12-2006

ELASTIC DEGRADABLE POLYURETHANES FOR BIOMEDICAL APPLICATIONS

Changhong Zhang
Clemson University, changz@clemson.edu

ELASTIC DEGRADABLE POLYURETHANES FOR BIOMEDICAL
APPLICATIONS

_____

A Thesis
Presented to
the Graduate School of
Clemson University

_____

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Bioengineering

_____

by
Changhong Zhang
December 2006

_____

Accepted by:
Dr. Xuejun Wen, Committee Chair
Dr. Thomas Boland
Dr. Yuehuei An

ABSTRACT

Several series of polyurethanes were synthesized with linear or crosslinked structures by using different synthesis routes. Two studies are mentioned: (1) the synthesis of degradable polyurethanes with linear structure and the investigation of the elasticity and cytophilicity of the materials as function of the chain extender, and (2) the synthesis and the investigation of the biocompatibility, degradation, hydrophilicity and mechanical properties of the polyurethane-based hydrogels with crosslinked structure.

In the first study, two types of biodegradable polyurethanes (PUs) were synthesized from methylene di-p-phenyl-diisocyanate (MDI), polycaprolactone diol (PCL-diol), and chain extenders of either butanediol (BD) or 2,2^r-(methylimino)diethanol (MIDE). The effects of two types of chain extenders on the degradation, mechanical properties, hydrophilicity, and cytophilicity of PUs were evaluated. In this study, we concluded that by changing the type of chain extender used during the synthesis of degradable PUs, the degradation rate, mechanical properties, hydrophilicity, and cytophilicity could be tailored for biomedical applications in different tissues.

In the second study, a series of degradable polyurethane based light-curable elastic hydrogels were synthesized from polycaprolactone diol (PCL-diol), polyethylene glycol (PEG), lysine diisocyanate (LDI), and 2-hydroxyethyl methacrylate (HEMA) through UV light initiated polymerization reaction. The use of PCL/PEG at different ratios, as well as the introduction of HEMA into polyurethane, allows the synthesis of a series of biocompatible elastic hydrogels with tunable physical and cytophilic properties.

This series of materials also allows for controlling cell attachment and growth by incorporating bioactive molecules during the light-curing process.

DEDICATION

This thesis is dedicated to my family and friends for their generous support.

ACKNOWLEDGEMENTS

I would like to express my gratitude to all those who gave me the possibility to complete this thesis. I thank Professor Xuejun Wen for his continuous guidance and support. I thank my other committee members, Dr. Tomas Boland and Dr. Yuehuei An, for their helpful suggestions and comments throughout my studies. I thank the Clemson-MUSC Bioengineering Department's faculty and students for their support.

TABLE OF CONTENTS

Page

Table of Contents (continued)

LIST OF FIGURES

List of Figures (continued)

LIST OF TABLES

CHAPTER 1

THESIS ROADMAP

Abstract

This thesis constitutes studies of the materials research of several novel polyurethanes with potential for biomedical use. Several series of polyurethanes were synthesized with linear or crosslinked structures by using different synthesis routes. This thesis includes two studies: (1) the synthesis of degradable polyurethanes with linear structure and the investigation of the elasticity and cytophilicity of the materials by changing chain extender, and (2) the synthesis of the polyurethane-based hydrogels with crosslinked structure and the investigation of the biocompatibility, degradation, hydrophilicity and mechanical properties.

In the first part of this thesis, two types of biodegradable polyurethanes (PUs) were synthesized from methylene di-p-phenyl-diisocyanate (MDI), polycaprolactone diol (PCL-diol), and chain extenders of either butanediol (BD) or 2,2ʳ-(methylimino)diethanol (MIDE). The effects of two types of chain extenders on the degradation, mechanical properties, hydrophilicity, and cytophilicity of PUs were evaluated. In this study, we concluded that by changing the type of chain extender used during the synthesis of degradable PUs, the degradation rate, mechanical properties, hydrophilicity, and cytophilicity could be adjusted for biomedical applications in different tissues.

In the second part of this thesis, a series of degradable polyurethane based light-curable elastic hydrogels were synthesized from polycaprolactone diol (PCL-diol), polyethylene glycol (PEG), lysine diisocyanate (LDI), and 2-hydroxyethyl methacrylate

(HEMA) through UV light initiated polymerization reaction. In this study, the use of

PCL/PEG at different ratios, as well as the introduction of HEMA into polyurethane,

allows the synthesis of a series of biocompatible elastic hydrogels with tunable physical

and cytophilic properties through light initiated polymerization. This series of materials

also allows for controlling cell attachment and growth by incorporating bioactive

molecules during the light-curing process.

CHAPTER 2

BACKGROUND

Introduction of Biomaterials and Scaffolds

The destruction or malformation of tissues and organs can be caused by trauma, primary disease or by medical intervention and treatment modalities. [1] A large part of modern medical practice targets the restoration of function by replacement of damaged or diseased tissues and organs, the replacement is by either artificial implants or transplantation of tissues. [2] Such interventions are hindered by factors such as immune rejection, limited supply and donor site morbidity.

In recent decades, tissue engineering has emerged as an alternative method for the regeneration of tissues and restoration of function of organs through implantation of cells/tissues grown outside the body or stimulating cell to grow into an implanted matrix.[3] While initial biomaterials were commonly inert, biomaterial research then focused on the bioactive materials that elicit action and reaction in the biological environment. Today, there is a move to new generation of materials to repair or replace diseased or damaged tissue, using controlled three-dimensional scaffolds in which cells can be seeded usually before implantation. The materials and scaffold can stimulate specific cellular response at the molecular level; they are also biodegradable and can be tailored to suit specific tissues. Ideally, the living tissue construct is functionally, structurally and mechanically equal to the tissue it has been designed to replace.[4]

There are a number of materials used for tissue engineering application; the materials can be subdivided into natural materials and synthetic materials. Examples of

natural materials include collagen, glycosaminoglycans (GAGs), chitosan and alginates. [5-8] The advantages of natural materials are that they have low toxicity and a low chronic inflammatory response. They can be combined into a composite with other natural materials or synthetic materials (thus possessing the mechanical strength of the synthetic material as well as the biocompatibility of the natural material) and can be degraded by naturally occurring enzymes. However, disadvantages include poor mechanical strength as well as a complex structure and, hence, manipulation becomes more difficult. They can easily be denatured and often require chemical modification, which can lead to toxicity. Examples of synthetic materials include the biodegradable polymers such as polyglycolide (PGA), polylactide (PLA) and polylactide-co-glycolide (PLG); non-degradable polymers such as polytetrafluoroethylene (PTFE), nylon and polyethylene terephthalate (PET); ceramics such as single crystal Al_2O_3 and polycrystalline Al_2O_3; bioactive glasses such as Bioglass (US Biomaterials).[9-13]

Techniques used to manufacture biomaterials into scaffolds are dependent on the properties of the material and its intended application. Scaffolds may be composed of polymers, metals, ceramics or composites. It is important to select a material that closely matches the properties of the tissue that it is to replace. For example, biomaterials intended to replace soft tissues such as skin, breast, eye, blood vessels and heart valves tend to be composed of natural and synthetic polymers. Replacement of hard tissues such as bone and dentine tends to use metals, ceramics, composites and polymers.[3, 14-18] For the three-dimensional scaffold with cells seeded before implantation, the clinical success of the scaffold is also largely dependent on the suitable supply of cells in the scaffold. There are a number of different sources of cells that could be used for tissue

repair and regeneration, including mature cells from the patient, 'adult' stem cells from the patient such as bone marrow stromal stem cells, and embryonic stem (ES) cells/embryonic germ (EG) cells.[19-21]

Introduction of Polyurethanes

Polyurethanes form a large family of polymeric materials with an enormous diversity of chemical composition and properties. These properties have contributed to their widespread application in many areas and use in a range of commodity products, such as polymers for clothing, automotive parts, furnishings, construction and paints for appliances. Compared to most polymers manufactured in industry, polyurethanes possess more complex chemical structures, typically comprising three monomers: a diisocyanate, a macroglycol, and a chain extender. These three parts in the polyurethane structure, enable one to create a virtually infinite number of materials with various physicochemical and mechanical characteristics. This unique composition makes the structure of polyurethanes quite different from that of other polymers. In fact, polyurethane polymers usually show a two-phase structure in which hard segment-enriched domains are dispersed in a matrix of soft segments. The hard segment-enriched domains are composed mainly of the diisocyanate and the chain extender, while the soft segment matrix is composed of a sequence of macroglycol moieties. For this reason, polyurethanes are often referred to as segmented block copolymers. This particular molecular architecture, as well as the intrinsic properties of each ingredient used for the synthesis of polyurethanes, contributes to the unique characteristics of this class of materials when compared to other polymers. [22]

Generally, there are three methods for the preparation of polyurethanes: the one-shot method, the prepolymer method, and the quasi-prepolymer method. [23-27] The prepolymer method and the quasi-prepolymer method are regrouped as 'two-step methods' or 'two-step polymerization'. In the one-shot method, all the ingredients are mixed simultaneously and the resulting mixture is directly allowed to polymerize. In the prepolymer method, the macroglycol is pre-reacted with an excess of poly-isocyanate. This prepolymer is then mixed with the rest of the ingredients during processing. In the quasi-prepolymer method, a part of the macroglycol is mixed with the poly-isocyanate and the rest of the polymer and the other constituents are mixed as a second phase. The streams thus obtained are finally mixed together at the end. Those final polyurethanes can be further categorized into two broad groups: thermoplastics and thermosets. Thermoplastics are defined as materials capable of being repeatedly softened by heat and hardened by cooling. Thermosets, on the contrary, are set into permanent shape by chemical crosslinking that occurs during or after forming. Once a thermoset has been hardened into the desired shape, the process is generally irreversible. In the case of polyurethanes synthesized in our laboratory, both thermoplastic and thermoset polyurethanes were obtained.

Polyurethanes exhibit many excellent properties for biomedical applications. For instance, one of the characteristic properties of polyurethanes is their mechanical flexibility combined with high tear strength, which can be achieved because of polyurethane's chemical versatility. These desirable properties attract the attention of developers of biomedical devices. In 1958, polyurethane materials were first introduced in biomedical applications; Pangman described a composite breast prosthesis covered

with a polyester-urethane foam. [28, 29] In the same year, Mandrino and Salvatore also used a rigid polyester-urethane foam called OstamerTM for in situ bone fixation. [30-33] Three years later, the application of polyester-urethane Polyurethane Estane® VC was proposed by Dreyer et al to be used as components for heart valves and chambers, and aortic grafts.[34, 35] In the mid-1960s, Cordis Corp. started to commercialize polyester-urethane diagnostic catheters.[36] In 1954, textile chemists at DuPont developed Lycra® spandex as a high-performance alternative to natural rubber in elastic thread. It was first introduced as a biomaterial in 1967 by Boretos and Pierce who obtained the polymer in solution directly from the DuPont spinning line that produced Lycra spandex yarn. This material was first used as the elastomeric components of a cardiac assist pump and its arterial cannulae.[37-39] The year 1971 marked the arrival of the earliest polyurethane specifically designed for medical use; Avcothane-51[TM], a polyurethane/slicone hybrid, was invented by AVCO-Everett Research Laboratory. In 1972, Biomer[TM], a version of Lycra® T-126 produced by Ethicon Corp. under a license from Dupont, was made available. Avcothane and Biomer were regarded as the first 'real' biomedical polyurethanes and have been studied intensively. Avcothane was used clinically in the first intra-aortic balloon pump (IAB), starting in about 1971, and is still in clinical use today in IABs. Biomer[TM] components were used in the 'Jarvik Heart' in 1982, the first artificial heart used for implantation. From that time, the research of polyurethanes in biomedical applications has been intensive, and currently polyurethanes have been applied in a number of biomedical tissue engineering areas such as pacemaker lead insulators, heart valves, vascular protheses, breast implants, gastric bubbles, drug release carriers, etc.[40-49]

While traditionally investigators have used polyurethanes as long-term implant materials [50] and have attempted to shield them from the biodegradation processes, recent work by investigators has utilized the flexible chemistry and diverse mechanical properties of polyurethane materials to design degradable polymers for applications as varied as neural conduits [51] to bone replacements.[52-55] These materials have for the most part taken advantage of hydrolytic mechanisms and have varied molecular structures to control rates of hydrolysis.[56]

The move to degradable polyurethane-based materials has required a change in the diisocyanates historically used for their synthesis. Generally, an aromatic diisocyanate was used for applications where degradation was not desired, such as pacemaker lead coverings, catheters, and wound dressings.[50] Partially because of the putative carcinogenic nature of aromatic diisocyanates,[57, 58] degradable polyurethanes are more frequently made from diisocyanates such as lysine-diisocyanate (LDI, 2,6 diisocyanato methyl caproate),[54, 59, 60] hexamethylene diisocyanate,[55, 61] and 1,4 diisocyanatobutane   whose ultimate degradation products are more likely to be non-toxic and biocompatible. [61]

Modification of the degradation rate is typically achieved through changes to he soft segment, and biodegradable PUs have been made using a variety of soft segments including polylactide or polyglycolic acid,[62-64] polycaprolactone (PCL), [51-55, 61] and polyethylene glycol (PEG). [53-55]

References:

1. Naumann, A., Rotter N., Tissue engineering of autologous cartilage in transplants in rhinology. Am. J. Rhinol, 1998. 12: p. 59-63.

2. Vacant J.P. and Langer R., Tissue engineering: the design and fabrication of living replavement devices for surgical reconstruction and transplantation. The Lancet, 1999. 354 (Suppl. 1): p. 32-34.

3. Vats. A, Tolley., NS, Polak, JM, Gough, JE, Scaffolds and biomaterials for tissue engineering: a review of clinical applications. Clin. Otolaryngol, 2003. 28: p. 165-172.

4. Stock U.A., V.J.P., Tissue engineering: current state and prospects. Annu. Rev. Med., 2001. 52: p. 443-451.

5. Eybl, E., Grimm, M., Grabenwoger, M., Bock, P., Muller, M. M., Wolner, E., Endothelial cell lining of bioprosthetic heart valve materials. J Thorac Cardiovasc Surg, 1992. 104(3): p. 763-9.

6. Gough, J.E., Scotchford, C. A., Downes, S., Cytotoxicity of glutaraldehyde crosslinked collagen/poly(vinyl alcohol) films is by the mechanism of apoptosis. J Biomed Mater Res, 2002. 61(1): p. 121-30.

7. Olde Damink L.L.H., Dijkstra, P.J., Van Luyn M.J.A, Cross-linking of dermal sheep collagen using a water soluble carbodiimide. Biomaterials, 1996. 17: p. 765-773.

8. Van Luyn M.J.A., Van Wachem, P.B., Relations between in vitro cytotoxicity and crosslinked dermal sheep collagen. J. Biomed. Mat. Res., 1992. 26: p. 1091-1110.

9.   Breuer, C.K., Mettler, B. A., Anthony, T., Sales, V. L., Schoen, F. J., Mayer, J. E., Application of tissue-engineering principles toward the development of a semilunar heart valve substitute. Tissue Eng, 2004. 10(11-12): p. 1725-36.

10. Wiesmann, H.P., Joos, U., Meyer, U., Biological and biophysical principles in extracorporal bone tissue engineering. Part II. Int J Oral Maxillofac Surg, 2004. 33(6): p. 523-30.

11. LeGeros, R.Z., Properties of osteoconductive biomaterials: calcium phosphates. Clin Orthop Relat Res, 2002(395): p. 81-98.

12. Fricain, J.C., Granja, P. L., Barbosa, M. A., De Jeso, B., Barthe, N., Baquey, C., Cellulose phosphates as biomaterials. In vivo biocompatibility studies. Biomaterials, 2002. 23(4): p. 971-80.

13. Hench L.L., Bioceramics. From concept to clinic. J. Am. Ceramic Soc., 1991. 74: p. 1487-1510.

14. Ellis, M.J., Chaudhuri, J. B., Poly(lactic-co-glycolic acid) hollow fibre membranes for use as a tissue engineering scaffold. Biotechnol Bioeng, 2006.

15. Zhang, Y., Cheng, X., Wang, J., Wang, Y., Shi, B., Huang, C., Yang, X., Liu, T., Novel chitosan/collagen scaffold containing transforming growth factor-beta1 DNA for periodontal tissue engineering. Biochem Biophys Res Commun, 2006. 344(1): p. 362-9.

16. Wang, T.W., Wu, H. C., Huang, Y. C., Sun, J. S., Lin, F. H., Biomimetic bilayered gelatin-chondroitin 6 sulfate-hyaluronic acid biopolymer as a scaffold for skin equivalent tissue engineering. Artif Organs, 2006. 30(3): p. 141-9.

17.   Ni, S., Chang, J., Chou, L., A novel bioactive porous CaSiO3 scaffold for bone tissue engineering. J Biomed Mater Res A, 2006. 76(1): p. 196-205.

18.   Danielsson, C., Ruault, S., Simonet, M., Neuenschwander, P., Frey, P., Polyesterurethane foam scaffold for smooth muscle cell tissue engineering. Biomaterials, 2006. 27(8): p. 1410-5.

19.   Vats A., T.N.S., Polak J.M.,, Stem cells: sources and applications. Clin. Otolaryngol. Allied Sci., 2002. 27: p. 227-232.

20.   Lloyd, D.A., Ansari, T. I., Gundabolu, P., Shurey, S., Maquet, V., Sibbons, P. D., Boccaccini, A. R., Gabe, S. M., A pilot study investigating a novel subcutaneously implanted pre-cellularised scaffold for tissue engineering of intestinal mucosa. Eur Cell Mater, 2006. 11: p. 27-33; discussion 34.

21.   Caplan, A.I., Embryonic development and the principles of tissue engineering. Novartis Found Symp, 2003. 249: p. 17-25; discussion 25-33, 170-4, 239-41.

22.   Vermette, P., Biomedical applications of polyurethanes. Tissue engineering intelligence unit ; 6. 2001, Georgetown, Tex. Austin, Tex.: Landes Bioscience ; Eurekah.com. 273.

23.   Frados J., SPI Plastics Engineering Handbook, 3rd ed. 1960, New York: Van Nostrand Reinhold.

24.   Frados J., SPI Plastics Engineering Handbook. 4th ed. 1976, New York: Van Nostrand Reinhold.

25.   Frados J., Plastics Engineering Handbook of the Society of the Plastic Industry Inc. 5th ed. 1991, New York: Van Nostrand Reinhold.

26. Saunders JH., F.K., Polyurethanes Chemistry and Technology. Part II: Technology. 1964, New York: Interscience Pub.

27. Axelrood SL. and H. CW., A one-shot method for urethane and urethane-urea elastomers. Ind Eng Chem, 1961. 53: p. 889-894.

28. Pangman WJ., Compound prosthesis. 1965: Unite State.

29. Pangman WJ., Compound prosthesis device. 1958: USA.

30. Mandarino, M.P., Salvatore, J. E., Polyurethane polymer; its use in fractured and diseased bones. Am J Surg, 1959. 97(4): p. 442-6.

31. Mandarino, M.P., Salvatore, J. E., Polyurethane polymer (ostamer): its use in fractured and diseased bones; experimental results. Surg Forum, 1958. 9: p. 762-5.

32. Mandarino, M.P., The use of a polyurethane polymer (ostamer) in fractured and diseased bones. Surg Clin North Am, 1960. 40: p. 243-51.

33. Mandarino, M.P., Salvatore, J. E., A polyurethane polymer (ostamer): its use in fractured and diseased bones. Report of thirty-five cases. Arch Surg, 1960. 80: p. 623-7.

34. Dreyer, B., Akutsu, T., Kolff, W. J., Testing of artificial heart valves. J Appl Physiol, 1959. 14(3): p. 475-8.

35. Dreyer, B., Akutsu, T., Kolff, W. J., Kolff, W. J., Aortic grafts of polyurethane in dogs. J Appl Physiol, 1960. 15: p. 18-22.

36. Pinchuk, L., A review of the biostability and carcinogenicity of polyurethanes in medicine and the new generation of 'biostable' polyurethanes. J Biomater Sci Polym Ed, 1994. 6(3): p. 225-67.

37. Boretos, J.W., Detmer, D. E., Donachy, J. H., Segmented polyurethane: a polyether polymer, II. Two years experience. J Biomed Mater Res, 1971. 5(4): p. 373-87.

38. Boretos, J.W., Pierce, W. S., Segmented polyurethane: a polyether polymer. An initial evaluation for biomedical applications. J Biomed Mater Res, 1968. 2(1): p. 121-30.

39. Bezon, E., Barra, J. A., Karaterki, A., Braesco, J., Pillet, J. C., Mondine, P., Mechanical system of left ventricle cannulation: control of tightness by experimental left ventricular assistance. Chirurgie, 1996. 121(6): p. 447-52.

40. Nyilas, E., Leinbach, R. C., Caulfield, J. B., Buckley, M. J., Austen, W. G., Development of blood-compatible elastomers. 3. Hematologic effects of Avcothane intra-aortic balloon pumps in cardiac patients. J Biomed Mater Res, 1972. 6(4): p. 129-54.

41. Nyilas, E., Ward, R. S., Jr., Development of blood-compatible elastomers. V. Surface structure and blood compatibility of avcothane elastomers. J Biomed Mater Res, 1977. 11(1): p. 69-84.

42. Nyilas, E., Development of blood compatible elastomers. II. Performance of Avcothane blood contact surfaces in experimental animal implantations. J Biomed Mater Res, 1972. 6(4): p. 97-127.

43. Lelah, M.D., Lambrecht, L. K., Young, B. R., Cooper, S. L., Physicochemical characterization and in vivo blood tolerability of cast and extruded Biomer. J Biomed Mater Res, 1983. 17(1): p. 1-22.

44. Sung, C.S., Hu, C. B., Merrill, E. W., Salzman, E. W., Surface chemical analysis of Avcothane and Biomer by Fourier transform IR internal reflection spectroscopy. J Biomed Mater Res, 1978. 12(6): p. 791-804.

45. Young, S., Pincus, G., Hwang, N. H., Dynamic evaluation of the viscoelastic properties of a biomedical polymer (biomer). Biomater Med Devices Artif Organs, 1977. 5(3): p. 233-54.

46. Graham, S.W., Hercules, D. M., Surface spectroscopic studies of Avcothane. J Biomed Mater Res, 1981. 15(3): p. 349-61.

47. Burke, A., Hasirci, N., Polyurethanes in biomedical applications. Adv Exp Med Biol, 2004. 553: p. 83-101.

48. Zdrahala, R.J., Zdrahala, I. J., Biomedical applications of polyurethanes: a review of past promises, present realities, and a vibrant future. J Biomater Appl, 1999. 14(1): p. 67-90.

49. Phillips, R.E., Smith, M. C., Thoma, R. J., Biomedical applications of polyurethanes: implications of failure mechanisms. J Biomater Appl, 1988. 3(2): p. 207-27.

50. Lamba, N.M.K., Woodhouse, K.A., Cooper, S.L., Polyurethanes in biomedical applications. 1998, Boca Raton, USA: CRC Press.

51. Borkenhagen, M., Stoll, R. C., Neuenschwander, P., Suter, U. W., Aebischer, P., In vivo performance of a new biodegradable polyester urethane system used as a nerve guidance channel. Biomaterials, 1998. 19(23): p. 2155-65.

52. Saad, B., Hirt, T.D., Welti, M., Uhlschmid, G.K., Euenschwander, P., Suter, U. W., Development of degradable polyesterurethanes for medical applications: in vitro and in vivo evaluations. J Biomed Mater Res, 1997. 36(1): p. 65-74.

53. Gorna, K., Gogolewski, S., Biodegradable polyurethanes for implants. II. In vitro degradation and calcification of materials from poly(epsilon-caprolactone)-poly(ethylene oxide) diols and various chain extenders. J Biomed Mater Res, 2002. 60(4): p. 592-606.

54. Skarja, G.A., Woodhouse, K. A., Synthesis and characterization of degradable polyurethane elastomers containing and amino acid-based chain extender. J Biomater Sci Polym Ed, 1998. 9(3): p. 271-95.

55. Cohn, D., Stern, T., Gonzalez, M. F., Epstein, J., Biodegradable poly(ethylene oxide)/poly(epsilon-caprolactone) multiblock copolymers. J Biomed Mater Res, 2002. 59(2): p. 273-81.

56. Santerre, J.P., Woodhouse, K., Laroche, G., Labow, R. S., Understanding the biodegradation of polyurethanes: from classical implants to tissue engineering materials. Biomaterials, 2005. 26(35): p. 7457-70.

57. Cardy, R.H., Carcinogenicity and chronic toxicity of 2,4-toluenediamine in F344 rats. J Natl Cancer Inst, 1979. 62(4): p. 1107-16.

58. Schoental, R., Carcinogenic and chronic effects of 4,4'-diaminodiphenylmethane, an epoxyresin hardener. Nature, 1968. 219(159): p. 1162-3.

59. Saad, B., Ciardelli, G., Matter, S., Welti, M., Hlschmid, G. K., Neuenschwander, P., Suter, U. W., Degradable and highly porous polyesterurethane foam as

biomaterial: effects and phagocytosis of degradation products in osteoblasts. J Biomed Mater Res, 1998. 39(4): p. 594-602.

60.   Zhang, J.Y., Beckman, E. J., Piesco, N. P., Agarwal, S., A new peptide-based urethane polymer: synthesis, biodegradation, and potential to support cell growth in vitro. Biomaterials, 2000. 21(12): p. 1247-58.

61.   Woo, G.L., Mittelman, M. W., Santerre, J. P., Synthesis and characterization of a novel biodegradable antimicrobial polymer. Biomaterials, 2000. 21(12): p. 1235-46.

62.   Bruin P., V.G.J., Nijenhuis A.J., Pennings A.J., Design and synthesis of biodegradable poly(ester-urethane)elastomer networks composed of non toxic building blocks. Makromol Chem Rapid Comm, 1988. 9: p. 589.

63.   Storey R.F., Hickey, T.P., Degradable polyurethane networks based on d, l-lactide, glycolide, α-caprolactone, and trimethylene carbonate homopolyester and copolyester triols. Polymer, 1994. 35: p. 830-838.

64.   Hitunen K., Seppala, J.V., Harkonen M., Effect of catalyst and polymerization conditions on the preparation of low molecular weight lactic acid polymers. Macromolecules, 1994. 30: p. 373-379.

CHAPTER 3

RESEARCH OBJECTIVES

Polyurethanes have great promise for biomedical applications due to their excellent mechanical properties and great chemical versability. The majority of research on biomedical polyurethanes in the past was focused on the development of nondegradable polyurethanes. In recent years, the study of degradable polyurethanes has examined its application in both hard tissue and soft tissue regeneration. Among the degradable polyurethanes, only few thermoplastic polyurethanes have been intensively investigated. To broaden the biomedical application of polyurethanes, our objective is not only to develop a series of thermoplastic (linear) but also thermoset (crosslinked structure) polyurethanes with adjustable degradation, cytophilicity and hydrophilicity. The research here is to study the properties of linear polyurethane as function of different chain extender during synthesis; furthermore, the properties of crosslinked polyurethanes as function of the soft segment will be highlighted. Our preliminary research on biomedical polyurethanes is addressed in this thesis.

PART I: CHAPTER 4

IMPROVING THE ELASTICITY AND CYTOPHILICITY OF BIODEGRADABLE

POLYURETHANE BY CHANGING CHAIN EXTENDER

Abstract

Two types of biodegradable polyurethanes (PUs) were synthesized from

methylene di-p-phenyl-diisocyanate (MDI), polycaprolactone diol (PCL-diol), and chain

extenders of either butanediol (BD) or 2,2-(methylimino)diethanol (MIDE). The effects

of two types of chain extenders on the degradation, mechanical properties, hydrophilicity,

and cytophilicity of PUs were evaluated. In vitro degradation studies showed that PU

containing MIDE has a higher degradation rate than PU synthesized using BD as a chain

extender. Mechanical testing on dry and wet samples demonstrated that PU containing

MIDE has a much higher elongation in the elastic region than PU containing BD. PU

containing MIDE is more hydrophilic and retains more liquid during in vitro culture.

Furthermore, preliminary cytocompatibility studies showed that both types of degradable

PU are nontoxic, and fibroblasts adhere better and proliferate faster on MIDE containing

PU than BD containing PU. To compare the cytocompatibility and degradation behaviors

of the synthesized PU with existing FDA approved biocompatible material, polylactide

(PLA), with a similar degradation rate, was used as negative control. Two types of PU

were shown to have similar cytocompatibility and degradation behaviors as those of the

PLA material. To verify the effectiveness of the cytotoxicity assay, latex was used as a

positive control. Latex samples showed toxicity to cultured cells as expected. In

conclusion, by changing the type of chain extender used during the synthesis of

degradable PUs, the degradation rate, mechanical properties, hydrophilicity, and cytophilicity can be adjusted for different tissue engineering applications.

Introduction

Biocompatible polymers are extensively investigated for applications in tissue and organ repair. More and more studies are focused on using biodegradable polymers for tissue engineering purposes, because nondegradable polymers may become detrimental due to their impediment of graft-host integration, mechanical impingement, and long-term foreign body reactions.[1-4] Many different categories of biodegradable polymers, including both natural and synthetic, have been used for tissue repair purposes, including collagen, chitosan, hyaluronic acid (HA), polyester, polyanhydride, polycarbonate, polyimide, polyamide, poly(amino acid), polyphosphazene, and so forth.[5-14] Although most of the currently investigated degradable polymers are well tolerated by cells in culture and in tissues, the mechanical properties of these polymers are not compatible with natural tissues. For example, most natural tissues, such as heart, blood vessels, skeletal muscle, tendon, and so forth, are very elastic and strong. The majority of degradable polymers are either too stiff/brittle with low elongation, or very soft with relatively low strength. With the increasing interest in engineering various tissues for the treatment of many types of injuries and diseases, a wide variety of degradable polymers with desirable mechanical, degradation, and cytophilic properties are needed. Because of its excellent mechanical properties and great chemical versatility,[15-22] elastic degradable PU shows promise as being a good candidate for most soft tissue regeneration, such as cardiac muscle,[23] blood vessel,[19, 24] skeletal muscle, tendon, ligament, and skin repair. In addition, elastic degradable PU is also

investigated for hard tissue regeneration, such as cartilage [22] and bone tissue repair.[21, 25] However, the majority of investigations in the past were focused on the development of nondegradable PUs for long-term implantation, such as pacemaker lead insulators, catheters, cardiovascular grafts, and so forth.[26] Relatively few investigations had been directed toward developing degradable PUs.[15-25, 27-31] Moreover, control of the degradation rate and cytophilicity of PUs has not been well studied. Different degradation profiles are required to promote the regeneration of specific tissues.[32-34] For example, if the degradation rate is too fast, the regenerated tissue may be exposed to physiological load too early as in bone, muscle, tendon, and ligament tissue repair, resulting in failure of the implants. If the degradation rate is too slow, stress shielding may occur on regenerating tissues and chronic inflammation may also be exaggerated.[32, 35, 36] To this end, this study is aimed at developing degradable PU with adjustable mechanical properties, degradation behaviors, and biocompatibility. Based on the fact that crystallinity, hydrophilicity, and so forth, may affect the mechanical properties, cytocompatibility, and degradation profile of the degradable polymers, the hypothesis of this study is that the mechanical properties, degradation profile, and cytophilicity of the degradable PU can be adjusted using a chain extender with more polarized, hydrophilic, and flexible characteristics. To this end, the effect of chain extenders on the properties of biodegradable PU was investigated. Methylene di-p-phenyl-diisocyanate (MDI) and polycaprolactone (PCL) based PU was used as a model degradable PU system; and butanediol (BD) and 2,2 -(methylimino)diethanol (MIDE) were utilized as two model chain extenders in this study. The effects of two types of chain extenders on the

degradation, mechanical property, hydrophilicity, and cytophilicity of degradable PUs were evaluated.

Materials and Methods

Materials

Methylene di-p-phenyl-diisocyanate (MDI), butanediol (BD), 2,2-(methylimino) diethanol (MIDE), and N,N-dimethylformamide (DMF) were obtained from Acros Organics Fine Chemicals (Geel, Belgium). Stannous octoate ($Sn(oct)_2$, $Sn[CH_3(CH_2)_3CH(C_2H_5)COO]_2$) and polycaprolactone diol (PCL-diol) with $Mn = 530$ were purchased from Sigma-Aldrich (St. Louis, MO). MDI was purified through vacuum distillation, while BD was distilled with calcium hydrogen in a vacuum to eliminate moisture. DMF was distilled over calcium hydrogen at atmospheric pressure under nitrogen protection. PCL530 was dehydrated in a vacuum oven at 60°C for 48 h. $Sn(oct)_2$ was purified by 4 Å molecular sieve with stirring overnight to get rid of the trace water prior to use.

Synthesis of Degradable PUs

Degradable PUs were synthesized using a two-step method.[35] Briefly, the stoichiometry of the PU synthesis reaction was approximately 2:1:1 of hard segment (diisocyanate)/soft segment (PCL-diol)/chain extender. The MDI was dissolved in 50 mL DMF and PCL-diol was added dropwise into the MDI solution. This mixture was allowed to react at 60°C for a period of 3 h. The solution was cooled to 25°C, 100 mL DMF was added, and then 5% (w/v) chain extender in DMF was added dropwise to the reaction

mixture and stirred for 18 h. After the reaction was finished, the polymer solution was

precipitated in distilled water, and dried in a vacuum oven at 60 ^{o}C for at least 48 h

before further use and characterization.

Chemical Structure of Two Types of Biodegradable PUs

The chemical structures of the two types of biodegradable PUs are shown in

Figure 1. Samples are designated with the first letter indicating the hard-segment type (M

= MDI), second letter indicating the soft-segment (P = PCL), the number indicating the

soft-segment molecular weight, and the final letter indicating the chain-extender (B = BD

and M = MIDE). Thus, MP530B indicates the 1:1 copolymer of MDI, PCL with

molecular weight 530, and BD as the chain extender, and MP530M symbolizes MIDE as

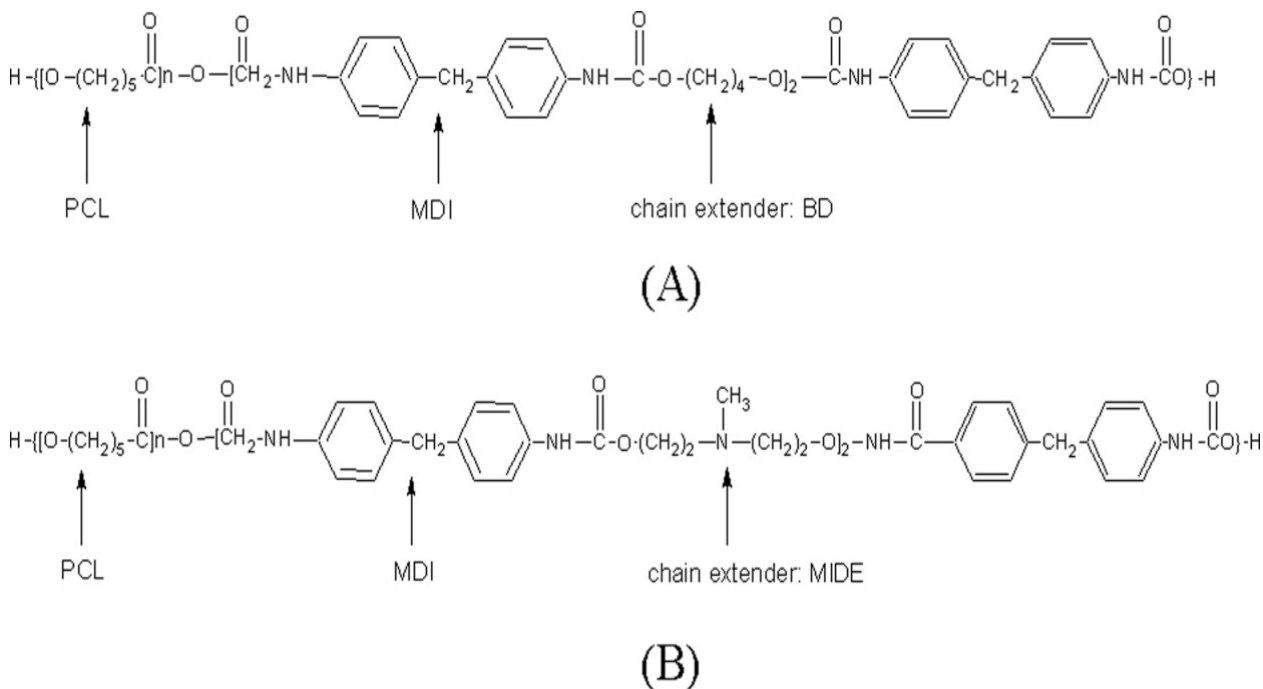the chain extender as shown in Figure 1.

(A)

(B)

Figure 1. Chemical structure of two types of degradable PUs. A: PU with BD as chain
extender. B: PU with MIDE as chain extender.

22

Preparation of PU Thick Films

Polymer films were prepared by solvent casting. The synthesized PUs were dissolved in tetrahydrofuran (THF) at a concentration of 4% (w/v). Polymer solution (12 mL) was then poured into leveled 5 cm PTFE casting plates and cast into thick films at room temperature. Casting plates were covered to prevent dust from contaminating the films and excessive fast casting, which may induce bubbles and result in surface defects. The cast films were removed from the casting plates and dried in a vacuum oven at 60°C for 4 h to remove residual solvent. The average thickness of the film was about 0.08-0.12 mm. Each film was then cut into 5 *12 mm2 rectangular strips for mechanical and degradation tests. Each strip was about 10 mg. For all the experiments PLA (Mw = 139 kDa, Birmingham Polymers, Pelham, AL) was used as the control.

Thermal Behavior Characterization

PU samples were dried under vacuum at room temperature prior to being sealed in an aluminum pan. Thermal analysis was performed in Mettler Differential Scanning Calorimetry (DSC) analyzer (DSC 822e), with a heating rate of 20°C /min under constant nitrogen flow. Polymer samples were heated to 70°C for 10 min, cooled to -100°C, maintained at this temperature for 10 min, and then tested over the range from -100 to 150°C.

Hydrophilicity Test

Hydrophilicity of the materials was examined by measuring the contact angle, the thickness change, and the weight change/swelling rate of the polymer films (n = 6 for

each type of measurement of each material). Water contact angles were measured using a home-made microscopy based contact angle analyzer. The wettability was examined by immersing the polymer films in aqueous solution. The thicknesses of the films were measured before and after being immersed in 0.1M phosphate buffered saline (PBS) for 24 h at 37°C. The film weight was measured before and after immersion in deionized water for 24 h at room temperature, and the film-swelling rate was calculated.

Mechanical Property Testing

Tensile tests were performed at a crosshead speed of 10 mm/min using an MTS 858 Mini Bionix tensile tester. The materials will be used in water-rich environments, such as culture media and live tissue. Therefore, the mechanical properties of the samples were tested in both dry and wet conditions. Wet samples were prepared by saturating them in 0.1M PBS for 12 h before testing. Six samples of each condition were measured to get an average tensile strength and elongation.

Cytocompatibility Testing

Polymer samples were spin-coated on 18 mm diameter coverglasses (n = 6 for each time point and each material). After being dried at room temperature in a vacuum oven for 48 h, samples were sterilized in 75% ethanol for 15 min, washed with sterile 0.1M PBS five times, then put into 12-well tissue culture plates. Two types of assay were carried out. A proliferation assay was used to examine the effect of the materials on cell adhesion and proliferation, and a cytotoxicity assay was used to examine the dead/live cell ratio after exposure to the materials. NIH 3T3 Fibroblasts (CRL-1658, ATCC,

Manassas, VA) were used as a model cell type. Cells ($1.6*10^4$) were seeded on each

sample. Five hours later, when cells were attached on the polymer surfaces, samples were

rinsed once and transferred into new 12-well tissue culture plates to continue culture in

DMEM/F-12 with 10% FBS. Cell proliferation and viability on the samples were

examined at 1, 3, 5, and 7 days using proliferation and cytotoxicity assays. Briefly, a

stable cytosolic lactate dehydrogenase (LDH) released from dead cells (cytotoxicity

assay) or lysed cells (proliferation assay) was coupled to a tetrazolium salt

(2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride, INT) and resulted in the

conversion of INT into a red formazan product. The concentration of red formazan

product was obtained by measuring the absorbance at 490 nm. The amount of color

present was proportional to the number of dead or lysed cells. PLA coated slides were

used as negative controls and latex was used as a positive control to verify the

effectiveness of the assay. Cell morphology for each sample was imaged at all the time

points before performing the two assays.

In Vitro Degradation

In vitro degradation of the polymer was evaluated by recording weight loss,

molecular weight changes, thermal behavior changes, and mechanical property changes

over time under static culture conditions in 0.1M PBS at 37°C. Each polymer strip was

placed in a small vial filled with 650 L 0.1M PBS (pH = 7.4) to perform the degradation

test. Strips of PLA were used as controls. The sealed vials were placed in the water bath

at 77°C. A higher temperature was used to accelerate the degradation rate. A

well-established relationship with different temperatures is available to convert the

degradation profile to 37°C.[34] At each time point, six vials of each type of material

were sampled, rinsed five times with distilled water, and vacuum-dried for 24 h before

weight loss, thermal behavior, and molecular weight loss were analyzed. Changes in the

weight average molecular weight and its distribution over time were determined by gel

permeation chromatography (GPC; Thermal Electron, San Jose, CA). The GPC data were

calibrated with polystyrene standards (EasiCal PS-1, PolymerLabs, Amherst, MA) with

molecular weights in a range of 580-7,500,000 Da. DMF was used as an eluting solvent.

The polymers were dissolved at 0.25% (w/v) in the GPC carrier solvent (pure DMF) and

20 L samples were injected. Changes in the thermal properties upon degradation were

monitored using a Mettler DSC analyzer (DSC 822e). DSC traces of PU before and after

degradation were plotted and the glass transition temperatures (Tg) were determined and

compared.

Results and Discussion

Thermal Analysis

In the development of polymers for biomedical applications, it is important to

know their thermal behaviors, which determine the physical properties of the materials.

For example, if the value of the glass transition temperature (Tg) of the polymer is above

that of body temperature, the polymer is rigid. In contrast, if the Tg values are below

body temperature, as in the PUs used in this study, this indicates the elastomeric nature of

the materials.

Figure 2 shows the DSC thermograms of the two types of PU samples. For PU samples with 530 g/mol PCL-diol, Tgs of 17 and 20°C for MP530B and MP530M, respectively, were observed. These were substantially higher than that of pure PCL (a Tg of -58°C was detected). The Tg values of PCL-based PU indicated a certain degree of hard and soft segment mixing. [37, 38] The slightly higher Tg of MP530M (20°C) than that of MP530B (17°C) indicates that MP530M had less phase separation between hard and soft segments than MP530B. [37, 38] The thermal behavior differences between MP530M and MP530B may have been caused by some of the extra tertiary nitrogen groups in chain extender MIDE. These extra nitrogen groups are able to form hydrogen bonds with the oxygen atoms of the ester linkage and therefore decrease the phase separation between hard and soft segments, which is consistent with the assumption that some hard segments are dissolved in the soft segment matrix phase of PU. [39, 40]
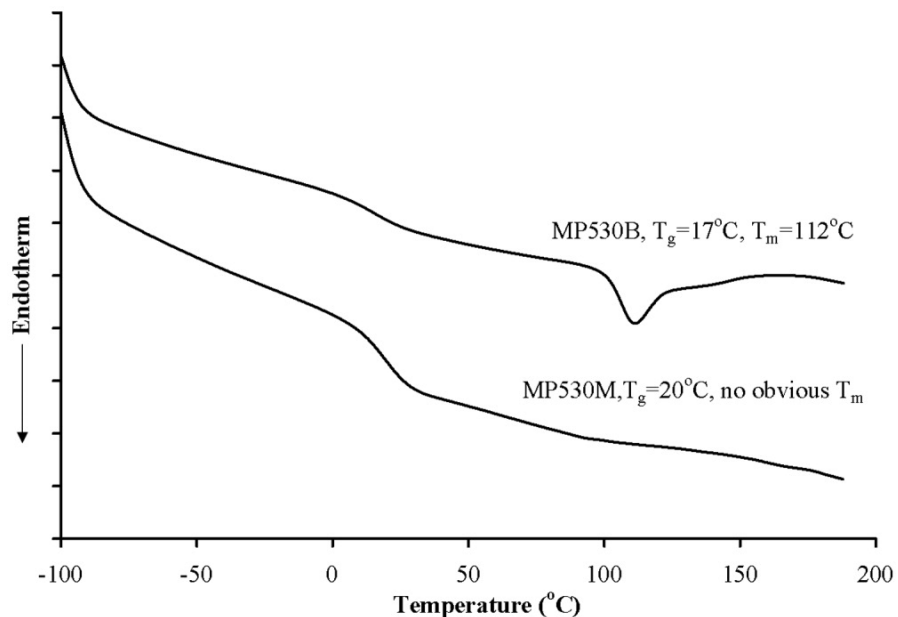

Figure 2. DSC thermograms of MP530B and MP530M. The curve on the top shows the thermal behavior of MP530B with Tg at 17°C and Tm at 112°C. The curve on the bottom shows the thermal behavior of MP530M with Tg at 20°C and no obvious Tm is observed.

Heat capacity (Cp), the amount of heat required to change the temperature of a material by one degree, is obtained by dividing the heat supplied by the temperature increase during the DSC measurement. In particular, the changes in the heat capacity of the two PU samples at the glass transition are 0.505 J/(kg K) for MP530B and 0.553 J/(kg K) for MP530M. The changes in the heat capacity at the glass transition are related to the mobility of the polymers in the rubbery state. The increase in Cp for MP530M over MP530B is attributed to the weakened hard-segment domain cohesion induced by the flexibility of the bonds connected to the tertiary nitrogen atom of the chain extender MIDE. Weakened hard-segment cohesion decreases the effectiveness of the hard-segment domain as a physical crosslinkage, thereby, increasing the mobility of the soft-segment phase and the Cp. Therefore, MP530M has stronger chain mobility than MP530B.

An endothermal peak, demonstrating the melting point (Tm), was observed for MP530B at around 110°C as shown in Figure 2. The melting peak is associated with long-range order in the hard segments. However, no endothermal peak at high temperature was observed for MP530M. The lack of the endothermal peak in MP530M indicates that hard segment ordering in MP530M is much less than that in MP530B.[41] The lack of the melting endothermal peak of MP530M may have been caused by the tertiary nitrogen atom and the pendant side methyl group in the chain extender MIDE backbone. Bonds connected to the nitrogen atom are more easily rotated than those connected to the carbon atom, a property attributed to the increased mixing of the side methyl groups with the soft segments, thus decreasing microphase separation of hard and soft segments.

Based on the thermal behaviors of these two degradable PU materials, both

materials possess elastomer characteristics at body temperature. Because of the decreased

microphase separation in MP530M, one can expect improved hydrophilicity and,

therefore, faster degradation rate. In addition, the elasticity of the material may be

improved as well, due to the homogenous mixture of soft and hard segments for PU

synthesized using MIDE as the chain extender. These hypotheses were further

demonstrated using hydrophilicity, degradation, and mechanical tests.

Hydrophilicity Analysis

Both MP530B and MP530M swell in water. The amount of water absorbed into

the materials was examined by measuring the contact angle, the thickness change of the

samples, and the weight change/swelling rate of the polymer films. The amount of water

absorption is highly dependent on the material's composition and surface properties.

Contact angle is dependent on polymer surface hydrophilicity, while swelling behavior

reflects the bulk hydrophilicity of the polymer. Lower contact angle or higher swelling

ratio indicates higher hydrophilicity.

As shown in Table I, compared with MP530B, MP530M has a lower contact

angle ($p > 0.05$), greater thickness change ($p < 0.05$) in PBS, and greater weight change

($p < 0.05$) in distilled water. These differences can be explained by the structure of

different chain extenders and the conformation of the polymer chains. Compared to BD,

MIDE has an extra tertiary nitrogen, which increases the relative amount of polar

segment, thus the hydrogen bonds between water molecules are easily formed. Moreover,

the side methyl group on MIDE increases the distance between the polymer chains, thus

resulting in higher chain extensibility and lower phase-separation in MIDE-based PUs.

The opportunities and spaces for the penetration of water molecules increased

accordingly. Therefore, MP530M has higher hydrophilicity than MP530B.

Table I. Properties of Two Types of Biodegradable Polyurethanes Synthesized From MDI, PCL530, and Chain Extenders of Either BD Or MIDE

| | Mechanical Properties | | | | | Hydrophilicity | | |
| | Tensile Strength (MPa) | | Elongation at Break (%) | | GPC Mn (g/mol) | | | |
Materials	Dry	Wet	Dry	Wet		Contact Angle (°)	Thickness Change (%)	Weight Change (%)
MP530B	63.8 ± 7.7	62.3 ± 8.3	797 ± 57.8	908 ± 90.1	74,400	75.4 ± 5.6	10.0 ± 1.1	1.6 ± 0.6
MP530M	40.9 ± 6.8	15.6 ± 3.4	756 ± 48.6	989 ± 94.5	80,500	73.6 ± 4.8	17.4 ± 1.4	20.9 ± 1.1

Several nondegradable PUs are commercially used as wound dressing materials

because of their ideal mechanical and hydrophilic properties.[26] It is anticipated that the

MIDE chain extender-based degradable PUs could be used for wound dressing purposes

as well. One of the major requirements of a wound dressing scaffold is that it have a

certain hydrophilicity to maintain an intermediate level of fluid retention, preventing

massive liquid loss from the wound site. Thus, MP530M may have great potential to be

produced into wound dressing scaffolds; work is in progress to test the material in animal

skin wound models.

Mechanical Testing

The tensile testing results for the BD-based and MIDE-based PUs are shown in

Table I and Figure 3. Because of the low microphase separation of hard and soft

segments in MIDE-based PU, MP530M possesses a much wider elastic region (with

500% of elongation) compared to that of MP530B (less than 60% in elongation). This

high elasticity of MP530M is important for engineering tissues with high elasticity, such as muscle, tendon, ligament, heart, and blood vessels. In addition, because these two types of polymers have a similar structure and chain extender content, similar elongation at the break point was found for both types of PUs [Figure 3(B)]. However, lower microphase separation resulted in a lower ultimate tensile strength (UTS) ($p < 0.05$) for MP530M than for MP530B because the amount of semicrystallized hard segment governs the UTS value. Although the tensile strength of MP530M is lower than that of MP530B, the strength is sufficient for tissue engineering applications. [42] The elongation ratio at break of two different materials were shown no significant difference ($p > 0.05$).
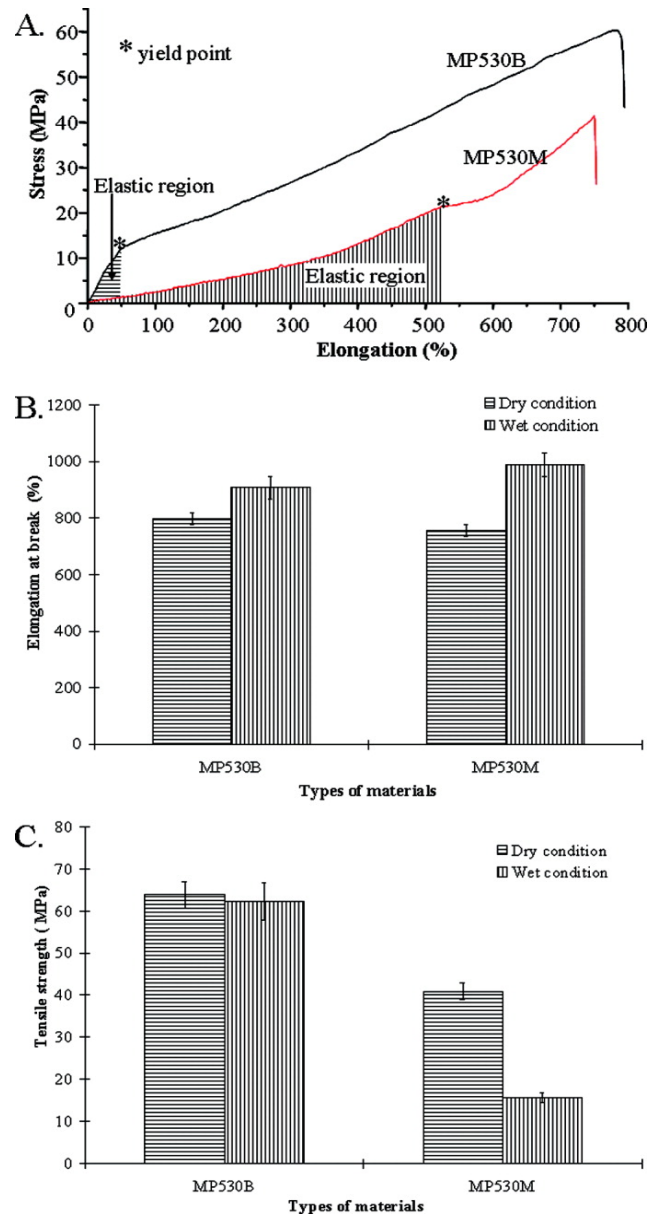
Figure 3. Mechanical properties of two types of PU. A: Typical stress-strain curves of MP530B and MP530M. * symbols show the yield points of two PUs. MP530M has much wider elastic region than that of MP530B, which may result from the homogenous mixture of hard and soft segments in MP530M as discussed in Thermal Analysis section. B: Elongation at break of MP530B and MP530M in dry or PBS saturated conditions are shown. In dry condition, there is no significant difference between two materials. However, in PBS saturated wet condition, MP530M has significantly higher elongation at break than MP530B (p < 0.05). The elongation at break for both materials is higher in wet condition than in dry condition (p < 0.05). C: UTS of MP530B and MP530M in dry and PBS saturated wet conditions. There is no significant UTS change between dry and wet conditions for MP530B. However, the UTS decrease significantly for MP530M in wet condition, which indicates the difference in hydrophilicity for two materials.

As also shown in Figure 3 (B, C), the type of chain extender affects the strength of PUs in both dry and wet states. There is no significant difference in tensile strength (p > 0.05, Table I) for MP530B in either dry or wet state. However, MP530B elongates more when wet (p < 0.05, Table I). In contrast, MP530M is very sensitive to water, and the tensile strength decreases significantly when exposed to water (p < 0.05, Table I). Two factors would be responsible for this effect on MP530M. One is the higher hydrophilicity of MIDE-based PU, which appears to have greater influence on the mechanical properties of MIDE-based PUs than those of BD-based PUs in the wet state. The other factor is the extra tertiary nitrogen groups in MIDE-based PUs, which have the ability to form extra hydrogen bonds with environmental water molecules. Moreover, the side methyl groups on MIDE increase the distance between polymer chains, thus increasing the flexibility of the polymer chain. Both factors may facilitate the penetration of water molecules into the polymer matrix, resulting in a decrease in the stress strength of the MIDE-based PU through the interruption of physical crosslinkages among polymer chains. Likewise, the elongation ratio of MIDE-based PU in the wet state is higher than that of BD-based PUs.

Cytocompatibility Testing

Both qualitative and quantitative evaluations were carried out to compare the cytocompatibility and cytophilicity of two PUs. The morphology of the NIH 3T3 fibroblasts grown on two PU materials and PLA and latex controls were monitored using an inverted light microscope equipped with a CCD camera. As shown in Figure 4, fibroblasts adhere and proliferate well on PLA, MP530B, and MP530M. Cells spread on the surface with a normal flattened appearance, indicating that MP530B and MP530M

have good cytocompatibility. Fibroblasts on MP530M reached confluence faster than MP530B and PLA, indicating improved cytophilicity with MP530M. To further confirm the qualitative observations, two quantitative assays were carried out: a proliferation assay to examine the effect of materials on the cell adhesion and proliferation, and a cytotoxicity assay to examine the dead/live cell ratio after exposure to the materials. As shown in Figure 5 (A), the total number of cells on the MP530M, MP530B, and PLA increased with the time in culture. However, the total number of cells on latex did not increase significantly, indicating that latex caused problems with DNA synthesis and therefore slowed proliferation. Figure 5(B) shows the percentage of dead cells in the culture at different time points. The lack of significant differences in the percentage of dead cells among MP530M, MP530B, and PLA groups indicates good cytocompatibility of MP530B and MP530M materials. In contrast, an increase in cell death was apparent after day 1 time point in the well with latex. The density of the cell nuclei was greatly decreased as shown in Figure 4, which indicated DNA damage of the cells. Cell proliferation was also greatly decreased after day 1. The data obtained from the latex group demonstrated that both the qualitative assay and quantitative assays work well in this study.
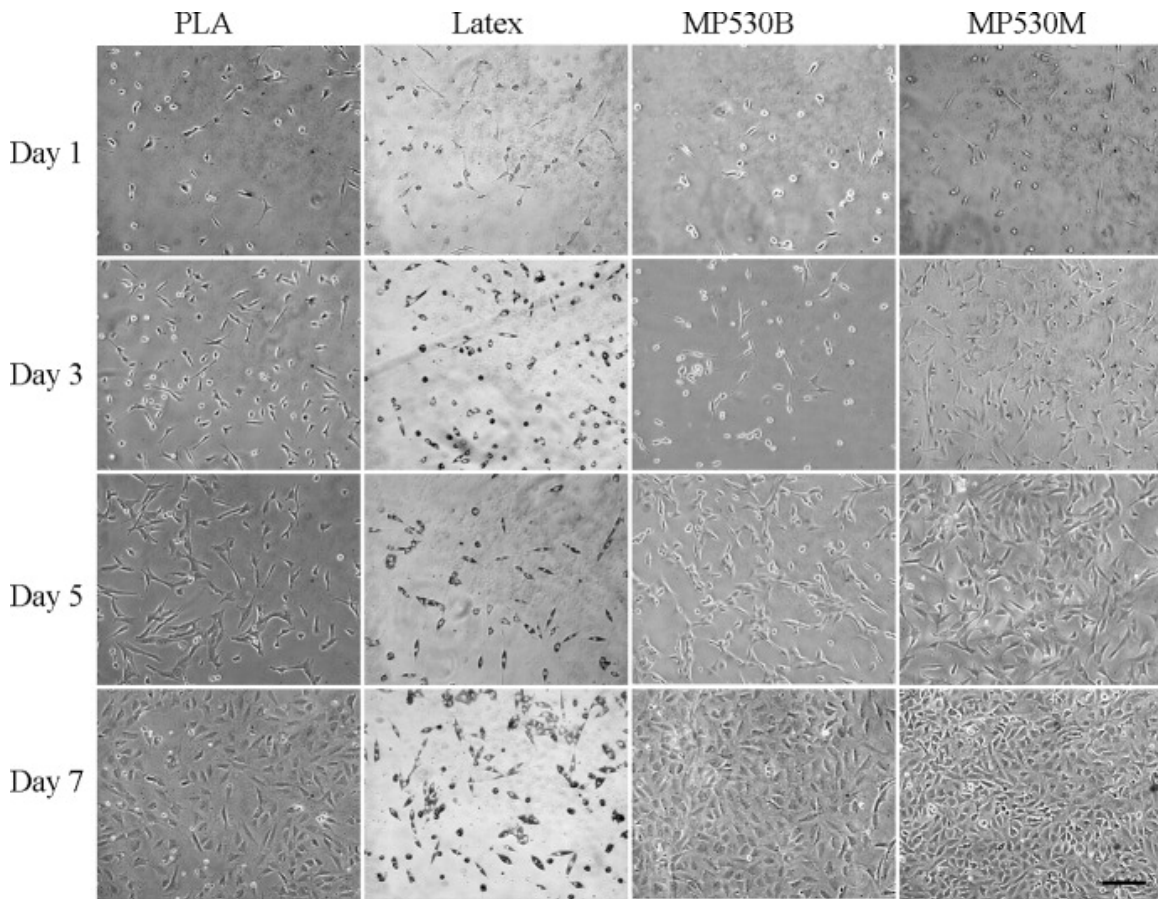
Figure 4. Light microscope images of NIH 3T3 fibroblasts grown on four types of materials for four different time points. Fibroblasts grew and spread well in monolayer on PLA, MP530B, and MP530M at all the time points, demonstrating good cytocompatibility of these materials. However, fibroblasts growing on positive latex control showed abnormal morphology, which indicates the cytotoxicity of latex. Scale bar = 100 um.

Figure 5. Quantitative assays on the proliferation and cytotoxicity of NIH 3T3 fibroblasts on MP530B, MP530M, PLA control, and latex control. A: Proliferation assay. B: Cytotoxicity assay.

The proliferation of fibroblasts on different materials was affected by cell-material interactions. There was no significant difference in proliferation of fibroblasts cultured on PLA controls and the two types of PUs from day 1 to day 3.

However, at day 5 cells divided faster on MP530M than on those on MP530B and PLA control. This may be explained by the higher surface hydrophilicity 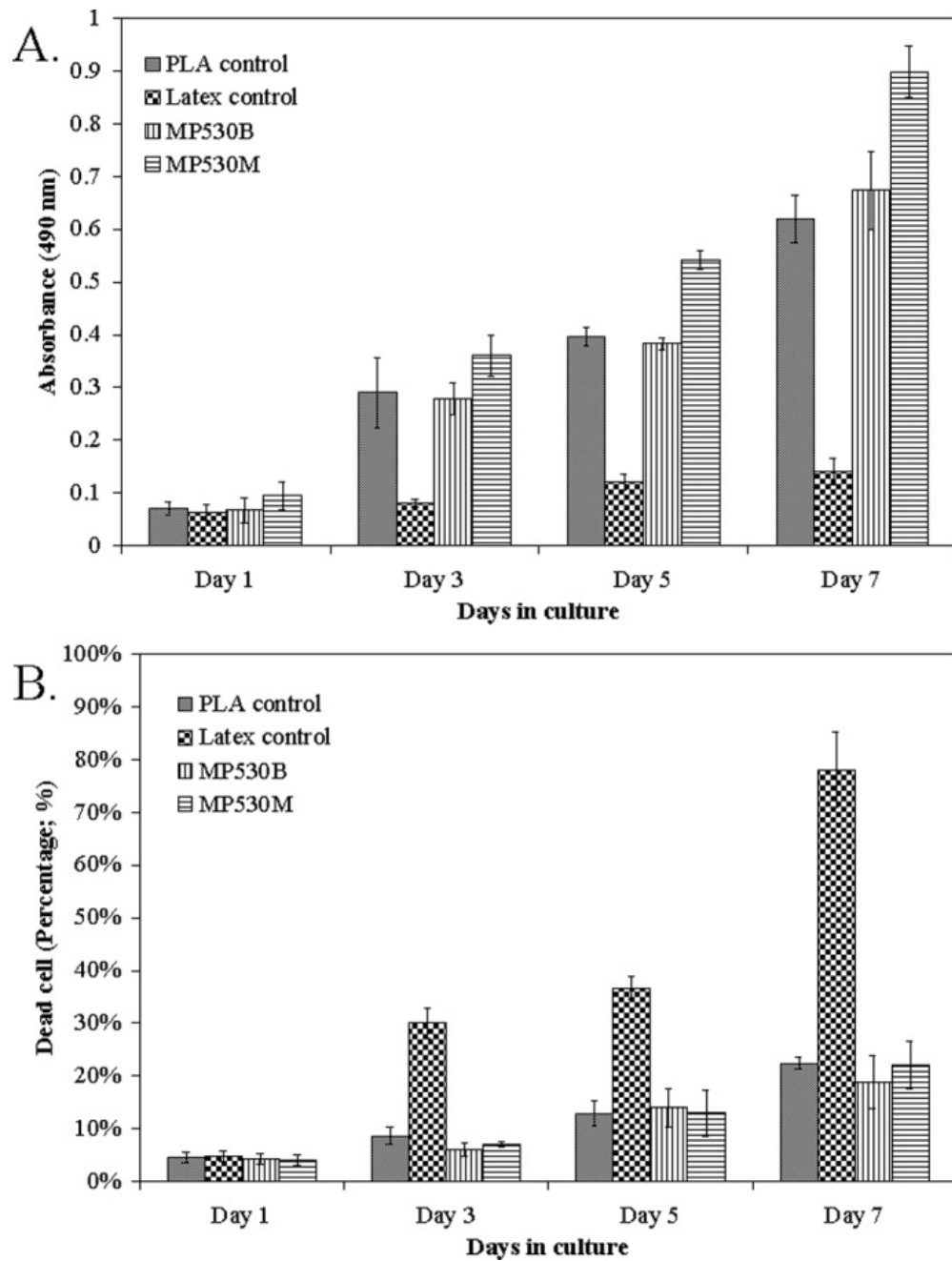of MP530M. This observation is in agreement with the observations of other groups of cell-polymer interactions where cell attachment increased with increasing material hydrophilicity.[43] In addition, it has been found that cells prefer to proliferate on the surface of polymers with slightly positively charged surfaces.[43] In MP530M, tertiary nitrogen atoms in MIDE give a relatively higher polarity than BD, which helps cell proliferation. Higher hydrophilicity and polarity of MP530M are responsible for faster cell proliferation. Although very high hydrophilicity may not promote cell growth, in a certain range (moderate hydrophilicity), with the increase of hydrophilicity, the cells attach and proliferate better.[43]

In Vitro Degradation

Degradation of a material means that the molecular weight, mass, and mechanical properties decay over time. Therefore, the degradation behaviors of MP530B and MP530M were examined by changes in molecular weight, thermal behavior, pH, weight, and mechanical behavior. For both MP530B and MP530M, molecular weight varied only a small amount during the first 2 days in culture at 77°C [Figure 6 (A)]. Molecular weight of MP530M dropped significantly on day 3. However, MP530B degraded significantly slower with the molecular weight remaining constant till day 7. After 7 days in 0.1M PBS culture at 77°C, MP530M lost about 20% of initial molar mass, while MP530B lost only 2%; 20 days later, MP530M lost about 58% of initial molar mass while MP530B lost 16%. Thus, MP530M has a 4-10 times faster degradation rate than MP530B. GPC data clearly demonstrated that MIDE-based PU has a faster degradation rate than BD-based

PU. PCL and most PCL-based polymers, such as PCL-based PU, degrade slowly, because of the high hydrophobicity of PCL, and therefore, are resistant to hydrolysis. This study has demonstrated that the introduction of MIDE increases the hydrophilicity and degradation rate when compared with BD-based PU. Comparing the chemical structure of the two chain extenders, MIDE contains extra tertiary nitrogen atoms whereas BD has no extra nitrogen. As described above, the side methyl groups in the MIDE backbone not only easily form hydrogen bonds with water, but also increase the distance between each polymer chain producing more amorphous regions. Both factors may result in the much faster degradation rate of MIDE based PU.
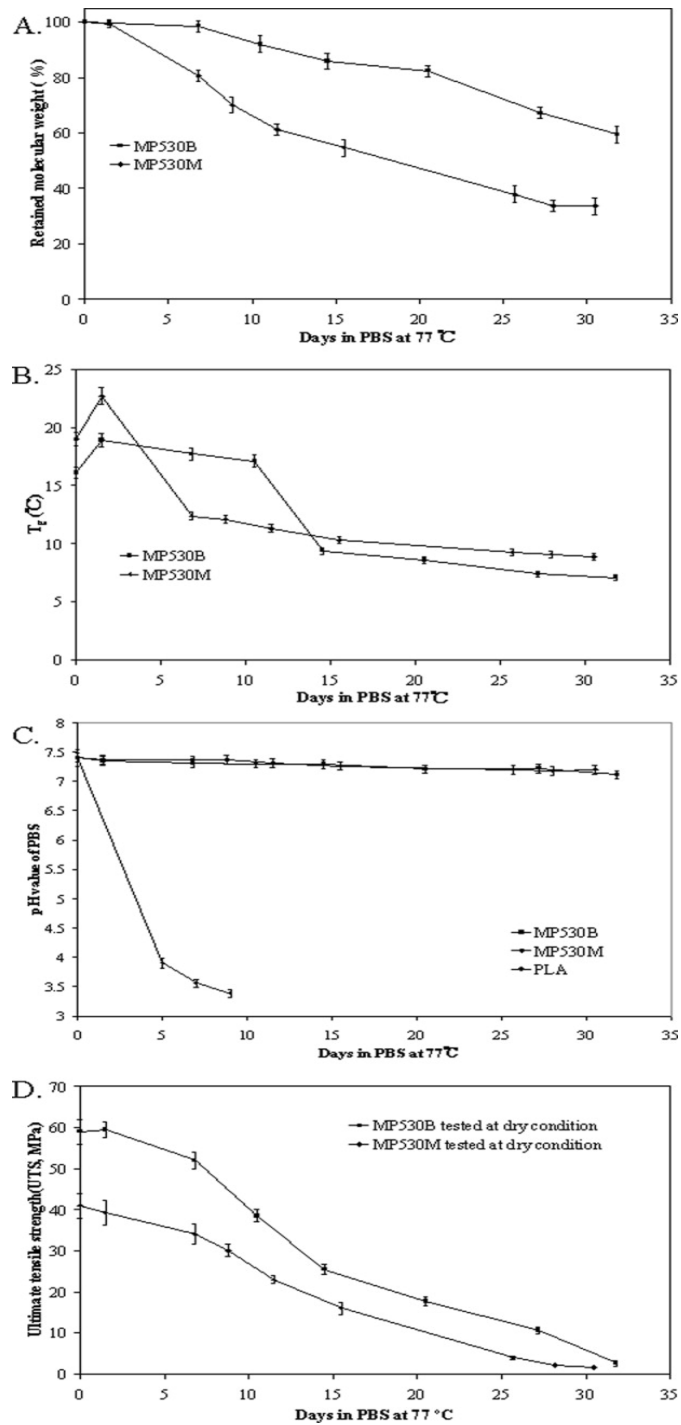
Figure 6. Degradation behavior of MP530B and MP530M in 0.1M PBS at 77°C. A: The average retained molecular weights of MP530B and MP530M change as a function of culture time. B: The glass transition temperatures change as a function of culture time. C: pH values of 0.1M PBS media where polymer degrade in change as a function of time. PLA samples were used as control. D: Mechanical properties of MP530B and MP530M change as a function of time.

For biomedical use, it is important to obtain the correlation between the degradation profile at 37 and 77°C. It was reported [34] that the degradation rate of MP530B at 37°C is about 1/20 of the degradation rate at 77°C. Thus, the degradation of MP530M at 37°C can be postulated from the degradation profile at 77°C. MP530M may lose 50% of its initial molecular weight after about 9-10 months at 37°C, while MP530B may degrade 3-4 times slower than MP530M. To further increase the degradation rate of elastic PU, hard segments or soft segments with more hydrophilic nature can be employed. Our lab has synthesized elastic degradable PU with half-life degradation ranging from 2 months to several years. Therefore, degradable PUs, some of the most promising materials for tissue engineering applications, with different degradation profiles can be developed and applied for engineering different types of tissues.

Figure 6(B) shows Tg changes during MP530B and MP530M degradation processes. There was an increase in Tg during the first 2 days, which may have been caused by the annealing effect when samples were initially put into 77°C. As the hydrolysis procedure was initiated, Tg decreased with the degradation time because molecular weight decreased during the degradation process. During degradation, the physical crosslinkages were destroyed, the percentage of short chains increased, and the phase separation increased, which resulted in the enhanced mobility of polymer chains. Thus the Tg decreased correspondingly.

As previously reported, polylactide- and polyglycolide-based degradable polymers may produce an acidic environment during degradation.[44-46] Degradation products of MP530M and MP530B are carboxylate and hydroxyl groups, which may produce an acidic environment. To examine whether MP530B and MP530M influence

the pH of culture media, pH values were recorded during polymer degradation at 77°C. The changes in pH of PBS culture media with culture time are illustrated in Figure 6(C). PLA was used as a control. Both MP530B and MP530M have little effect on pH of the culture media. For example, MP530M, with a faster degradation rate than MP530B, caused the pH to decrease no more than 0.25 points after 32 days of degradation at 77°C, which is equal to 9-10 months of degradation at 37°C. In contrast, the pH values of PLA cultured media markedly decreased in the first 10 days [Figure 6(C)]. Therefore, the degradation products of MP530B and MP530M do not significantly cause the pH value change in the culture media in vitro.

The changes in UTS of MP530B and MP530M as a function of in vitro degradation time are shown in Figure 6(D). Although MP530M has a lower tensile strength than MP530B, they both have a similar slope of tensile strength loss during the degradation. The tensile strengths of MP530B and MP530M were examined at both dry and PBS saturated wet conditions as shown in Figure 7. As shown in Figure 3(C), the influence of water on the tensile strength of MP530M is far stronger than on MP530B before degradation process. The wet MP530M sample had only 30% of the tensile strength of the dry sample. The influence of water on the tensile strength of MP530B is much lower, which is caused by the higher hydrophilicity of MP530M than MP530B. Therefore, it is easier for water molecules to attack the bonds of the MP530M, which undermines the physical interaction among polymer chains. This results in lower tensile strength of the wet samples when compared to the wet MP530B samples. However, during the degradation, hydrophilic groups in MP530M were released, and therefore the influence of the water on MP530M became weaker. As shown in Figure 7(A), the

differences between the dry and wet MP530M samples in UTS value became less and

less during the degradation process.
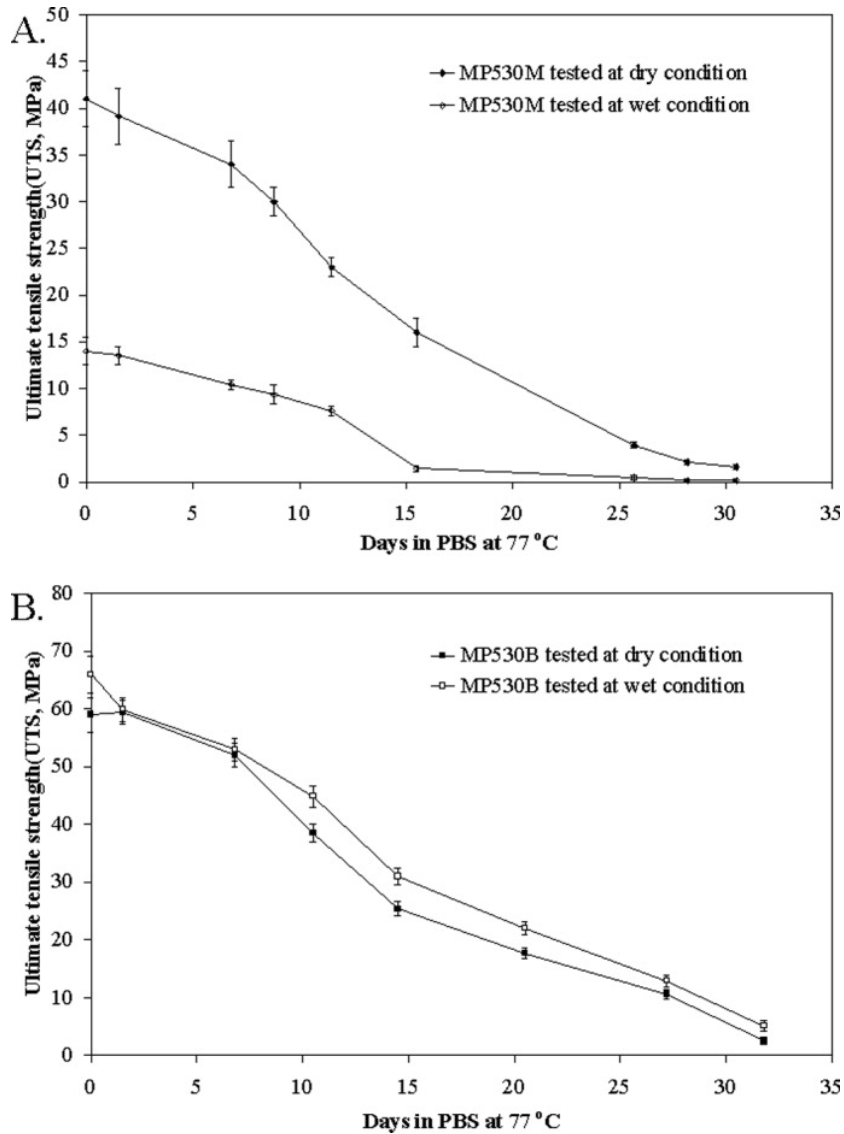
Figure 7. UTS of MP530M (A) and MP530B (B) as a function of hydrolysis time in 0.1M PBS at 77°C. Curves with solid symbols were tested under dry condition, and curves with hollow symbols were tested under PBS-saturated condition.

Conclusions

This study aimed at developing degradable PU with adjustable mechanical

properties, degradation behaviors, and biocompatibility. The hypothesis is that the

mechanical properties, degradation profile, and cytophilicity of the degradable PU can be

adjusted using a chain extender with more polarized, hydrophilic, and flexible characteristics. MDI and PCL based PUs were investigated as a model degradable PU system. BD and MIDE were studied as two model chain extenders. In vitro degradation studies showed that PU containing MIDE possesses a higher degradation rate than PU synthesized using BD as a chain extender. Mechanical testing of dry and wet samples demonstrated that PU containing MIDE has a much higher elongation in the elastic region than PU containing BD. PU containing MIDE is more hydrophilic and retains more liquid during the in vitro culture. Furthermore, preliminary cytocompatibility studies showed that both types of degradable PU are nontoxic, and fibroblasts adhere better and proliferate faster on MIDE containing PU than BD containing PU. In conclusion, by changing the types of chain extender used during degradable PU synthesis, the degradation rate, mechanical properties, hydrophilicity and cytophilicity can be adjusted for different tissue engineering applications.

References

1. Ertel, S.I., Kohn, J., Evaluation of a series of tyrosine-derived polycarbonates as degradable biomaterials. J Biomed Mater Res, 1994. 28(8): p. 919-30.

2. Sawhney, A.S., Hubbell, J. A., Rapidly degraded terpolymers of dl-lactide, glycolide, and epsilon-caprolactone with increased hydrophilicity by copolymerization with polyethers. J Biomed Mater Res, 1990.24(10):p. 1397-411.

3. Radder, A.M., Leenders, H., Van Blitterswijk, C. A., Interface reactions to PEO/PBT copolymers (Polyactive) after implantation in cortical bone. J Biomed Mater Res, 1994. 28(2): p. 141-51.

4. Engelberg, I., Kohn, J., Physico-mechanical properties of degradable polymers used in medical applications: a comparative study. Biomaterials, 1991. 12(3): p. 292-304.

5. Courtman DW, Pereira, CA., Kashef V, McComb D, Lee JM, Wilson GJ., Development of a pericardial acellular matrix biomaterial: Biochemical and mechanical effects of cell extraction. J Biomed Mater Res, 1994. 28: p. 655-666.

6. Ma L, G.C., Mao Z, Zhou J, Shen J, Hu X, Han C., Collagen/chitosan porous scaffolds with improved biostability for skin tissue engineering. Biomaterials, 2003. 24: p. 4833-4841.

7. Silver FH, Pins, G., Cell growth on collagen: A review of tissue engineering using scaffolds containing extracellular matrix. J Long Term Eff Med Implants, 1992. 2: p. 67-80.

8. Aigner J, Tegeler, J., Hutzler P, Campoccia D, Pavesio A, Hammer C, Kastenbauer E, Naumann A., Cartilage tissue engineering with novel nonwoven

structured biomaterial based on hyaluronic acid benzyl ester. J Biomed Mater Res, 1998. 42: p. 172-181.

9.   Heller J, Ng, SY., Fritzinger BK, Roskos KV, Controlled drug release from bioerodible hydrophobic ointments. Biomaterials, 1990. 11(235-237.).

10.   Domb AJ., Biodegradable polymers derived from amino acids. Biomaterials, 1990. 11(686-689).

11.   Leong KW, D'Amore, PD., Marletta M, Langer R., Bioerodible polyanhydrides as drug-carrier matrices. II. Biocompatibility and chemical reactivity. J Biomed Mater Res, 1986. 20: p. 51-64.

12.   Ambrosio AM, Allcock, HR., Katti DS, Laurencin CT., Degradable polyphosphazene/poly(-hydroxyester) blends: Degradation studies. Biomaterials, 2002. 23: p. 1667-1672.

13.   Nagaoka S, A.K., Okuyama Y, Kawakami H., Interaction between fibroblast cells and fluorinated polyimide with nano-modified surface. Int J Artif Organs, 2003. 26: p. 339-345.

14.   Risbud MV, Bhonde, RR., Polyamide 6 composite membranes: Properties and in vitro biocompatibility evaluation. J Biomater Sci Polym Ed, 2001. 12: p. 125-136.

15.   Fromstein, J.D., Woodhouse, K. A., Elastomeric biodegradable polyurethane blends for soft tissue applications. J Biomater Sci Polym Ed, 2002. 13(4): p. 391-406.

16.   Van Minnen B, S.B., van Leeuwen MB, van Kooten TG, Bos RR., A long-term in vitro biocompatibility study of a biodegradable polyurethane and its degradation products. J Biomed Mater Res A, 2005. 76(2): p. 377-385.

17. Liu C, G.Y., Qian Z, Fan L, Li J, Chao G, Tu M, Jia W., Hydrolytic degradation behavior of biodegradable polyetheresteramide-based polyurethane copolymers. J Biomed Mater Res A, 2005. 75: p. 465-471.

18. Van Minnen B, L.M., Stegenga B, Zuidema J, Hissink CE, van Kooten TG, Bos RR., Short-term in vitro and in vivo biocompatibility of a biodegradable polyurethane foam based on 1,4-butanediisocyanate. J Mater Sci Mater Med, 2005. 16: p. 221-227.

19. Guan J, F.K., Sacks MS, Wagner WR., Preparation and characterization of highly porous, biodegradable polyurethane scaffolds for soft tissue applications. Biomaterials, 2005. 26: p. 3961-3971.

20. Yang TF, Chin, WK., Cherng JY, Shau MD., Synthesis of novel biodegradable cationic polymer: N,N-diethylethylenediamine polyurethane as a gene carrier. Biomacromolecules, 2004. 5: p. 1926-1932.

21. Gorna K, Gogolewski, S., Preparation, degradation, and calcification of biodegradable polyurethane foams for bone graft substitutes. J Biomed Mater Res A, 2003. 67: p. 813-827.

22. Grad S, K.L., Gorna K, Gogolewski S, Alini M., The use of biodegradable polyurethane scaffolds for cartilage tissue engineering: Potential and limitations. Biomaterials, 2003. 24: p. 5163-5171.

23. McDevitt, TC, Woodhouse, KA., Hauschka SD, Murry CE, Stayton PS., Spatially organized layers of cardiomyocytes on biodegradable polyurethane films for myocardial repair. J Biomed Mater Res A, 2003. 66: p. 586-595.

24. Galletti G, Ussia, G. , Farruggia F, Baccarini E, Biagi G, Gogolewski S., Prevention of platelet aggregation by dietary polyunsaturated fatty acids in the biodegradable polyurethane vascular prosthesis: An experimental model in pigs. Ital J Surg Sci, 1989. 19: p. 121-130.

25. Zhang J, Doll, BA., Beckman EJ, Hollinger JO., A biodegradable polyurethane-ascorbic acid scaffold for bone tissue engineering. J Biomed Mater Res A, 2003. 67: p. 389-400.

26. Cohen, I.K., Diegelman, R. F., Lindblad, W. J., Wound healing : biochemical and clinical aspects. 1st ed. 1992, Philadelphia: Saunders. xxv, 630.

27. Farso Nielsen F, Karring, T., Gogolewski S., Biodegradable guide for bone regeneration. Polyurethane membranes tested in rabbit radius defects. Acta Orthop Scand, 1992. 63: p. 66-69.

28. Galletti G, Gogolewski, S., Ussia G, Farruggia F., Long-term patency of regenerated neoaortic wall following the implant of a fully biodegradable polyurethane prosthesis: Experimental lipid diet model in pigs. Ann Vasc Surg, 1989. 3: p. 236-243.

29. Pavlova M, D.M., Biocompatible and biodegradable polyurethane polymers. Biomaterials, 1993. 14: p. 1024-1029.

30. Warrer K, Karring, T., Nyman S, Gogolewski S., Guided tissue regeneration using biodegradable membranes of polylactic acid or polyurethane. J Clin Periodontol, 1992. 9(9, Part 1): p. 633-640.

31.   Ganta, SR, Piesco, NP., Long P, Gassner R, Motta LF, Papworth GD, Stolz DB, Watkins SC, Agarwal S., Vascularization and tissue infiltration of a biodegradable polyurethane matrix. J Biomed Mater Res A, 2003. 64: p. 242-248.

32.   Sutherland, K., Mahoney, J. R., Coury, A. J., Eaton, J. W., Degradation of biomaterials by phagocyte-derived oxidants. J Clin Invest, 1993. 92(5): p. 2360-7.

33.   Gogolewski, S., Pennings, A. J., Lommen, E., Wildevuur, C. R., Small-caliber biodegradable vascular grafts from Groningen. Life Support Syst, 1983. 1 Suppl 1: p. 382-5.

34.   Gisselfalt, K., Edberg, B., Flodin, P., Synthesis and properties of degradable poly(urethane urea)s to be used for ligament reconstructions. Biomacromolecules, 2002. 3(5): p. 951-8.

35.   Vermette, P., Biomedical applications of polyurethanes. Tissue engineering intelligence unit ; 6. 2001, Georgetown, Tex. Austin, Tex.: Landes Bioscience ; Eurekah.com. 273.

36.   Ewing JW. , Arthroscopy Association of North America, Bristol-Myers/Zimmer (Firm). Articular Cartilage and Knee Joint Function : Basic Science and Arthroscopy. 1990, Raven: New York. p. 35-69.

37.   Couchman PR., Prediction of glass-transition temperature for compatible blends formed from homopolymers of arbitrary degree of polymerization. Compositional variation of glass-transition temperatures. Macromolec. , 1980. 13: p. 1272-1276.

38.   Koberstein JT, Leung, LM., Compression-molded polyurethane block copolymers. II. Evaluation of microphase compositions. Macromolecules, 1992. 23: p. 6205-6213.

39. Srichatrapimuk, V.W., Cooper, S.L.J., Infrared thermal analysis of polyurethane block polymers. J of Macromolecular Sci.. Physics, 1978. B15: p.267-311.

40. Senich GA, MacKnight, WJ., Fourier transform infrared thermal analysis of a segmented polyurethane. Macromolecules, 1980. 13: p. 106-110.

41. Takahara, A., Tashita, J., Kajiyama, T., Takayanagi, M., Effect of aggregation state of hard segment in segmented poly(urethaneureas) on their fatigue behavior after interaction with blood components. J Biomed Mater Res, 1985.19(1): p.13-34.

42. Dunn, M.G., Avasarala, P. N., Zawadsky, J. P., Optimization of extruded collagen fibers for ACL reconstruction. J Biomed Mater Res, 1993. 27(12): p. 1545-52.

43. Webb K, Hlady, V., Tresco PA., Relative importance of surface wettability and charged functional groups on NIH 3T3 fibroblast attachment, spreading, and cytoskeletal organization. J Biomed Mater Res, 1998. 41: p. 422-430.

44. Hollinger, J., O., Biomedical applications of synthetic biodegradable polymers. 1995, Boca Raton: CRC Press. 247.

45. Athanasiou, K.A., Niederauer, G. G., Agrawal, C. M., Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. Biomaterials, 1996. 17(2): p. 93-102.

46. Penco, M., Marcioni, S., Ferruti, P., D' Antone S., Deghenghi, R., Degradation behaviour of block copolymers containing poly(lactic-glycolic acid) and poly(ethylene glycol) segments. Biomaterials, 1996. 17(16): p. 1583-90.

PART II: CHAPTER 5

SYNTHESIS AND CHARACTERIZATION OF BIOCOMPATIBLE, DEGRADABLE,

LIGHT-CURABLE, POLYURETHANE-BASED ELASTIC HYDROGELS

Abstract

A series of degradable polyurethane based light-curable elastic hydrogels were

synthesized from polycaprolactone diol (PCL-diol), polyethylene glycol (PEG), lysine

diisocyanate (LDI), and 2-hydroxyethyl methacrylate (HEMA) through UV light initiated

polymerization reaction. LDI was used as hard segment and PCL and/or PEG were used

as soft segments. By changing the PCL to PEG ratio during the pre-polymer synthesis,

polyurethanes with different soft segmental structures, hydrophilicity, and cytophilicity

were obtained after light-initiated polymerization. The chemical structures of the

synthesized polymers were characterized using differential scanning calorimetry (DSC),

thermogravimetric analysis (TGA) and Fourier transform infrared spectroscopy (FTIR).

Physical properties such as swelling, mechanical properties, and in vitro degradation

were evaluated. Materials containing a higher ratio of PEG exhibit higher water

absorbance, higher degradation rate in vitro, and lower mechanical strength in the

hydrated state. Mouse embryonal carcinoma-derived clonal chondrocytes were used as a

model cell type to study the cytocompatibility of the synthesized polymers. Chondrocyte

attachment, proliferation rates, and morphologies all varied with changes in the PCL/PEG

ratio. With a higher PEG ratio, lower cell attachment and proliferation were observed. To

improve the cell attachment and proliferation on high PEG content hydrogels, bioactive

molecules, such as peptides and proteins, were conjugated or immobilized in the gel

matrix during the light-curing process. In this study, a short peptide, Arg-Gly-Asp-Ser (RGDS), was used as a model biomolecule and incorporated into the gels during the light-curing process and improved cell growth was observed. In summary, the use of PCL/PEG at different ratios, as well as the introduction of HEMA into polyurethane, allows the synthesis of a series of biocompatible elastic hydrogels with tunable physical and cytophilic properties through light initiated polymerization. This series of materials also allows for controlling cell attachment and growth by incorporating bioactive molecules during the light-curing process.

Introduction

A material that is to be used for tissue repair requires a wide range of physical and biological properties including biocompatibility, biodegradability, strength, and elasticity. [1] Due to their excellent biocompatibility, chemical versatility and superior mechanical properties, polyurethanes have been extensively investigated for biomedical applications, including cardiovascular devices such as vascular prostheses, intra-aortic balloons, cardiac valves and insulating sheaths for pacemakers, membranes for dialysis, craniofacial reconstruction, breast implants, etc. [2-8] Most polyurethane materials utilized for these applications are nondegradable.

In recent years, interest in using resorbable or degradable polyurethanes for tissue regeneration has been continuously increasing. Several series of degradable polyurethanes have been developed for applications including cardiovascular repair, ligament reconstruction, cancellous bone regeneration, and controlled drug delivery, among others. [9-11] Most degradable polyurethanes were developed by the introduction of labile moieties, such as caprolactone, [12] lactides, [13] hydroxybutyric acid, [14],

saccharide [15], or amino acids [16], as either soft segments or chain extenders. The labile bonds can be broken in vivo either enzymatically or chemically, in most cases by hydrolysis. In some studies, polyethylene glycol (PEG) was introduced into the soft segment to adjust the susceptibility of the polymer chains to hydrolysis [17].

Since polycaprolactone (PCL) and its degradation products are nontoxic and it has been approved by the Food and Drug Administration (FDA) and evaluated for many tissue repair and drug delivery applications, polycaprolactone diol (PCL-diol) is one of the most frequently used building blocks for soft segments of degradable polyurethanes. [9,17-20] However, its hydrophobicity and semi-crystalline structure determine its very slow degradation profile. [9] In those studies, [9, 17-20] polyurethanes with a PCL segment exhibited improved elasticity, tensile strength and yield strength when compared to PLC due to the formation of hydrogen bonds among polyurethane chains.

Due to its chemical functionality, super flexible hydrophilic chain motion, non-toxicity and non-immunogenicity, [21-22], PEG is another candidate for the soft segment of polyurethanes. PEG is widely used for tissue engineering, drug delivery, surface modification of implants, etc. [21, 23-27]. For instance, because its hydrophilic surface has a low interfacial free energy in contact with body fluids, PEG has been used for surface modification of a blood contacting construct to decrease protein adsorption and platelet adhesion for cardiovascular application. [28] PEG has been functionalized with dimethacrylate groups and crosslinked to form hydrogels via light-initiated polymerization and tested for cartilage tissue repair. [21] PEG can be incorporated into the polyurethane structure to form hydrogel materials that inhibit protein adsorption and cell adhesion by dramatically increasing the hydrophilicity of the materials. Reduced

protein or cell adhesion allow to limit unwanted protein and cell adhesion and therefore provide great opportunities for functionalizing these materials with specific biomolecules to promote the attachment of desirable cell types, and the same time, wards-off unwanted cell types.

Hydrogels, an insoluble network of polymer chains that swell in aqueous solution, are a class of materials that have shown great promise for tissue repair due to their tissue-like water content, permeability, adjustable functionality, and low coefficient of friction. [29]. Many types of tissues in the body are in the form of hydrogels. Native cartilage, for instance, is a durable elastic tissue, with an extracellular matrix consisting mostly of proteoglycans and type II collagen. The extracellular matrix of cartilage is hydrogel in nature [30] and cartilage is able to withstand large loads by virtue of the matrix's strength and the exudable water held within the tissue. Thus, hydrogels are especially attractive for cartilage tissue regeneration due to their excellent diffusive permeability, minimal irritation to surrounding tissues, low surface friction and wear that related to the strong water retention of the hydrophilic network. [31]. However, most biocompatible degradable hydrogels developed to date are not strong enough or elastic enough for immediate physiological loading in cartilage repair.

To overcome the disadvantages associated with currently available soft hydrogels, we are endeavoring to develop a highly elastic biocompatible and degradable hydrogel for cartilage repair. Since most cartilage defects are irregular in shape and size, instant polymerization causing the gel to assume the shape of the defect offers a great advantage for customizing the scaffolds or even for computer-aided scaffold fabrication with precise

temporal and spatial control. [32, 33]. To this end, a series of photopolymerizing elastic hydrogels were developed and characterized in this study.

Materials and Methods

Materials

Lysine diisocyanate (LDI) was obtained from Kyowa Hakko Koygo Co., Ltd, Tokyo, Japan. Stannous octoate ($Sn(oct)_2$, $Sn[CH_3(CH_2)_3CH(C_2H_5)COO]_2$) and polycaprolactone diol (PCL-diol) with $M_n = 530$ (PCL530) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). 2-hydroxyethyl methacrylate (HEMA), dimethyl sulfoxide (DMSO), and polyethylene glycol (PEG600) with average molecular weight 600 (Mw=600) were purchased from Fisher Scientific International. UV light-initiator Irgacure 2959 was kindly provided by Ciba Specialty Chemicals, Inc. LDI was purified by vacuum distillation, while DMSO was distilled with calcium hydrogen (CaH_2) at atmospheric pressure under nitrogen protection. PCL530 and PEG600 were dehydrated in a vacuum oven at 60 °C for 48 hours, then 4 Å molecular sieve was added to maintain dryness at room temperature. $Sn(oct)_2$ and HEMA were dried by 4 Å molecular sieve with stirring at room temperature overnight to eliminate the trace water. Irgacure 2959 was used without further purification.

UV light generator (American Ultraviolet Company, Santa Ana, CA, USA) was used to generate the UV light used to initiate polymerization. The light intensity used was $800 mW/cm^2$ at a wavelength of 365 nm. Irradiation from the generator was transmitted through a quartz optical guide fiber (diameter: 5mm; length: 1m) to the pre-polymers.

Synthesis of LDI based biodegradable hydrogel

Pre-polymers of UV light curable polyurethanes with different PCL-PEG molar ratios were synthesized using a traditional two-step method [2]. Briefly, the stoichiometry of the synthesis reaction was approximately 2:1:1 of hard segment (LDI):soft segment (PCL530, PEG600, or their mixture):chain terminator (HEMA). Three different PCL530 to PEG600 ratios were studied as model materials, i.e., 100% PCL530, a mixture of equal parts PCL530 and PEG600, and 100% PEG600. LDI was dissolved in DMSO at room temperature. A calculated amount of PCL530-PEG600 mixture was added dropwise into the LDI solution with vigorous stirring, then 0.1% wt $Sn(oct)_2$ was added as catalyst. The mixture was allowed to react at 80 °C for 3 hours under nitrogen protection. The resulting mixture was cooled to room temperature, and a calculated amount of HEMA with 0.1 wt% 4-methoxyphenol (MEHQ, inhibitor) was added to the reaction mixture and stirred for another 24 hours in the dark under dry oxygen atmosphere protection to prevent the self-crosslinking reaction. The final pre-polymers were viscous liquids and can be preserved in the dark at 4 °C. The pre-polymer was degassed using nitrogen to remove the oxygen before final UV light polymerization, then 0.04 wt% UV light initiator Irgacure 2959 was added and mixed homogeneously. This viscous mixture was poured into Teflon molds and exposed to UV light for 20 seconds to obtain either elastic rod or film samples. Samples were then rinsed in 75% ethanol solution for 24 hours and vacuum dried for use in future experiments. The resulting specimens were clear elastomers. The polymerization reaction procedure and chemical structures of the final product are illustrated in Figure 1.
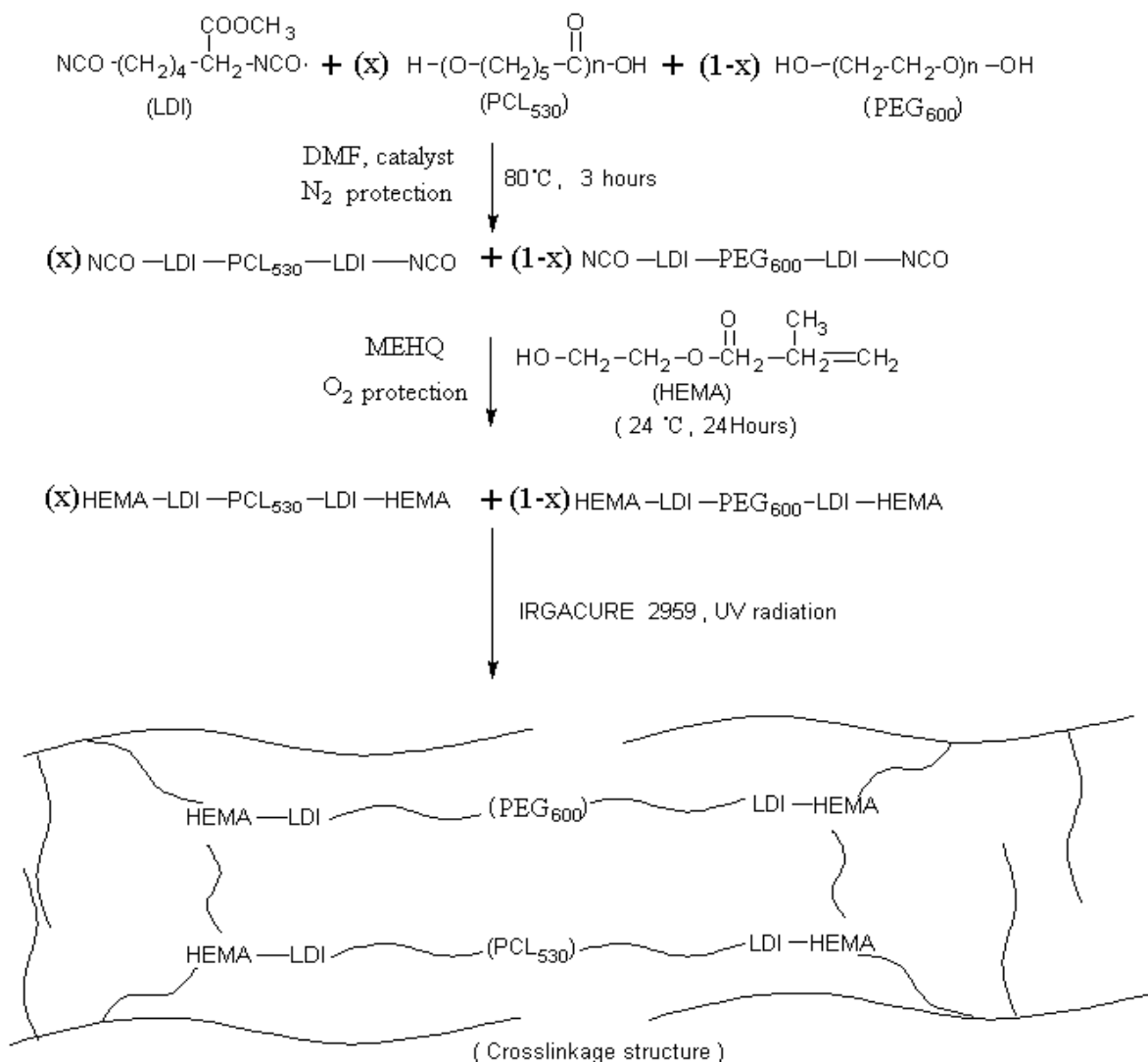
Figure 1: Schematic diagram shows the preparation of LDI, PEG600, PCL530 and HEMA based light curable degradable hydrogels. The PCL530:PEG600 ratio was adjusted for synthesizing materials with different hydrophilicity.

For the model hydrogels with bioactive peptides, RGDS (American Peptide Company Inc., Sunnyvale, CA, USA) at a final concentration of 2 µmol/ml was crosslinked into the hydrogel during the UV light-initiated polymerization.

Chemical structures of two types of biodegradable polyurethanes

Samples were designated with the first letter indicating the hard-segment type (L= LDI), the second to the fourth letters indicating the polycaprolactone with 530 molecular

weight (PCL530) in the soft segment (PCL=PCL530), the number following the fourth letter indicating the molar percentage of PCL530 in the soft segment, and the final letter indicating the chain terminator (E= HEMA). For instance, LPCL50E indicates the polymer with LDI as hard segment, mixture of 50:50 molar ratio of PCL530 and PEG600 as soft segment, and HEMA as chain terminator. LPCL0E symbolizes that soft segment of polyurethane is 100% PEG600 and LPCL100E symbolizes that the soft segment of polyurethane contains 100% PCL530.

Sample Preparation

The solid cylindrical samples for mechanical testing were UV light polymerized in a Teflon cylindrical mold with inner diameter   and height 10 mm; film samples for other characterizations and evaluations were UV light polymerized in a Teflon Petri dish with thickness between 0.6 mm before hydration.

Thermal Behavior Characterization

Characterization of the thermal behavior of the polymers was performed with a Mettler Differential Scanning Calorimetry (DSC) analyzer (DSC 822e), with a heating rate of 20 °C/min under constant nitrogen flow. Samples were heated to 70 °C for 10 minutes, cooled to -100 °C, maintained at this temperature for 10 minutes, and then tested over a range from -100 °C to 150 °C. Prior to being sealed into an aluminum pan, samples were dried under vacuum at room temperature for 24 hours.

Thermogravimetrical analysis (TGA) under nitrogen atmosphere was carried out on a Mettler TGA analyzer (SDTA851e), with a heating rate of 10 °C/min. Samples were heated from room temperature to 800 °C. The mass of sample used was approximately 10

mg to avoid significant temperature gradients. Prior to being tested, samples were dried under vacuum at room temperature for more than 24 hours.

Examination of Chemical Composition

Attenuated total internal reflective-Fourier transform infrared spectroscopy (ATR-FTIR, Research series 100, Madison Instrument, Inc., Madison, WI, USA) was used to characterize the chemical composition of synthesized polymers. Polymer films were dehydrated in a vacuum oven at 60 °C for 36 hours and then placed on the sample holder in the nitrogen protected chamber for FTIR characterization at room temperature.

Swelling Test

Swelling property of the materials was measured by weight change of the sample in the film form (4 mm × 4 mm × 0.6 mm) before and after soaking in double deionized water for 12 hours at room temperature. Filter paper was used to remove the water on the surface of samples before weight measurements. Six samples of each material were measured. The weight swelling ratio is determined by the weight of the swollen sample divided by the weight of the dry sample.

Mechanical Property Testing

Samples used for mechanical testing were in solid cylinder form (10 mm height, 8 mm diameter before being hydrated) and were hydrated in 0.1 M PBS for 12 hours before mechanical testing. Compressive mechanical tests were performed on an MTS Synergie 100 materials tester (MTS System Co., Eden Prairie, MN, USA) with a maximum load of 50 N and a 1 mm/second crosshead speed. The elastic modulus was calculated from the initial slope of the linear stress-strain curve, and the yield strength was calculated at the point of 10% compression rate. Five samples were analyzed for each material.

Cytocompatibility Testing

Hydrated polyurethane films were cut into round pieces (16 mm in diameter) and placed in 12-well plates (n=6 for each time point and each material). Samples were sterilized with 75% ethanol solution for 30 min and then left in excess sterilized PBS solution overnight and washed five times to replace the ethanol before cell seeding.

Mouse embryonal carcinoma-derived clonal chondrogenic ATDC5 cells were used as a model cell line to study the compatibility of materials to cartilage cells.   Cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 medium (Sigma) containing 5% fetal bovine serum (FBS, Invitrogen, Gaithersburg, MD), 10 ug/ml human transferrin (Sigma), 3×10^{-8} M sodium selenite (Sigma), and 50 units/ml penicillin and 50 ug/ml streptomycin. For induction of chondrogenesis, human insulin (Sigma) at 10 ug/ml was added to the culture. The cells were cultured at 37 °C in a water-jacketed incubator with 5% CO_2. At the time of the experiments, cells were trypsinized and re-suspended at a concentration of 1.0×10^6 cells/ml; 1 μl cell suspension (10^3 cells) was seeded on each polyurethane film and 1 ml culture media added. At each time point, alamarBlue assay (Invitrogen, Gaithersburg, MD) was used to measure the cell proliferation following the manufacturer recommended protocols. Briefly, cell metabolic activity results in the chemical consumption of alamarBlue. The reduction of the alamarBlue in culture media can be measured by the change of the indicator combined with alamarBlue. At each time point, culture media was aspirated and 1 ml culture media with 10% (v/v) alamarBlue was added to each well. After 3 hours in culture, 100 μl media was transferred to 96-well UV transparent microplates (Costar) for UV absorption measurement (n=6 for each material at each time point). The change in

UV absorption reflected the metabolic activity of the living cells in a 3-hour period, thus indirectly reflecting the amount of living cells in each well. Empty wells were used as negative control. Cell morphology for each sample was imaged at 2 weeks of culture.

In-vitro Degradation

In vitro degradation of the polyurethane materials was evaluated by recording the weight loss of samples over time in 0.1 M PBS at 37 °C.   Samples in membrane form (8-10 mg) were placed in a small vial filled with 500 µL 0.1 M PBS (pH=7.4) to perform the degradation test (n=6 for each material). Those vials were placed in a 37 °C water bath with gentle shaking at 30 cycle/min to simulate dynamic in vivo tissue environment. At each time point, six vials of each type of material were sampled, rinsed for two hours with double deionized water, and vacuum-dried for 24 hours before weight loss was analyzed. The PBS in each vial was also collected for measurement of pH.

Results

Thermal Analysis

In the development of polymers for biomedical applications, it is important to know their thermal behaviors, which determine the physical properties of the materials. For example, if the value of the glass transition temperature (T_g) of the polymer is above that of body temperature, the polymer is rigid. In contrast, if the T_g values are below body temperature, this indicates the elastomeric nature of the materials. Figure 2A shows the DSC thermograms of the three model materials studied. All three materials displayed glass transition temperatures below body temperature, suggesting that these polyurethanes exhibited elastomeric natures at body temperature. Possesssing an

60

elastomeric nature at body temperature is an important quality for polymers in soft tissue engineering.

(A)

LPCL100E, $T_g = 15^o$C, no T_c

LPCL50E, $T_g = 3^o$C, $T_c = 178^o$C

LPCL0E, $T_g = -6^o$C, $T_c = 189^o$C

Endotherm

Temperature (^{o}C)

(B)

Weight Remain (%)

Temperature (^{o}C)

LPCL100E

LPCL50E

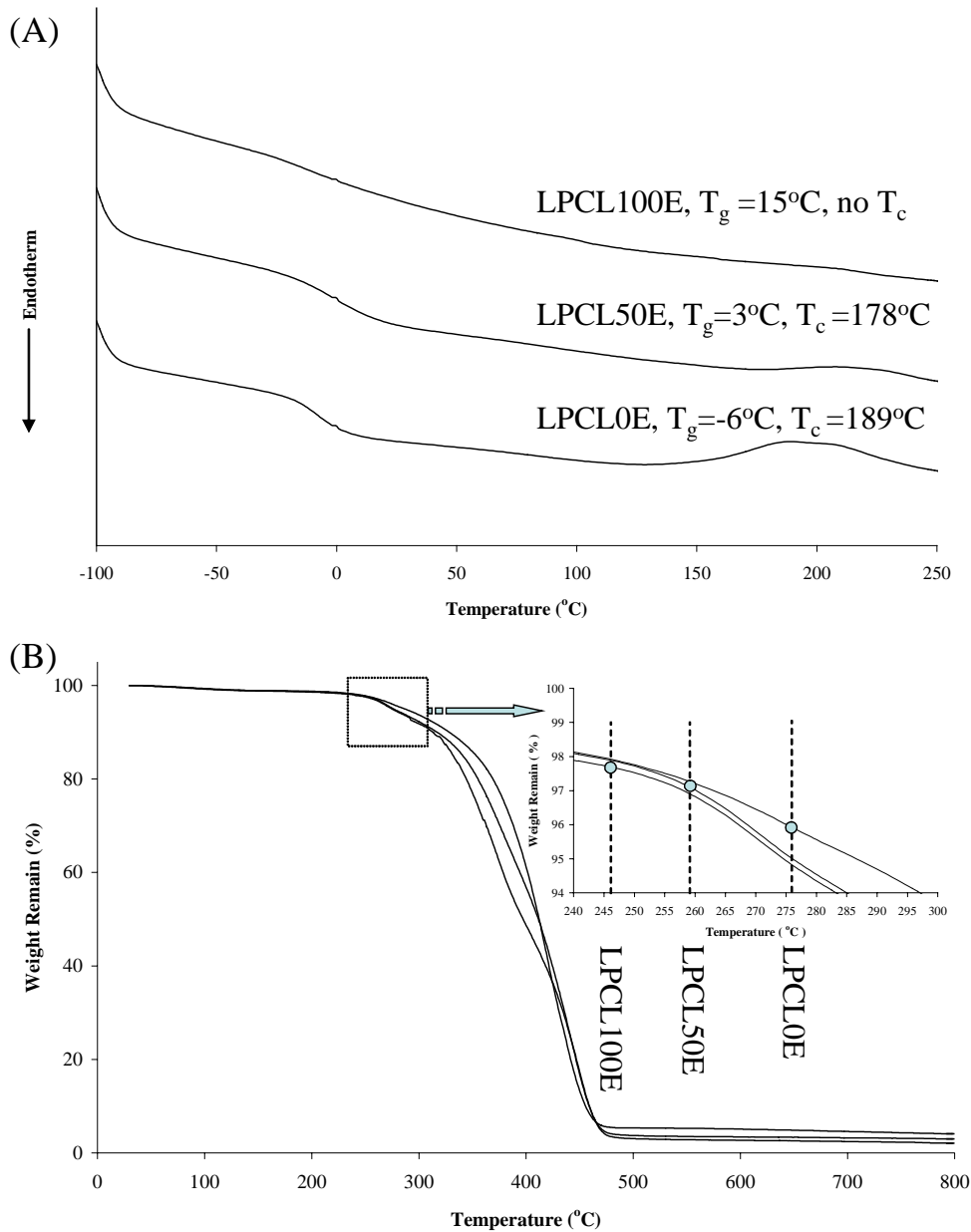LPCL0E

Weight Remain (%)

Temperature (^{o}C)

Figure 2: Thermal behaviors of LPCL100E, LPCL50E and LPCL0E. (A). DSC thermograms of three materials. The top curve shows the DSC curve of LPCL100E with T_g at 15 °C and no T_c is observed. The middle curve shows the DSC curve of LPCL50E with T_g at 3 °C and T_c at 178 °C. The bottom curve shows the DSC curve of LPCL0E with T_g at -6 °C and T_c at 189 °C. (B). Thermogravimetric analysis (TGA) curves of LPCL100E, LPCL50E, and LPCL0E with onset temperature at 246 °C, 259 °C, and 276 °C, respectively. Small insert shows the TGA curves at 240 °C to 300 °C.

All three types of polyurethane have the same soft segment percentage (60 wt%) and hard segment percentage (40 wt%); however, as the PCL530 content decreased in the soft segment, lower T_g values were observed. For instance, T_g of LPCL100E is 15 °C, while T_g of LPCL50E is 3 °C, and T_g of LPCL0E is –6 °C.   Because Tg values indicate the degree of hard and soft segment phase mixing, [34,35] the highest T_g (LPCL100E =15 °C) among the three materials indicates that the polymer with 100% PCL530 in the soft segment had the highest phase mixing. The T_g of LPCL0E (–6 °C) is 9 °C lower than that of LPCL50E (3 °C), indicating that increasing the content of PEG600 in the soft segment causes less phase separation among soft and hard segments in the polymer matrix.

The T_g change caused by the change of PCL:PEG ratio in the soft segment can be explained by the different chemical structure   of PCL530 and PEG600. PCL530 with extra ester groups containing pendant carbonyl groups (-C=O) exhibits higher extra-molecular distance and molecular mobility when compared to PEG600. Moreover, these carbonyl groups in the soft segment can form strong hydrogen bonds with urethane groups in the hard segments. Therefore, the increased molecular mobility and the hydrogen bonds caused higher phase mixing and higher T_g in PCL530 based polyurethanes. On the other hand, in the materials containing PEG600, the linear main chain in PEG containing the repetitive ether structure (–C-O-C-) has high rotation flexibility, but low mobility of the whole polymer chain, and can be easily rotated and arranged into an orderly structure. Therefore, the PEG600 region in the soft segment exhibits the tendency to form orderly arrangement with lower chain mobility, which causes the phase separation. The formation of the ordered region in soft segments can be

indirectly observed in DSC thermogram, which shows the exothermic peaks (T_c) for

LPCL0E (189 °C) and LPCL50E (178 °C) (Figure 2A). Compared to LPCL0E, the

strength of the T_c peak of LPCL50E became weaker due to the decreased amount of

ordered area in the soft segment; and the disappearance of exothermic peak of

LPCL100E can be attributed to the highly disordered soft segment region and the higher

degree of phase mixing.

TGA curves of three materials are shown in Figure 2B. The onset temperatures of

thermal decomposition for LPCL100E, LPCL50E and LPCL0E are 246 °C, 259 °C and

276 °C, respectively. The onset temperatures decrease with the decrease of the PEG600

content in the soft segment, which reflects the increase of the thermal stability of the

materials. The increased thermal stability can be attributed to the orderly arranged soft

segment area and higher phase separation in polymers containing PEG600, which is

consistent with the DSC results showing that LPCL100E and LPCL50E possess
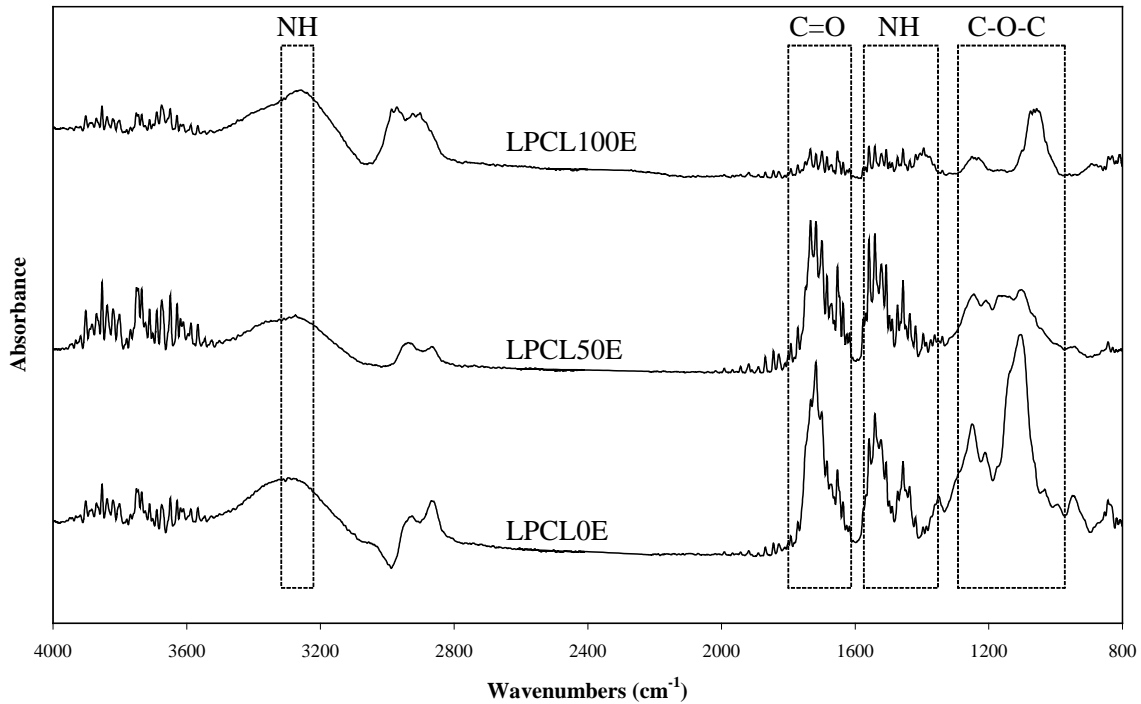
exothermic processes at higher temperatures.

Figure 3: FTIR spectra of three types of polyurethane with different ratios of PCL530 and PEG600 in the soft segment. The region from 1780 to 1600 cm^{-1} indicates to the C=O stretching absorption, the region from 1580 to 1300 cm^{-1} indicates to the –NH- with hydrogen bonds and the region from 1310-1000 cm^{-1} indicates to –C-O-C- stretching absorption.

Figure 3 shows the FTIR spectra of three types of polymers with different ratios of PCL530 and PEG600 in the soft segment. All three polymers display the peaks of free amide group (NH) stretching absorption at 3280–3252 cm^{-1}, carbonyl group (C=O) stretching absorption at 1780-1600 cm^{-1}, hydrogen bonded NH stretch absorption at 1580-1330 cm^{-1}, and ether group (–C-O-C-) stretching absorption at 1310-1000 cm^{-1} [36]. Due to the overlap of the carbonyl group of LDI, HEMA and PCL, the carbonyl group displays a broad stretching absorption at 1780 -1600 cm^{-1}. In LPCL100E spectra, the carbonyl stretch absorption exhibits weaker and broader peaks when compared to the other two polymers because of the higher phase mixing of soft segment and hard

segment. The molecular interaction (hydrogen bonds) of carbonyl groups and amide groups causes the decreased strength of the carbonyl group's absorption. This increase in hard and soft segment mixing also results in the broad hydrogen bonded amide group's stretch absorption at 1580-1330 cm^{-1} in LPCL100E, which is consistent with the thermal analysis results indicating that the material with higher content of PCL530 in the soft segment exhibited higher phase mixing. The ether group stretch absorption at 1310-1000 cm^{-1} exhibits a different shape due to the different ratio of PCL and PEG in the soft segment. Ether group absorptions from LDI, HEMA, PEG and PCL are overlapped in this region. In LPCL0E spectra, ether group absorptions display sharp peaks with a small shift to high wavelength. This may be due to the higher content of ether groups in soft segments and the formation of the ordered phase separated structure, in which the soft segment containing 100% PEG600 caused the formation of a linear structure that enhanced absorption of the ether groups and shifted the peaks to higher wavelengths. However, compared to the ether group absorptions in LPCL0E, the ether groups in LPCL50E showed wider and weaker peaks. This can be explained by the decreased ether group content in the soft segment due to the decreased PEG content in LPCL50E; moreover, the enhanced molecular interaction between PCL and PEG segments undermines the orderly structure and increases the hard-soft segment phase mixing, which caused the wider ether group absorption peaks. However, compared to LPCL50E, ether groups in LPCL100E showed relatively sharper absorption peaks and the shifts to lower wavelength. This sharper absorption in LPCL100E may contribute to the 100% percent of PCL in soft segments and there is no interaction with PEG600. The shifts to lower wavelengths may be due to the higher phase mixing of hard and soft segments in

LPCL100E. Compared to LPCL0E, due to the lower content of ether groups in LPCL100E, the ether group absorption peaks in LPCL100E are relatively lower. Therefore, FTIR curves of the three materials reflect the differences in the chemical structure, such as soft segment content, phase separation, and the molecular interaction inside the polymers.

Swelling Behavior

Swelling behaviors of three materials were measured by water retention at room temperature. All three polymers absorb water; however, the water retention ratio differs dramatically among the three materials. With the increase of the PEG content in the soft segment from 0% to 100%, the water retention ratio increases from $3.2\pm1.4\%$ to $66.0\pm2.5\%$. The reason PEG-free material possesses water retention property is that carbonyl groups (C=O) exist in this hard segment. LDI and HEMA can form weak hydrogen bonds with environmental water molecules, which causes hydrophilicity of the material. However, hydrophilicity resulting from the hard segment is insignificant when compared with the water retention ability from PEG600, which possesses repetitive ether (–C-O-C-) groups on the linear chains and can form strong hydrogen bonds with water molecules. In contrast, PCL exhibits very low hydrophilicity due to the existence of a high percentage of water repellent carbonic bonds (-C-C). Therefore, changing the ratio of PEG and PCL can adjust the ratio of water repellent and water absorbent groups in soft segments, and based on this principle, the hydrophilicity of the elastic biodegradable polyurethanes can be finely tuned accordingly for different biomedical applications. For example, polymers used for gene delivery system are generally required to be highly hydrophilic, [11, 37] polymers used for wound dressing material need moderate

hydrophilicity, [38] while polymers applied as the scaffold for the anterior cruciate ligament (ACL) reconstruction require low hydrophilicity. [9] Therefore, synthesis of elastic polyurethanes with adjustable hydrophilicity would expand their utility as candidate materials for a wide range of biomedical applications.
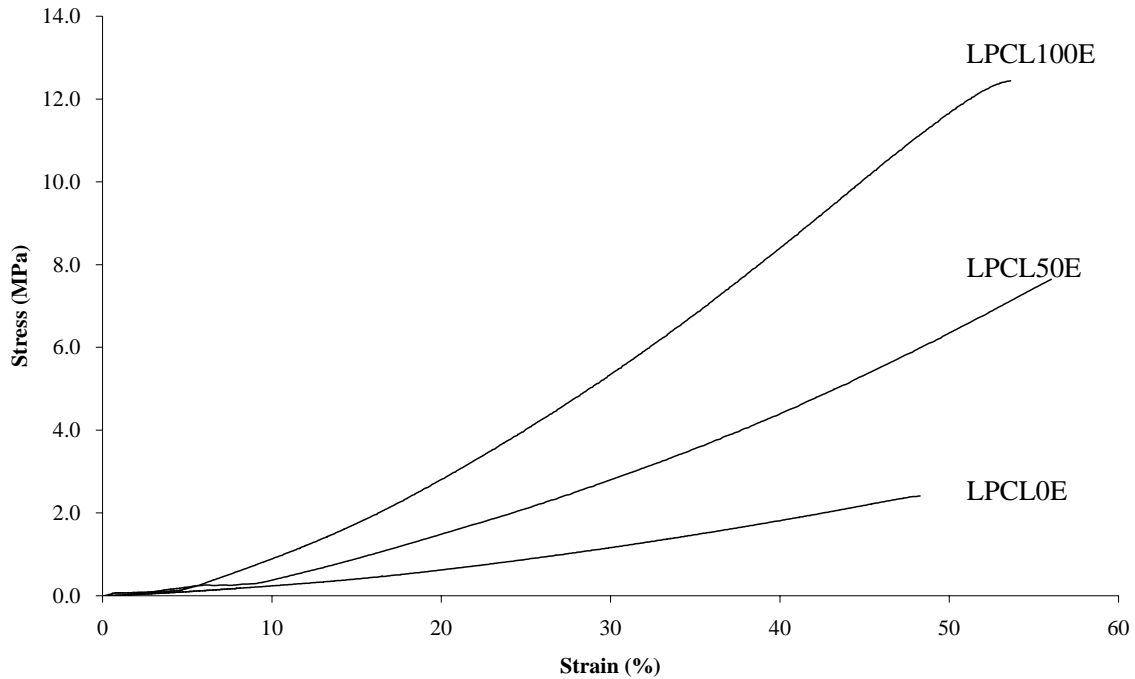
Mechanical Testing

Figure 4: Mechanical properties of the hydrated samples. Solid samples were made 10 mm in height and 8 mm in diameter. Fifty Newton compression force was applied at 1 mm/second crosshead speed.

As shown in Figure 4, three polymers with different PCL: PEG600 ratios show significant differences in mechanical properties under hydrated condition (P<0.05). Figure 4 shows typical stress-strain curves of three polymers under compression. The yield strengths (at 10% compression rate) of LPCL0E, LPCL50E, and LPCL100E are 0.24±0.03 MPa, 0.43±0.07 MPa, and 0.88±0.12 MPa, respectively. The elastic moduli of LPCL0E, LPCL50E, and LPCL100E are 16.8±3.3 MPa, 26.6±3.9 MPa, and 34.2±2.6 MPa, respectively. Although two hydrogels (LPCL0E and LPCL50E) are significantly

weaker than non-hydrogels in this series of materials, two hydrogels are significantly stronger than most biodegradable hydrogels developed so far. Since LP100E, LP50E and LP0E have similar hard segment contents, the PCL:PEG ratios in soft segments were responsible for the differences in mechanical properties. LPCL100E, with the lowest water retention (3.2±1.4%), exhibited the highest mechanical properties, while LPCL0E, with the highest water retention (66.0±2.5%), exhibited the lowest mechanical properties. Water molecules combined with PEG segments through strong hydrogen bonds decreased the molecular force among polymer chains, thus the polymer chains mobility were increased accordingly. As a result, the mechanical properties were decreased with the increase of PEG content in soft segments.

Cytocompatibility Testing

To examine the cytocompatibility and cell-biomaterial interaction, chondrogenic ATDC5 cells were cultured on the surfaces of LPCL0E, LPCL50E and LPCL100E membranes. Cell attachment and proliferation were tested by alamarBlue assay after 7 days in culture. To obtain the maximum sensitivity of the alamarBlue assay, only a small number of chondrocytes (1000 cells/sample; each sample was 16 mm in diameter) were initially seeded on each material. Empty polystyrene wells were used as control.
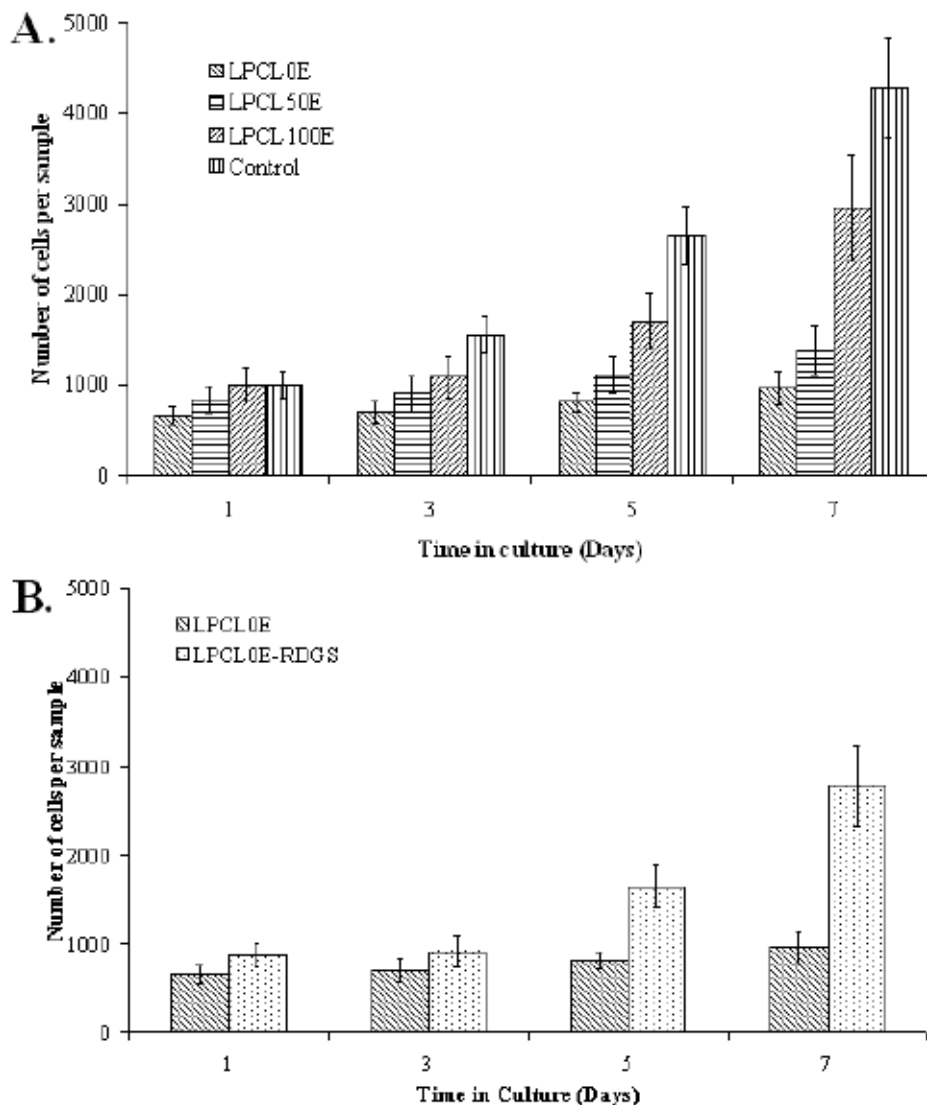
Figure 5:   (A) alamarBlue assays on the chondrogenic cell attachment and proliferation on LPCL0E, LPCL50E, LPCL100E and polystyrene (negative control). Chondrocytes on hydrophilic LPCL0E and LPCL50E exhibited lower attachment and proliferation rates. (B) alamarBlue assays of the chondrogenic cell attachment on LPCL0E and LPCL0E with immobilized RGD in the hydrogel network (LPCL0E-RGDS hydrogel) show significantly higher cell attachment and proliferation.

Cell attachment was examined at twenty-four hours after cell seeding. After gently washing culture wells and changing the media, alamarBlue was added to the culture for three hours and the number of the cells on each sample was calculated through the conversion of the intensity of absorbance into cell numbers based on a calibration curve obtained with a known number of ATDC5 cells. There were significant differences

between the numbers of cells attached on the three samples (P<0.05) (Figure. 5A). As the

PEG600 content increased, the number of attached cells on the material decreases. Cells

showed the highest attachment on LPCL100E and lowest on LPCL0E, as expected. It is

well-documented that PEG can prevent protein adsorption and as well as inhibit cell

attachment, [21, 25] which allows development of biomaterials that promote desirable

cell attachment but ward off unwanted cell attachment by immobilizing a specific ligand

on the surface or into the scaffolds. [21, 25] We used Arg-Gly-Asp-Ser (RGDS) as a

model ligand to be immobilized into this series of hydrogels during the light-curing

process. We found a significant increase in cell attachment as shown in Figure 5B when

compared with the material without ligand immobilization.

There were significant differences on the cell proliferation on the three materials

as well. The proliferation rates after 7 days in culture for cells on LPCL100E, LPCL50E

and LPCL0E are 316±37%, 165±32% and 145±22% respectively, while cell numbers in

the polystyrene well increased by 452±33% (Figure 5A). The differences in cell

proliferation rate can also be observed from cell morphology images as shown in Figure

6, in which cell density on LPCL100E was much higher than on either LPCL50E or

LPCL0E. The differences in cell attachment and proliferation rate can be attributed to the

effects of PEG600 in soft segments. PEG segments tend to orient their chains to the

hydrated environment due to their high mobility in water and PEG chains readily

accumulate on the surface of materials, which then provide high excluding volume and

flexible hydrophilic chain motion to repel proteins/cells that approached the surface. It is

not surprising that cell attachment and proliferation are lower on materials with higher

PEG content. [22, 39] However, this can be easily overcome by immobilizing specific
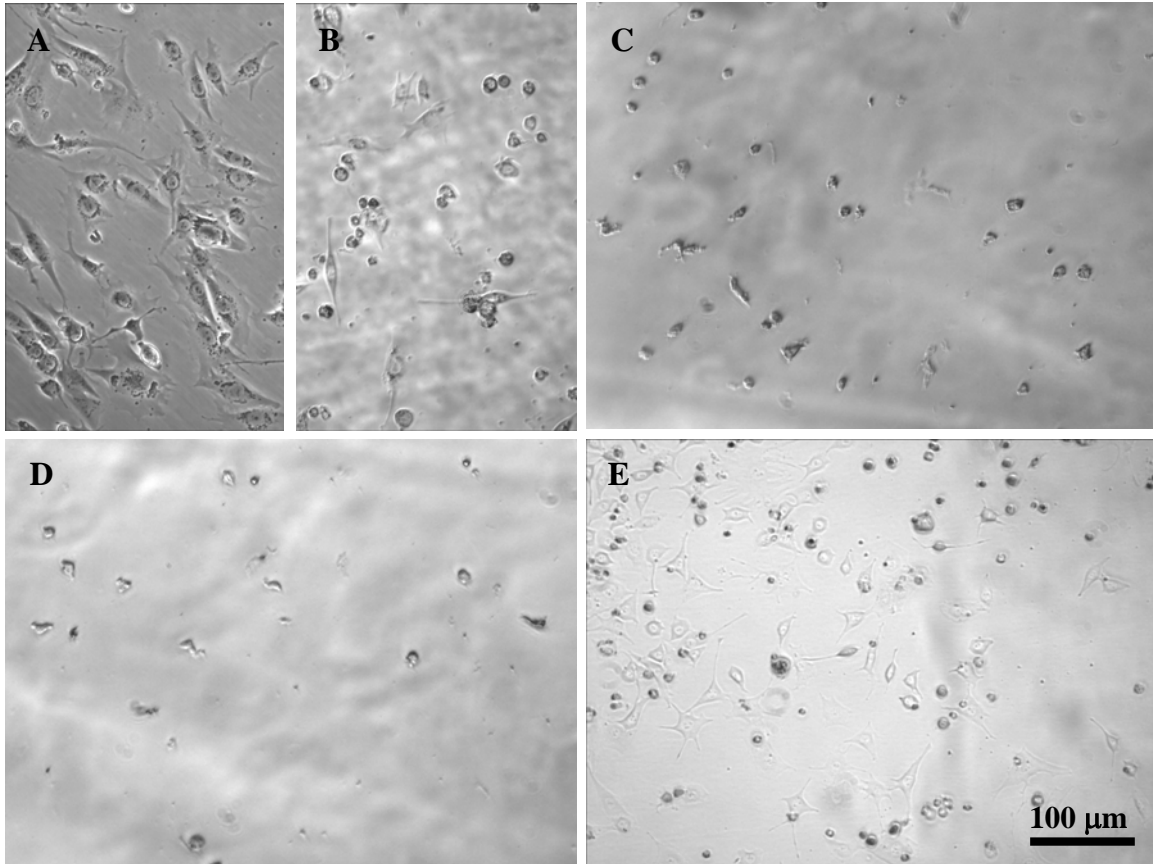
ligands into the polymers (Figure 5B).

Figure 6: Phase contrast microscopic image of chondrocytes grown on (A) polystyrene, (B) LPCL100E, (C) LPCL50E, (D) LPCL0E, and (E) LPCL0E-RGDS after 7 days in culture.

As shown in Figure 6, a much higher number of chondrogenic cells on the two

hydrogels, LPCL50E and LPCL0E, exhibit a rounded shape than on either LPCL100E or

polystyrene. This can probably be explained by the polymer-cell-media interactions on

the cell growth. On the surfaces of those hydrogels with the similar environment to what

chondrocytes normal reside in living tissue, mobile polymer chains can effectively

interact with large volume of water, and form a dynamic environment that limits

chondrocytes spreading out and therefore cells showing rounded shape in tissue or on these hydrogels.
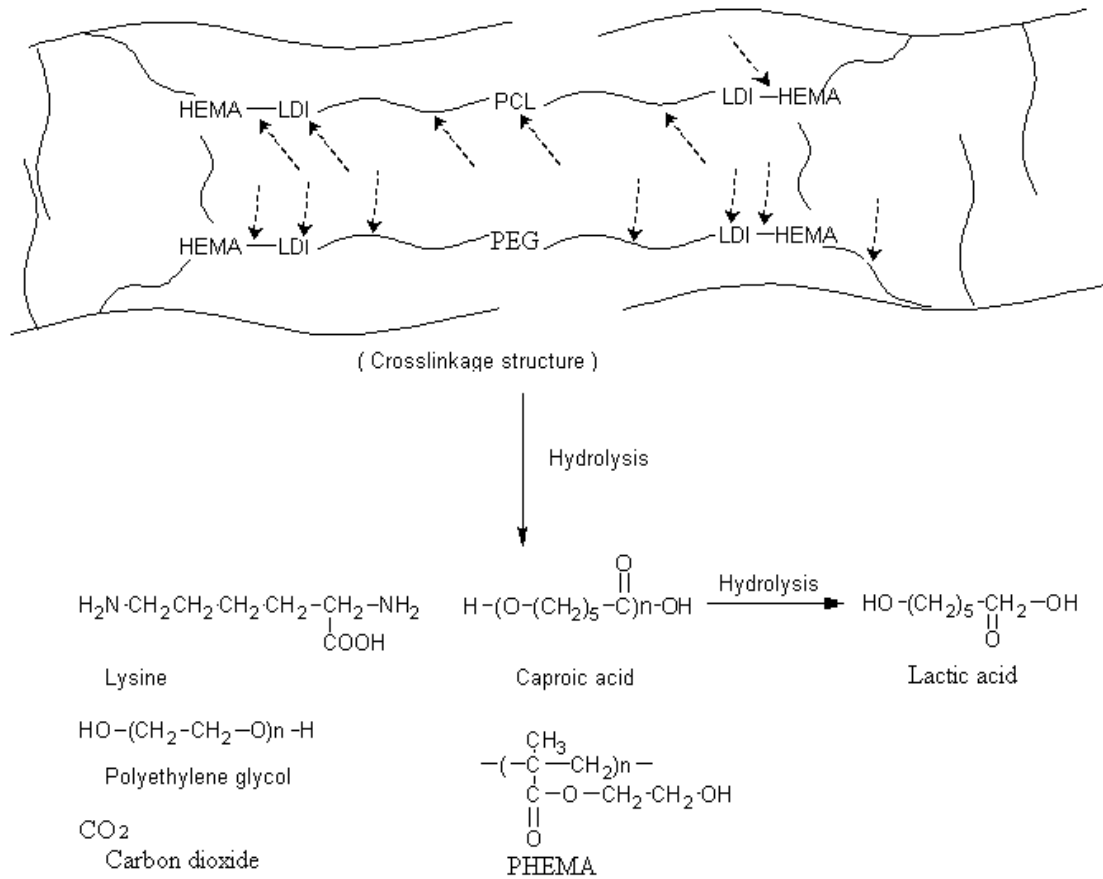
In-vitro Degradation

Figure 7: Schematic representation of polyurethane degradation into lysine, PCL, PEG, HEMA, CO_2 and caproic acid or lactic acid.
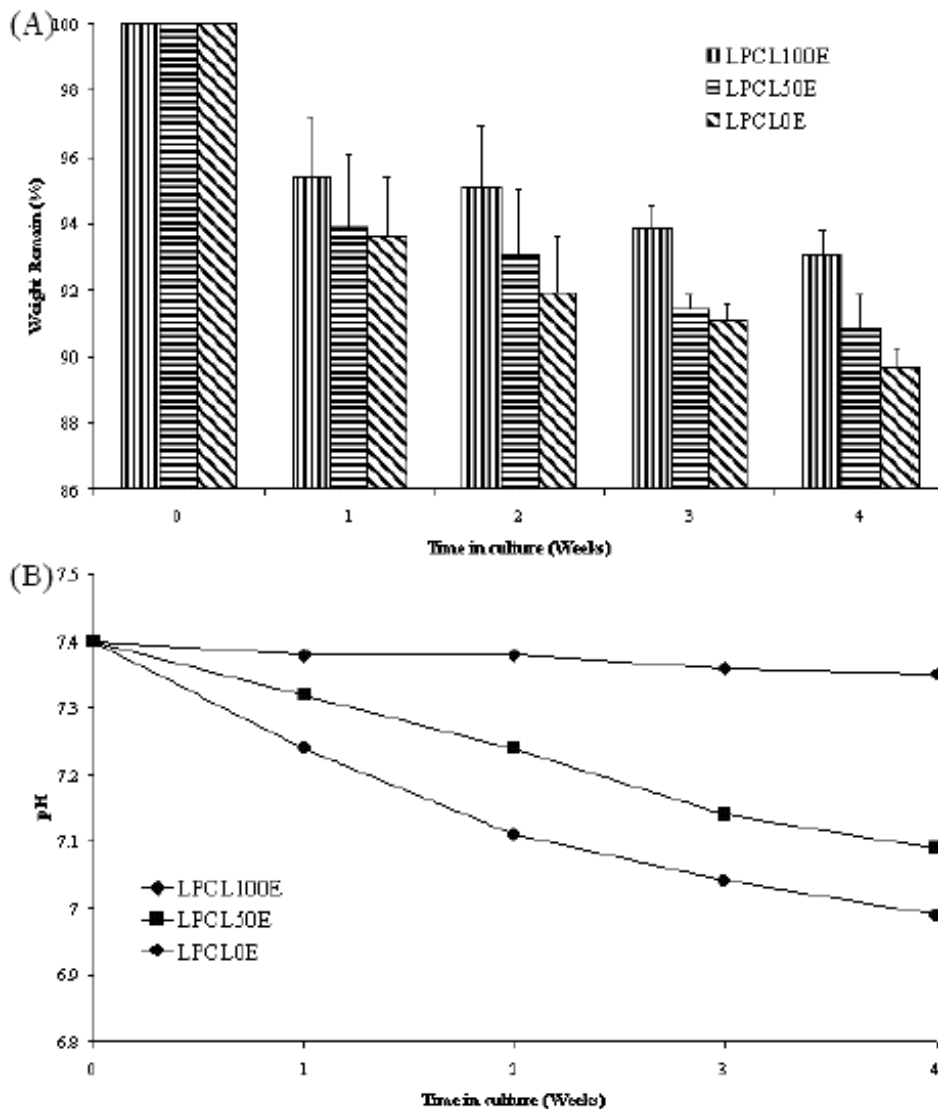
Figure 8: In vitro degradation behavior of three materials at 37 $^{\circ}$C with gentle shaking. (A) Weight loss and (B) change in pH value of culture media .

Since this series of polymers are comprised of LDI, HEMA, PCL, and/or PEG, the degradation process is considered to be hydrolysis of the ester groups and the urethane groups among those polyurethanes hard and soft segments. Ester groups exist in LDI, PCL and the urethane groups formed during the polymer synthesis, in which LDI were polymerized with PCL, PEG and HEMA. As illustrated in Figure 7, the degradation products of the polymerized hydrogels during hydrolysis are lysine, PEG, poly 2-hydroxyethyl methacrylate (PHEMA), carbon dioxide (CO_2), and caproic acid or lactic

acid. All of these have been demonstrated non-toxic in humans. Attack of the water molecules to the ester groups and urethane groups results in the weight loss of the polymers and the environmental pH value changes due to the formation of CO_2 and organic acid. In vitro degradation rates were measured by the weight loss of the sample films in 0.1 M PBS solution at multiple time points at 37 °C with gentle shaking to mimic in vivo environment. pH values of the PBS solution were measured at each time point to examine the influence of the degraded products on the culture environment. As shown in Figure 8A, the three types of polyurethanes exhibit different degradation rates: LPCL100E displays the slowest degradation rate while LPCL50E and LPCL0E display faster degradation rates, which is consistent with the pH value change shown in Figure 8B. The fastest degrading polyurethane, LPCL0E, exhibits the highest pH value change (0.41) at 4 weeks while the slowest degrading polyurethane, LPCL100E, exhibits only a slight change (0.02) at 4 weeks. The decrease in pH is attributed to the release of small molecules such as caproic acid or lactic acid and the dissolution of the CO_2 in the PBS solution. Since LPCL100E contains the highest ester group content in the soft segment, it shows a lower degradation rate than LPCL50E and LPCL0E. This can be explained by the hydrolysis mechanism of this series of polymers. Since their degradation is a hydrolysis procedure, water penetration and diffusion will cause the hydrolysis of ester groups and urethane groups in the polymers. Compared to LPCL50E and LPCL0E, LP100E has lowest hydrophilicity, thus the water molecules exhibited the lowest penetration and diffusion ability to enter the polymer chains, which results in the lowest degradation rate during the hydrolysis process. Therefore, with the increase of the PEG content in soft segment, the degradation rate increased significantly.

Discussion

Cartilage is very important in load bearing and in maintaining the appropriate shapes of many organs. Three types of cartilages can be found in the body: hyaline cartilage lining joints, elastic cartilage found in the nasal septum, the pinna of the ear, and the walls of the auditory and eustachian canals and larynx, and fibrocartilage found in intervertebral disks. Many diseases lead to damage of cartilage tissue characterizing chondrodystrophies, such as arthritis, achondroplasia, costochondritis, herniated disk, and so on. Sports and accidents are also major causes of cartilage injuries, including meniscus injuries, and articular cartilage damage. Due to the avascular nature of cartilage, it has very limited capacity for self-repair, and currently, there is no treatment available to completely regenerate damaged cartilage. For example, the mainstay treatment for articular cartilage reconstruction is still arthrodesis (joint fusion) and arthroplasty (joint replacement). Other approaches, such as transplantation of osteochondral allografts or chondrocytes to replace the damaged cartilage arthroscopically and have shown improved outcome. Approximately 95,000 total knee replacements and 41,000 other surgical procedures to repair cartilaginous defects of the knee are performed annually in the United States. [40] However, the repairs are far from satisfactory. Tissue engineering cartilage by combining biomaterials and cells may present a cure for cartilage damage.

A large variety of materials, including natural and synthetic polymers, have been investigated both in vitro and in vivo for cartilage engineering. Natural materials, including agarose, alginate, hyaluronic acid, gelatin, fibrin glue, collagen, etc., have been employed as porous scaffolds, fibers, microbeads, hydrogel, glue, and other forms for cartilage repair. However, their weak mechanical properties, such as fragility, and low

reproducibility severely limit their clinical usefulness. Synthetic materials, with the advantages of reproducible mechanical property, tunable biochemical behavior, and controllable degradation, were extensively investigated as well. Polymers, such as polyester, poly(anhydrides), poly(phosphazenes), and poly(ethylene glycol) based polymers, have been explored for cartilage engineering. Due to the hydrogel nature of cartilage, polymers in hydrogel are form good candidates for cartilage repair, and they have been extensively investigated in recent years. [41-49]

Due to their unique properties, hydrogels have been explored for a wide range of biomedical applications, including drug delivery, [50], protein delivery, [51], gene delivery, [52] wound dressing, [53] water elimination for edema, [54], cosmetic reconstruction, [55] contact lenses, [56] and tissue engineering. [49, 57-59] One major challenge in employing hydrogels for load-bearing connective tissue repair in cartilage, tendon, intervertebral disk, ligament, and so on, is insufficient mechanical strength. For instance, articular cartilage contains ~70% water and bears loads up to 100 MPa, [60] but most hydrogels, either synthetic or derived from natural sources, can be easily broken by pressing with a finger or pulling with the hand, indicating that they are much weaker than native cartilage tissue. Although Stammen et al reported a poly(vinyl alcohol) (PVA) based hydrogel with compressive modulus up to 18 MPa [61], and Gong et al developed a double network hydrogel with a similar range of compressive modulus, [31] these hydrogels are nondegradable and can only be used as a permanent non-physiological replacement for damaged cartilage or a temporary solution to improve joint function and delay total joint replacement. Gong and associates then developed a double-network degradable hydrogel, but this had a compression modulus less than 4 MPa. [62] The

degradable elastic hydrogels developed in this study have elastic moduli ranging from 16.8±3.3 MPa to 26.6±3.9 MPa, which are very close to the properties of native cartilage. We expect to improve the mechanical properties when chondrocytes are cultured in these materials and exposed to physiological dynamic forces.

The series of elastic hydrogels we described in this work are based on a backbone of degradable polyurethane. A series of degradable polyurethanes was recently developed for biomedical applications which had excellent mechanical properties and great chemical versatility. [9, 12, 16, 17, 63] Elastic degradable polyurethane shows promise as being a good candidate for most soft tissue regeneration, including cardiac muscle, [64] blood vessel, [65, 66] skeletal muscle, tendon, ligament, and skin repair. In addition, elastic degradable polyurethane has also been investigated for hard tissue regeneration, such as cartilage [67] and bone tissue repair [67]. However, most currently applicable degradable polyurethanes were based on toluene diisocyanate (TDI), methylenediphenyl diisocyanate (MDI) or hexamethylene diisocyanate (HDI) as the hard segment and ethylene glycol (EG) or ethylene diamine (ED) as the chain extender. These polyurethanes were found to release toxic byproducts during degradation. For example, MDI based polyurethanes release in vivo 4,4'-methylenedianiline which causes hepatitis in humans. HDI, TDI and ED based polyurethanes liberate diamine during degradation which has been proved to be toxic to the human liver and kidney. [1, 68] In recent years, a type of diisocyanate based on lysine (LDI) has been developed for biodegradable polyurethane synthesis. [63, 69] In these polyurethanes LDI was used as hard segment, its degradation products in vivo were considered to be lysine and no significant toxicity or carcinogenicity to tissue upon implantation was found. [10, 70]

The hydrophilic nature of the PEG motifs in this series of polyurethane based hydrogels allows protein adsorption and cell attachment to be controlled. The immunogenicity and antigenicity are also very minimal. [40] This unique characteristic allows fine tuning of the bioactivity of the hydrogel by selectively immobilizing specific functional biomolecules on the surface of the hydrogel or throughout the bulk of the gel through instant polymerization, such as UV light-induced, or through temperature induced polymerization. [71, 72] In this study, we used RGDS as a model biomolecule immobilized into the hydrogels, and we demonstrated increased cell attachment. Therefore, this elastic polymer system allows for development of a bioactive hydrogel system to selectively allow certain cell adhesion, but ward off unwanted cell attachment by immobilizing specific bioactive molecules. In summary, our work described here can be used as a base for developing hydrogels with precisely tunable properties for different tissue repair.

Conclusion

In conclusion, a series of degradable polyurethane based light-curable elastic hydrogels were synthesized from polycaprolactone diol (PCL-diol), polyethylene glycol (PEG), lysine diisocyanate (LDI), and 2-hydroxyethyl methacrylate (HEMA) through UV light initiated polymerization reaction. By changing the ratio of PCL to PEG during the pre-polymer synthesis, polyurethanes with different soft segmental structures, hydrophilicity, and cytophilicity were obtained after light-initiated polymerization. The chemical structures of the synthesized polymers were characterized using differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and Fourier transform infrared spectroscopy (FTIR). Physical properties such as swelling, mechanical

properties, and in vitro degradation were evaluated. Materials containing a higher proportion of PEG exhibit higher water absorbance, higher degradation rate in vitro, and lower mechanical strength in the hydrated state. Chondrocytes exhibit different attachment and proliferation rates and different morphologies on the materials with different PCL:PEG ratios. With a higher PEG ratio, lower cell attachment and proliferation were observed. To improve the cell attachment and proliferation on high PEG content hydrogels, bioactive molecules, such as peptides and proteins, could be readily conjugated or immobilized in the gel matrix during the light-curing process. To demonstrate that, a short peptide, Arg-Gly-Asp-Ser (RGDS), was used as a model biomolecule and incorporated into the gels during the light-curing process, and improved cell growth was observed. In summary, the use of PCL:PEG at different ratios, as well as the introduction of HEMA into polyurethane, allows the synthesis of a series of biocompatible elastic hydrogels with tunable physical and cytophilic properties through light initiated polymerization. This series of materials also allows for controlling the cell attachment and growth by incorporating bioactive molecules during the light-curing process.

References

1. Arshady R. Desk reference of functional polymers: syntheses and applications. Washington, DC: American Chemical Society; 1997. xviii, 820 p.

2. Vermette P, Griesser HJ, Laroche G, Guidoin R. Biomedical Applications of Polyurethanes. Austin, TX: Landes Bioscience; 2001. 220-241 p.

3. Brody GS. The meme implant: Reconstruction of the breast using polyurethane-coated prostheses. Plastic and Reconstructive Surgery 1984;73:420-421.

4. Guidoin R, Sigot M, King M. Biocompatibility of the Vascugraft: Evaluation of a novel polyester urethane vascular substitute by an organotypic culture technique. Biomaterials 1992;13:281-288.

5. Liu SQ, Kodama M. Porous polyurethane vascular prostheses with variable compliance. Journal of Biomedical Materials Research 1992;26:1489-1502.

6. Martz H, Beaudoin G, Paynter R. Physicochemical characterization of hydrophilic microporous polyurethane vascular graft. Journal of Biomedical Materials Research 1987;21:399-412.

7. Stokes K, Cobian K. Polyether polyurethanes for implantable pacemaker leads. Biomaterials 1982;3:225-231.

8. Sung P, Fraker AC. Corrision and degradation of a polyurethane/Co-Ni-Cr-Mo pacemaker lead. Journal of Biomedical Materials Research 1987;21:287-297.

9. Gisselfalt K, Edberg B, Flodin P. Synthesis and properties of degradable poly(urethane urea)s to be used for ligament reconstructions. Biomacromolecules 2002;3(5):951-8.

10. Saad B, Hirt TD, Welti M, Uhlschmid GK, Neuenschwander P, Suter UW. Development of degradable polyesterurethanes for medical applications: In vitro and in vivo evaluations. Journal of Biomedical Materials Research 1997;36:65-74.

11. Yang TF, Chin WK, Cherng JY, Shau MD. Synthesis of novel biodegradable cationic polymer: N,N-diethylethylenediamine polyurethane as a gene carrier. Biomacromolecules 2004;5(5):1926-32.

12. Zhang C, Zhang N, Wen X. Improving the Elasticity and Cytophilicity of Biodegradable Polyurethane by Changing Chain Extender. Journal of Biomedical Materials Research B 2006;In press.

13. Storey RF, Wiggins JS, Puckett AD. Hydrolyzable poly(ester-urethane) networks from L-lysine diisocyanate and D,L-lactide/-caprolactone homo- and copolyester triols. Journal of Polymer Science Part A: Polymer Chemistry 1994;32(12):2345-2363.

14. Hirt TD, Neuenschwander P, Suter UW. Synthesis of degradable, biocompatible, and tough block-copolyesterurethanes. Macromolecular Chemistry and Physics 1996;197(12):4253-4268.

15. Zhang JY, Beckman EJ, Hu J, Yang GG, Agarwal S, Hollinger JO. Synthesis, Biodegradability, and Biocompatibility of Lysine Diisocyanate glucose Polymers. Tissue Engineering 2002;8:771-785.

16. Skarja GA, Woodhouse KA. Structure-Property Relationships of Degradable Polyurethane Elastomers Containing an Amino Acid-Based Chain Extender. Journal of Applied Polymer Science 1999;75:1522-1534.

17. Fromstein JD, Woodhouse KA. Elastomeric biodegradable polyurethane blends for soft tissue applications. J Biomater Sci Polym Ed 2002;13(4):391-406.

18. Abraham GA, Marcos-Fernandez A, Roman JS. Bioresorbable poly(ester-ether urethane)s from L-lysine diisocyanate and triblock copolymers with different hydrophilic character. J Biomed Mater Res A 2006;76(4):729-36.

19. Ping P, Wang W, Chen X, Jing X. Poly(epsilon-caprolactone) polyurethane and its shape-memory property. Biomacromolecules 2005;6(2):587-92.

20. Ng KW, Hutmacher DW, Schantz JT, Ng CS, Too HP, Lim TC, Phan TT, Teoh SH. Evaluation of ultra-thin poly(epsilon-caprolactone) films for tissue-engineered skin. Tissue Eng 2001;7(4):441-55.

21. Moffat KL, Marra KG. Biodegradable poly(ethylene glycol) hydrogels crosslinked with genipin for tissue engineering applications. J Biomed Mater Res B Appl Biomater 2004;71(1):181-7.

22. Lee JH, Lee HB, Andrades JD. Blood Compatibility of Polyethylene Oxide Surfaces. Progress in polymer science 1995;20:1043-1079.

23. Hoque ME, Hutmacher DW, Feng W, Li S, Huang MH, Vert M, Wong YS. Fabrication using a rapid prototyping system and in vitro characterization of PEG-PCL-PLA scaffolds for tissue engineering. J Biomater Sci Polym Ed 2005;16(12):1595-610.

24. Leach JB, Schmidt CE. Characterization of protein release from photocrosslinkable hyaluronic acid-polyethylene glycol hydrogel tissue engineering scaffolds. Biomaterials 2005;26(2):125-35.

25. Bhadra D, Bhadra S, Jain P, Jain NK. Pegnology: a review of PEG-ylated systems. Pharmazie 2002;57(1):5-29.

26. Kelner A, Schacht EH. Tailor-made polymers for local drug delivery: release of macromolecular model drugs from biodegradable hydrogels based on poly(ethylene oxide). J Control Release 2005;101(1-3):13-20.

27. Dziubla TD, Lowman AM. Vascularization of PEG-grafted macroporous hydrogel sponges: a three-dimensional in vitro angiogenesis model using human microvascular endothelial cells. J Biomed Mater Res A 2004;68(4):603-14.

28. Silver JH, Myers JW, Lim F, Cooper SL. Effect of polyol molecular weight on the physical properties and haemocompatibility of polyurethanes containing polyethylene oxide macroglycols. Biomaterials 1994;15:695-704.

29. Peppas NA, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm 2000;50(1):27-46.

30. Riley SL, Dutt S, De La Torre R, Chen AC, Sah RL, Ratcliffe A. Formulation of PEG-based hydrogels affects tissue-engineered cartilage construct characteristics. J Mater Sci Mater Med 2001;12(10-12):983-90.

31. Yasuda K, Ping Gong J, Katsuyama Y, Nakayama A, Tanabe Y, Kondo E, Ueno M, Osada Y. Biomechanical properties of high-toughness double network hydrogels. Biomaterials 2005;26(21):4468-75.

32. Elisseeff J, Anseth K, Sims D, McIntosh W, Randolph M, Yaremchuk M, Langer R. Transdermal photopolymerization of poly(ethylene oxide)-based injectable hydrogels for tissue-engineered cartilage. Plast Reconstr Surg 1999;104(4):1014-22.

33.   Burdick JA, Frankel D, Dernell WS, Anseth KS. An initial investigation of photocurable three-dimensional lactic acid based scaffolds in a critical-sized cranial defect. Biomaterials 2003;24(9):1613-20.

34.   Koberstein JT, leung LM. Compression-molded polyurethande block copllymers. II. Evaluation of microphase compositions. Macromolecules 1992;23:6205-6213.

35.   Couchman PR. Predication of glass-transition temperature for compatible blends formed from homopolymers of arbitrary degree of polymerization. Compositional variation of glass-transition temperatrues. Macromolecules 1980;13:1272-1276.

36.   Luo N, Wang DN, Ying SK. Hydrogen-Bonding Properties of Segmented Ployether poly(urethane urea) Copolymer. Macromolecules 1997;30:4405-4409.

37.   Jabbari E. Release characteristics of a model plasmid DNA encapsulated in biodegradable poly(ethylene glycol fumarate)/acrylamide hydrogel microspheres. J Microencapsul 2004;21(5):525-38.

38.   Hackam DJ, Ford HR. Cellular, biochemical, and clinical aspects of wound healing. Surg Infect (Larchmt) 2002;3 Suppl 1:S23-35.

39.   Bryant SJ, Anseth KS. The effects of scaffold thickness on tissue engineered cartilage in photocrosslinked poly(ethylene oxide) hydrogels. Biomaterials 2001;22(6):619-26.

40.   Frenkel SR, Di Cesare PE. Degradation and repair of articular cartilage. Front Biosci 1999;4:D671-85.

41.   Saim AB, Cao Y, Weng Y, Chang CN, Vacanti MA, Vacanti CA, Eavey RD. Engineering autogenous cartilage in the shape of a helix using an injectable hydrogel scaffold. Laryngoscope 2000;110(10 Pt 1):1694-7.

42. Passaretti D, Silverman RP, Huang W, Kirchhoff CH, Ashiku S, Randolph MA, Yaremchuk MJ. Cultured chondrocytes produce injectable tissue-engineered cartilage in hydrogel polymer. Tissue Eng 2001;7(6):805-15.

43. Elisseeff J, McIntosh W, Fu K, Blunk BT, Langer R. Controlled-release of IGF-I and TGF-beta1 in a photopolymerizing hydrogel for cartilage tissue engineering. J Orthop Res 2001;19(6):1098-104.

44. Risbud M, Ringe J, Bhonde R, Sittinger M. In vitro expression of cartilage-specific markers by chondrocytes on a biocompatible hydrogel: implications for engineering cartilage tissue. Cell Transplant 2001;10(8):755-63.

45. Fisher JP, Jo S, Mikos AG, Reddi AH. Thermoreversible hydrogel scaffolds for articular cartilage engineering. J Biomed Mater Res A 2004;71(2):268-74.

46. Holland TA, Tabata Y, Mikos AG. Dual growth factor delivery from degradable oligo(poly(ethylene glycol) fumarate) hydrogel scaffolds for cartilage tissue engineering. J Control Release 2005;101(1-3):111-25.

47. Ramaswamy S, Wang DA, Fishbein KW, Elisseeff JH, Spencer RG. An analysis of the integration between articular cartilage and nondegradable hydrogel using magnetic resonance imaging. J Biomed Mater Res B Appl Biomater 2006;77(1):144-8.

48. Sontjens SH, Nettles DL, Carnahan MA, Setton LA, Grinstaff MW. Biodendrimer-based hydrogel scaffolds for cartilage tissue repair. Biomacromolecules 2006;7(1):310-6.

49. Yamaoka H, Asato H, Ogasawara T, Nishizawa S, Takahashi T, Nakatsuka T, Koshima I, Nakamura K, Kawaguchi H, Chung UI and others. Cartilage tissue

engineering using human auricular chondrocytes embedded in different hydrogel materials. J Biomed Mater Res A 2006.

50. Stoy VA. New type of hydrogel for controlled drug delivery. J Biomater Appl 1989;3(4):552-604.

51. Ishihara M, Obara K, Nakamura S, Fujita M, Masuoka K, Kanatani Y, Takase B, Hattori H, Morimoto Y, Ishihara M and others. Chitosan hydrogel as a drug delivery carrier to control angiogenesis. J Artif Organs 2006;9(1):8-16.

52. Li Z, Ning W, Wang J, Choi A, Lee PY, Tyagi P, Huang L. Controlled gene delivery system based on thermosensitive biodegradable hydrogel. Pharm Res 2003;20(6):884-8.

53. Yates DW, Hadfield JM. Clinical experience with a new hydrogel wound dressing. Injury 1984;16(1):23-4.

54. Sannino A, Esposito A, De Rosa A, Cozzolino A, Ambrosio L, Nicolais L. Biomedical application of a superabsorbent hydrogel for body water elimination in the treatment of edemas. J Biomed Mater Res A 2003;67(3):1016-24.

55. Mazzoli RA, Raymond WRt, Ainbinder DJ, Hansen EA. Use of self-expanding, hydrophilic osmotic expanders (hydrogel) in the reconstruction of congenital clinical anophthalmos. Curr Opin Ophthalmol 2004;15(5):426-31.

56. Mely R. Therapeutic and cosmetic indications of lotrafilcon a silicone hydrogel extended-wear lenses. Ophthalmologica 2004;218 Suppl 1:29-38; discussion 45-6.

57. Stabenfeldt SE, Garcia AJ, Laplaca MC. Thermoreversible laminin-functionalized hydrogel for neural tissue engineering. J Biomed Mater Res A 2006;77A(4):718-725.

58. Risbud MV, Karamuk E, Schlosser V, Mayer J. Hydrogel-coated textile scaffolds as candidate in liver tissue engineering: II. Evaluation of spheroid formation and viability of hepatocytes. J Biomater Sci Polym Ed 2003;14(7):719-31.

59. Risbud M, Endres M, Ringe J, Bhonde R, Sittinger M. Biocompatible hydrogel supports the growth of respiratory epithelial cells: possibilities in tracheal tissue engineering. J Biomed Mater Res 2001;56(1):120-7.

60. Marcinko DE, Dollard MD. Physical and mechanical properties of joints (the pathomechanics of articular cartilage degeneration). J Foot Surg 1986;25(1):3-13.

61. Stammen JA, Williams S, Ku DN, Guldberg RE. Mechanical properties of a novel PVA hydrogel in shear and unconfined compression. Biomaterials 2001;22(8):799-806.

62. Nakayama A, Kakugo A, Gong JP, Osada Y, Takai M, Erata T, Kawano S. High Mechanical Strength Double-Network Hydrogel with Bacterial Cellulose. Advanced Functional Materials 2004;14(11):1124-1128.

63. Zhang JY, Beckman EJ, Piesco NP, Agarwal S. A new peptide-based urethane polymer: synthesis, biodegradation, and potential to support cell growth in vitro. Biomaterials 2000;21(12):1247-58.

64. McDevitt TC, Woodhouse KA, Hauschka SD, Murry CE, Stayton PS. Spatially organized layers of cardiomyocytes on biodegradable polyurethane films for myocardial repair. J Biomed Mater Res A 2003;66(3):586-95.

65. Galletti G, Ussia G, Farruggia F, Baccarini E, Biagi G, Gogolewski S. Prevention of platelet aggregation by dietary polyunsaturated fatty acids in the biodegradable polyurethane vascular prosthesis: an experimental model in pigs. Ital J Surg Sci 1989;19(2):121-30.

66. Guan J, Wagner WR. Synthesis, characterization and cytocompatibility of polyurethaneurea elastomers with designed elastase sensitivity. Biomacromolecules 2005;6(5):2833-42.

67. Grad S, Kupcsik L, Gorna K, Gogolewski S, Alini M. The use of biodegradable polyurethane scaffolds for cartilage tissue engineering: potential and limitations. Biomaterials 2003;24(28):5163-71.

68. Planck H. Polyurethanes in biomedical engineering II : proceedings of the 2nd International Conference on Polyurethanes in Biomedical Engineering, Fellbach/Stuttgart, June 18-19, 1986. Amsterdam ; New York: Elsevier; 1987. ix, 272 p.

69. Hettrich W, Becker R. New isocyanates from amino acids. Polymer 1997;38:2437-2445.

70. Bruin P, Smedinga J, Pennings AJ. Biodegradable lysine diisocyanate-based poly(glycolide-co--caprolactone)-urethane network in artificial skin. Biomaterials 1990;11:291-295.

71. DeLong SA, Moon JJ, West JL. Covalently immobilized gradients of bFGF on hydrogel scaffolds for directed cell migration. Biomaterials 2005;26(16):3227-34.

72. Hern DL, Hubbell JA. Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing. J Biomed Mater Res 1998;39(2):266-76.

CHAPTER 6

FUTURE DIRECTIONS

1. Synthesis of inter-penetrated network (IPN) of polyurethane with natural

    materials such as gelatin for the improvement of the biomaterial's cytophilicity

    and mechanical properties.

2. Research on the potential of the degradable polyurethane-based highly elastic

    hydrogel and a computer-controlled dynamic mini-compression bioreactor for use

    in cartilage tissue engineering applications.

3. Development of polyurethane hydrogels to enable the encapsulation and sustained

    release of the DNA. This DNA-polyurethane complex is expected to offer an

    increased resistance to nuclease degradation and have the potential to enhance the

    gene therapeutic effects in clinical application.

4. Application of polyurethane hydrogels to reduce post-surgical adhesion

    formation.