70]. Ovine mesenchymal stem cells have also been shown to proliferate on the P4HB-coated PGA scaffolds [73], as have human umbilical cord cells [74], and ovine myofibroblasts and endothelial cells [75].

P4HB has been said to be not only biocompatible but also often extremely well-tolerated in vivo [4]. The authors also suggest that P4HB implants are unlikely to cause any adverse pharmacological effects due to their relatively small sample size, slow release and rapid metabolism.

6.1. Endogenous levels of 4-hydroxybutyrate

The final degradation products upon hydrolysis of the ester linkages of poly(γ-butyrolactone) are γ-BL and 4-hydroxybutyric acid both of which are essentially the
same thing in vivo since in the body, \( \gamma \)-BL rapidly undergoes ring opening catalysed by the enzyme \( \gamma \)lactonase to give 4-hydroxybutyric acid.

It has been known for some time that 4-hydroxybutyric acid is endogenous in the human brain [76], but more recent findings have shown that it can be found in a number of other organs and in blood samples; however, care must be taken in the interpretation of results since tissue samples that are not fresh or frozen immediately after death have been shown to give increased readings. Fresh human blood has been found to contain 0.17–1.51 mg/l of 4-hydroxybutyrate [77]; however, some other studies have been unable to detect traces of 4-hydroxybutyrate in healthy individuals [78–80]. In another study, recoveries greater than 100% in samples of blood and urine spiked with 4-hydroxybutyrate have been attributed to endogenous concentrations [81]. The concentration of 4-hydroxybutyrate in autopsy blood has been shown to increase over the time between death and autopsy [78] and the authors suggest it is most likely due to enzymatic conversion of succinic acid, \( \gamma \)-aminobutyrate and putrescine. The concentration of 4-hydroxybutyrate in post-mortem blood stored for 10 days at 4°C was 6.06 ± 4.27 mg/l but only 4.55 ± 3.88 mg/l when stored for the same period frozen at −20°C [78] and a positive correlation was found between concentration and post-mortem interval. Human liver samples have also been shown to exhibit the same post-mortem increase [80]. Endogenous 4-hydroxybutyrate concentrations of ~0.25 mg/l have been found in human urine and have been shown to increase in concentration up to 404% in 6 months depending on storage conditions [82]. Human sigmoid colon samples explanted from the gastrointestinal tract contained endogenous 4-hydroxybutyrate at concentrations of 2.59–4.19 mg/kg [83].

Succinic semialdehyde dehydrogenase deficiency (SSADH) is a rare hereditary ailment where succinic semialdehyde is not converted to succinic acid but rather follows an alternative pathway to form 4-hydroxybutyrate [84]. A review of SSADH cases [84], showed elevated cerebrospinal fluid concentrations of 4-hydroxybutyrate from 65 to 230 times the normal levels (46.7 ± 4.3 mg/l) in combination with three times the normal \( \gamma \)-aminobutyrate concentration and a low concentration of glutamine. Significant behavioural problems were evident in 42% of patients, and most were said to experience global development delays and 50% experienced seizures [84].

6.2. Exogenous 4-hydroxybutyrate

There is a history of human consumption of \( \gamma \)-BL and it has been used as a flavouring ingredient [85], as a sedative [86], for bodybuilding [87,88], in the treatment of alcoholism and opiate dependence [89,90]. It is naturally occurring in some food, has been used as a drug of dependence [87,91–94] and is characterised as a ‘date-rape’ drug [95,96]. Hence, there are a number of references detailing the metabolism of \( \gamma \)-BL in the human body as well as numerous toxicity studies and they have previously been reviewed [85].

The half-life of \( \gamma \)-BL in plasma is less than a minute [85] before conversion to 4-hydroxybutyric acid by \( \gamma \)lactonase, and the maximum concentration of 4-hydroxybutyrate in plasma occurs 20–60 min after oral administration [97] before ~2–5% is excreted in urine [98], and the majority has been shown to be metabolised and exhaled as CO\(_2\) within 150 min [99]. Automobile drivers in the Netherlands have had readings of 4-hydroxybutyric acid as high as 2000 mg/l in urine and as high as 194 mg/l in blood [95]. The reason the drivers had 4-hydroxybutyric acid in their system is because it gives the user a “high” when taken orally. It is commonly reported that it is abused for its euphoric effects, as well as for reported properties of increasing muscle mass and sexual pleasure [95].

When a 25 mg/kg dose of sodium 4-hydroxybutyrate was administered orally to a human volunteer, 4-hydroxybutyrate was detectable in urine at 30 min, was highest at 1 h (30.3 mg/l), and was undetectable after 4 h (<2 mg/l) showing that it is rapidly absorbed and eliminated [98]. The dose–response curve for 4-hydroxybutyrate is purportedly steep, where 40–50 mg/kg can cause somnolence leading to arousable sleep and 60–70 mg/kg can cause coma for 1–2 h [97].

There have been a number of studies and reviews that show 4-hydroxybutyrate to have therapeutic value in the treatment of alcoholism [100–105] and opiate dependence [90,102]. Doses of 4-hydroxybutyrate have been shown effective in suppressing the effects of alcohol withdrawal without serious side-effects [100]. The authors speculate that the mechanism of action of 4-hydroxybutyrate is an interference with the release of dopamine and serotonin which are the main modulators of the ethanol reward system [100]. The mechanism has been reviewed in more detail [104] and is thought to be of a substitutional nature.

Bodybuilders have used 4-hydroxybutyrate as a dietary supplement since research was published showing it increases production of certain growth hormones in healthy humans, yet other research shows it does not build muscle mass in rats, dogs or alcoholics [105,106]. The recommended daily dosage for bodybuilders is 1.4–2.8 g of 4-hydroxybutyrate with some reportedly taking considerably more [96].

There are reports of people who have displayed an addiction to 4-hydroxybutyrate or its precursor \( \gamma \)-BL [87,91–93]. A typical case involves a 36-year-old man who imbibed ~5 g \( \gamma \)-BL every 2 h around the clock for 6 months [87]. Upon forced removal from the use of \( \gamma \)-BL he displayed withdrawal symptoms such as tremors,
sweating, hallucinations and delirium; however, he was asymptomatic after 3 days treatment.

One must bear in mind that an implanted P4HB scaffold weighing only a few grams would release 4-hydroxybutyrate over a long period and would not have significant pharmacological effects. For example, 2 g of pure P4HB implanted in a 70 kg person which degraded in 2 months would average just under 0.5 mg/kg/day of 4-hydroxybutyrate, which is negligible considering endogenous concentrations in blood have been measured to be 0.17–1.51 mg/l [77], endogenous gastrointestinal concentrations of 2.59–4.19 mg/kg [83], and therapeutic doses of 50–100 mg/kg/day have been used in the treatment of alcoholism [100].

6.3. Genotoxicity studies of γ-butyrolactone

The Flavour and Extract Manufacturers’ Association (FEMA) Expert Panel has reviewed more than 50 genotoxicity studies of γ-BL and concluded: “…that γ-butyrolactone (4-hydroxybutanoic acid lactone) is not mutagenic and that isolated positive results performed in non-standard assays at high solution concentrations are not compelling evidence of genotoxic potential. The negative response in repeated Salmonella mutagenicity assays…supports the Panel’s conclusion that exposure to 4-hydroxybutanoic acid lactone exhibits little potential for interaction with DNA.” [85]

7. Conclusion

Oligomers and co-oligomers containing γ-BL have recently been shown to be chemosynthetically possible precursors for biodegradable polyesters and polyurethanes, which have many potential uses in the field of biomaterials. Bacterially derived poly(4-hydroxybutyrate) has been evaluated for use in a number of biodegradable scaffolds both in vitro and in vivo with promising results showing it to be a strong, flexible and biocompatible polymer with a relatively rapid degradation profile. This suggests that this hitherto little-explored branch of the biodegradable polyester family may provide extremely valuable novel biomaterials for drug delivery and tissue engineering, thus warranting further investigation.

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