Chapter Three

Biodegradable Elastomeric Polymers and MEMS in Tissue Engineering

Richard Tran, Jagannath Dey, Dipendra Gyawali, Yi Zhang and Jian Yang

Department of Bioengineering, University of Texas at Arlington, 501 West First Street, Arlington, Texas 76019, USA.

3.1 INTRODUCTION

Within the past decade, researchers in the field of tissue engineering have recognized the need for new materials with soft and elastic properties. As a result, many groups have focused on the synthesis, characterization, and application of materials with a wide range of biodegradable and elastomeric properties.[1] The combination of these polymers with Micro–Electro–Mechanical Systems (MEMS) technologies has sparked a new area of research with increasing practical applications.[2] The following chapter discusses important design criteria for creating polymers with elastomeric properties, recently researched biodegradable elastomers, and the use of MEMS in combination with biodegradable elastomers in tissue engineering applications.

3.1.1 Tissue Engineering

Currently, the only effective and permanent treatment to restore lost tissue function is transplantation. Although the success rate for organ replacement therapy has improved, the number of patients awaiting transplantation continues to increase, and the supply of transplantable organs does not meet the current demand.[3] In addition, complications can occur from chronic immune rejection and the required life–long immunosuppressive drug regimen. Due to the growing demand for transplantable organs, a heavy burden is placed on the healthcare industry and the national economy. For example, patients suffering from liver failure cost the United States over $9 billion annually since 1992.[4]

Biomaterials for MEMS, edited by M. Chiao and J.-C. Chiao

www.panstanford.com
978-981-4241-47-2

Better alternatives need to be developed that are less invasive and more cost effective to provide the needed tissue.[5] As defined by Langer and Vacanti, tissue engineering, or regenerative medicine, is "an interdisciplinary field that applies the principles and methods of engineering and life sciences toward the understanding and development of biological substitutes to restore, maintain, and improve human tissue functions." By combining the fundamental principles and methods from chemistry, engineering, and biological sciences, the major goal of tissue engineering is to restore damaged or diseased tissue.[1]

The field of tissue engineering has progressed for almost 30 years. Due to the great potential of this field, much attention has been attracted to help overcome major healthcare needs.[6] Research groups in the field have attempted to recreate a variety of mammalian tissue. For example, ectodermal-, endodermal-, and mesodermal-derived tissue such as the nerve, cornea, skin, liver, pancreas, cartilage, bone, muscle, urethra, bladder, and blood vessels have been investigated.[7–15]

The foundation of tissue engineering relies on four key elements: cells, scaffolds, signals, and bioreactors.[16,17] In the general scheme for tissue engineering, cells are seeded onto a three–dimensional (3D) scaffold, a tissue is cultivated *in vitro*, then proper signals are supplemented to the system, and finally the construct is implanted into the body as a prosthesis.[17] The general scheme for the key elements involved in the tissue engineering paradigm is illustrated in Fig. 3.1.

The cells used in tissue engineering applications can be isolated from either an autologous, allogenic, or xenogenic source. The cells may be tissue specific, stem cells, or progenitor cells. Scaffolds, which provide a substrate for cell growth, can be composed of either a natural or synthetic material, and fabricated into a fibrous, foam, hydrogel, or capsule architecture. Signals can be introduced to enhance cell proliferation, differentiation, and vascularization of the construct. Bioreactors mimic the conditions inside the body, and provide many benefits towards a successful design. For example, bioreactors allow for an increase in the volume of cells that can be cultured *in vitro*, enhance mass transport, and add

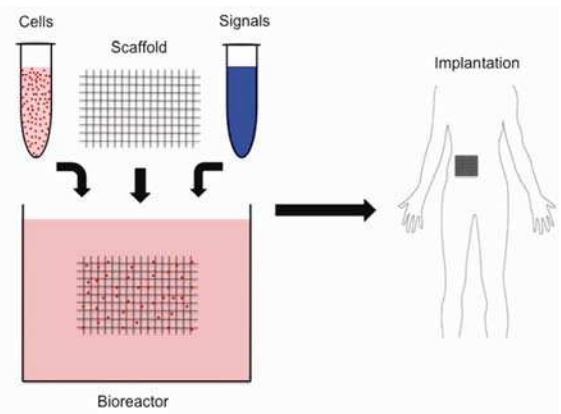

Figure 3.1. The key elements involved in the classic tissue engineering paradigm.

Table 3.1 The controllable parameters from the key elements of the tissue engineering paradigm.

Cells	Scaffold	Bioreactor
Source	Architecture	Nutrients/Oxygen Content
Type	Materials	Growth Factors
Density	Pore Size/Shape	Dynamic Flow Rate
Genetic Manipulation	Bioactive Molecules	Tension/Compression
Gene Expression	Mechanical Properties	Pulsatile Stress
	Degradation Rate	Shear Stress

mechanical cues to stimulate cell differentiation and growth.[17,18] Thus, controlling the parameters from the key elements of the tissue engineering paradigm can ultimately influence the outcome of a cell–scaffold–bioreactor system (Table 3.1).

Despite much of the recent success in tissue engineering, key challenges remain to be addressed. Along with the difficulty in finding an appropriate cell source, the lack of suitable scaffolding biomaterials and the current graft engineering design strategies challenge the success of the field. For example, one major obstacle limiting the success of tissue engineering is compliance mismatch. The current scaffolds cannot be fully integrated with their surrounding tissues because of their incompliant molecular structures and mechanical properties. Thus, further consideration in regards to matching scaffold mechanical properties to the native tissues must be taken into consideration.

3.1.2 Mechanical Considerations for Tissue Engineering Scaffolds

All the tissue cells in the body are located in a unique 3-D extracellular matrix (ECM) environment. The ECM supplies important biochemical signals and functions, facilitates nutrient and waste exchange, guides cellular organization and differentiation, and provides mechanical integrity to the cells.[16,19] In order to sufficiently emulate the natural ECM, a successful scaffold design should include several key requirements. The ideal scaffold should be biocompatible, biodegradable, have an interconnected pore structure, possess a large surface area, allow for adequate cell loading, encourage cell attachment and proliferation, facilitate nutrient and waste exchange, and possess the appropriate mechanical properties for the intended target application.[6,20] Materials used in scaffold fabrication can be divided into four groups: metals, ceramics, polymers, and composites.[21] The content of this discussion will be limited to synthetic polymers. Unlike other materials, synthetic polymers have received great attention because of their controllable material properties such as strength, processability, degradation, microstructure, and permeability. [18]

Many of the soft tissues in the body have soft and elastomeric properties.[22] In order to successfully engineer these tissues, the use of a mechanically compliant

biodegradable scaffold will be required. Engineered scaffolds must be strong enough to withstand the mechanical demands asserted upon them when implanted into the body, and must be able to retain their mechanical properties over time.[23] The utilization of an elastomeric scaffold is advantageous in that it can sustain and recover from multiple deformations without causing irritation to the surrounding tissue in a mechanically demanding environment.[24,25]

Another advantage of elastomeric scaffolds is their ability to be used with mechanical conditioning regimens to promote improved tissue formation. By gradually transferring stress from the degrading synthetic matrix to the newly forming tissue, scaffolds with applied cyclic mechanical strains have been shown to increase collagen and elastin production in vascular smooth muscle cells, and enhance the mechanical properties of the tissue engineered constructs in cardiac applications.[26,27] Research groups have also shown that mechanical signals aid in the development of tissue engineered cartilage.[28]

3.2 DESIGN CRITERIA FOR BIODEGRADABLE ELASTOMERIC POLYMERS

In order to fabricate constructs with the appropriate mechanical properties, many important design criteria must be met when creating the starting materials for the intended target application. The following section will discuss the design requirements and concerns that should be taken into consideration when creating an elastic material for soft tissue engineering applications.

3.2.1 Polymerization Mechanisms

The two main forms of polymerization for elastic polymers are polycondensation and polyaddition reactions. Polycondensation reactions have stepwise growth kinetics, and are characterized by the formation of by-products during synthesis. For example, a diol can be reacted with a diacid to produce a polyester with water as a by-product.

Polyaddition reactions display chain-growth kinetics, and require the use of an initiator. Chain initiation, propagation, and termination are steps that characterize a polyaddition reaction. Through this general mechanism, the average molecular weight of the polymer increases during the reaction. High molecular weight polymers and/or crosslinked polymers can be produced in a polyaddition reaction.

3.2.2 Methods to Incorporate Elasticity

The two methods to incorporate the elasticity are physical crosslinking and chemical crosslinking. Certain segments of polymer chain will form a crystalline structure, which will serve as a means for physical crosslinking. In the case of polyurethanes, the clusters of hard segments act as "pseudo cross-links", and allow the material to behave as an elastomer.[29] When the temperature is raised,

the hard segment clusters disassociate, and the material can be made to flow. When subsequently cooled, the clusters reform and the material will again exhibit elastomeric properties. Some ABA triblock co-polymers will also show elastomeric properties.[30] For example, the thermal liable crosslinks in an ABA triblock co-polymer can aggregate to form physical crosslinking between polymer chains.

Chemical crosslinking joins the polymer chains together into a network linked by covalent. Unlike physical crosslinks, the chemical crosslinks are generally irreversible, and display greater mechanical strength and elasticity. It is well known that natural ECM components such as collagen and elastin are crosslinked polymers. The crosslinking provides these natural materials with their elastic nature. Due to this phenomenon, researchers have utilized the concept of crosslinking in the creation of elastomers to meet the versatile needs in tissue engineering and other biomedical applications.

For polycondensation mechanism, in order to create a polymer with a 3D elastomeric network structure, at least one of the monomers chosen should be multifunctional. In addition to providing the needed functional groups for chain extension, a multifunctional monomer provides valuable unused functional groups, which can be used in later post-processing to create a 3D crosslinked network. Thus, by creating crosslinked network structure, a material with elastic properties can be obtained.

3.2.3 Design Concerns

The three major concerns when designing a biodegradable elastomer for biomedical applications are the biocompatibility, mechanical properties, and degradation rate of the material. In the following section, a brief introduction of these three properties and how they affect each other are discussed.

3.2.3.1 Biocompatibility

Biocompatibility is a term used to describe the ability of a material to perform with an appropriate host response in a specific application. For the materials used in biomedical applications, the biocompatibility should always be put as the first concern. There are several factors that can affect the biocompatibility of a material. For example, the hydrophilicity or hydrophobicity of a material can greatly influence its biocompatibility. It has been demonstrated that the degree of hydrophilicity/hydrophobicity should be balanced to achieve optimal cell affinity.[31,32]

The acidity of a material can also influence its overall biocompatibility. Certain functional groups located on the polymer chain have the ability to greatly change the pH of the surrounding area. In addition to the chemistry of the bulk material causing pH changes, certain materials will degrade into acidic products to alter the pH of the immediate area. This deviation in pH from the body's normal values can create a cytotoxic effect, which can later lead to adverse reactions.

The use of certain chemicals during the synthesis of elastomers can also cause biocompatibility issues. In the case of elastomers created through a polyaddition mechanism, one factor to influence the material's biocompatibility is the toxicity of initiator used during synthesis. In other situations, redox initiators and photoinitiators used to crosslink the polymer have been shown to be toxic to cells when used in large concentrations. Thus, the amount of initiator used for crosslinking should be strictly controlled, and the residual remaining initiator not used during the crosslinking mechanism should be removed.

3.2.3.2 Important Mechanical Properties

During the mechanical test of a polymer, the stress–strain curve obtained is used to define many important parameters of a material's mechanical properties. The stress–strain curve is a graphical representation of the relationship between the amount of stress applied and the resulting strain of the sample.

The tensile strength of a material is the maximum amount of tensile stress that can be subjected to the material before failure. Normally for an elastomer, there is no yield point, and as a result, the peak stress of the stress–strain curve should appear at the break point. The compressive strength is usually obtained experimentally by means of a compression test, and is the value of uniaxial compressive stress reached when the material fails completely.

The elongation, also known as the stretch ratio, is a measure of the largest deformation of the material before failure during the tensile test. A higher elongation indicates the capability of a material to deform. The elastic modulus is also a very important parameter, and is used to determine the stiffness of a material. Depending on the type of mechanical test being performed, the three different types of modulus that can be obtained are the Young's modulus, shear modulus, and bulk modulus. The Young's modulus is the most commonly obtained for an elastomer in biomedical applications. It is defined as the ratio of stress over strain, and can be derived from the slope of the initial linear region of the stress–strain curve. The recovery from deformation is also a parameter used to characterize elastomers. Many of the tissues in the body are fully elastic within a certain deformation. The time to recover from deformation for elastomers should also be considered when characterizing elastomers.

3.2.3.3 Degradation Rate

In most cases, the degradability of an elastomer is due to the hydrolyzable bonds in the polymer network. *In vitro* degradation studies are always used to predict the degradation rate.[33] Enzymatic degradation and oxidative degradation are also two possible ways for the degradation of elastomer.[34,35] In some previous works, *in vitro* enzymatic degradation studies were carried out to evaluate the property of the polymer.[36]

The process of hydrolysis is mainly dependent upon the amount of water penetration into the network structure. Normally, a more hydrophobic material

will have a lower degradation rate. Moreover, a lower glass transition temperature (T_g) will also affect the degradation rate due to the increased water diffusion rate into the material. In order to prevent any changes in the elasticity of a material, it is important to maintain the T_g below the normal body temperature. In the case of an elastomer, both the T_g and mechanical properties are affected by the degree of crosslinking. A higher crosslinked elastomer will normally have a slower degradation rate, stronger mechanical strength and smaller elongation rate.

3.3 BIODEGRADABLE ELASTOMERIC POLYMERS

3.3.1 Polyesters

The use of elastomers in medical applications originates back to the beginning of the rubber industry. Since then, numerous materials have played a major role in medical technology.[37] Polyesters are the most widespread category of polymers used in biomedical applications. The ester bond is important because it allows for degradation through hydrolytic cleavage in the presence of water. Unlike enzymatic degradation, this form of degradation is advantageous because of the minimal site–to–site and patient–to–patient variations.

A polymer used in tissue engineering applications should show good degradability and biocompatibility when presented *in vivo*. Due to these requirements, glycolic and lactic acid based poly(α–hydroxy acids) such as PLLA and PLGA have gained attention in the past few decades as suitable polyesters for various medical applications. Their use can be seen in drug delivery systems, scaffolds for tissue regeneration, resorbable sutures, staples, and orthopedic fixation devices.[38]

However, these α–hydroxyl acid polymers are inappropriate for soft tissue applications because of their stiff nature. Due to this major drawback, researchers are advancing towards a new category of polyesters whose mechanical properties can be tuned for particular soft tissue engineering applications such as blood vessels, heart valves, ligaments, and tendons. Polyesters that possess elastic properties to meet the requirements for soft tissue engineering are shown in Table 3.2. The following section will focus on the polyester elastomers that have been used in the field of soft tissue engineering.

3.3.1.1 Polyhydroxyalkanoates (PHAs)

In the early 1920's, the bacteria *bacillus megaterium* was recognized for producing poly(3–hydroxybutyrate) (PHB), which is the most common polymer among the polyester class. Since then, more than 150 different monomer combinations have been used in the formation of different polymers within the PHA family.[39] Four different pathways have been revealed for the synthesis of PHA through the process of biosynthesis, which has been mentioned in detail elsewhere.[40] Due to advancements in the field of genetic engineering, researchers have also used plants as the production house for PHB-related polymers.[41]

Table 3.2 Mechanical properties of polyester elastomers in recent research.

	Mechanical Properties		
Polymer Name	Youngs Modulus(MPa)	Elongation at Break(%)	Tensile Strength (MPa)
PHB	2500	3	36
P4HB	70	1000	50
PGS	0.056–1.2	40–448	0.2–0.5
PGSA	0.048–1.37	47–170	0.54–0.5
POC	1.85–13.98	117–502	2.93–11.15
PEC	0.25–1.91	140–1505	0.51–1.51
PPSC	0.6–1.23	226–432	0.87–2.12
POM	Not reported	3.86–14.34	7.32–25.6
PAMC	0.05–1.8	55–450	0.29–0.88
CUPE	4.14–38.35	222.66–337.558	14.6–41.07

Several groups have also reported the chemical synthesis of poly(3–hydroxyalkanoates) (P(3HB)) through the process of a ring opening of β–butyrolactone (BL) in the presence of aluminum, zinc, and tin based catalysts.[42–44] However, these reactions did not yield high molecular weight polymers. To overcome this limitation, Hori *et al.* utilized the distannoxane complexes as an excellent catalyst for the ring-opening polymerization of (*R*)–b–butyrolactone ((*R*)–BL) and BL to produce P[(*R*)–3HB] and P(3HB) of high molecular weights and in high yields.[45]

By using different combinations of various monomers, researchers have successfully produced PHAs with a wide range of mechanical properties and degradation profiles. For example, poly(3–hydroxybutyrate) is a stiff polymer with a Young's Modulus of 2500 MPa and 3% elongation where as poly(4–hydroxybutyrate) is an elastic polymer with a Young's Modulus of 70 MPa and 1000% elongation. In terms of their biocompatibility, PHA elastomers are biosynthetic polymers and require serious consideration on their purity.[46]

In the early 1990's, Akhtar *et al.* reported a prolonged acute inflammatory response and severe chronic inflammatory response from PHA films implanted*in vivo*.[47] William *et al.* proposed the idea of using a depyrogenation technique through the use of an oxidizing agent that resulted in the reduction in the amount of endotoxins. In addition, William and co-workers also understood the problems associated with the use of solvents while extracting the polymer. The group found that a higher purity could be obtained if the polymer was extracted with hexane or acetone instead of the traditional chlorinated solvents. In order to support their study, the research group performed *in vivo* tests by placing several different types of implants such as microspheres, tubes, and pellets subcutaneously in mice. The histological results revealed the formation of a thin fibroblast capsule (four to six cell layers), and the absence of macrophages at the implant sites.[46]

Medical device companies have extensively investigated P4HB due to its potential as scaffold material for engineering various tissues. For example, Tepha Inc. is evaluating this member of the PHA family in order to meet all the standards set by the US Food and Drug Administration. In two independent studies lead by Stock and co-workers, it has been demonstrated that this elastomer is a potential candidate for engineering heart valves and for blood vessel augmentation.[48,49]

3.3.1.2 Poly(glycerol–sebacate) (PGS)

In the late 1990's, Nagata and co-workers reported their work on the synthesis and characterization of polymer based on sebacic acid and glycerol. By reacting glycerol and sebacic acid, they achieved their goal in creating an environmentally friendly plastic that can be degraded by soil bacteria.[50] PGS is synthesized through a polycondensation reaction, which produces degradable ester bonds throughout the polymer backbone to solve any degradation issues. However, material property challenges still remained due to the non-elastic nature of the polymer.

In 2002, Wang *et al.* realized that the monomers of this polymer are biocompatible, which opened the door for its use in biomedical applications.[24] After further study of the synthesis procedure and chemical structure, Wang and co-workers realized that Nagata *et al.* were using a 2:3 molar ratio of glycerol and sebacic acid in the reaction, which resulted in the total consumption of all the available functional groups. In contrast, Wang *et al.* divided the reaction into two steps to preserve some of the functional groups for later processing. Thus, a 1:1 molar ratio of glycerol and sebacic acid was used to obtain a linear pre-polymer. This initial step preserved the pendant hydroxyl groups in the PGS pre-polymer, which was later used to form an elastic 3D crosslinked network through ester bond formation.[24] Through this novel idea, a new trend of scaffolding materials was initiated in the field of soft tissue engineering.

PGS is a soft (Young's Modulus of 0.282 ± 0.025 MPa) and elastic (elongation of $267 \pm 59.4\%$) material that has potential for engineering soft tissue such as arteries, veins, and nerves.[24,51] A study by Sundback *et al.*, utilizing PGS for neural reconstruction, showed that PGS can be a good scaffolding material with a desirable biocompatibility.[51] The study also proved that a normal morphology and acceptable growth rate of Schwaan cells could be obtained when compared to PLGA, which is widely used in neural reconstruction.

In a study by Chen *et al.*, the mechanical properties of PGS were evaluated by varying the degree of crosslinking in order to match the mechanical properties of myocardial tissues. They further demonstrated that PGS is bioresorbable through hydrolysis and enzymatic degradation. The degradation rate of PGS can be fine tuned in order to meet the requirement for the construction of heart patches.[52]

3.3.1.3 Poly(glycerol sebacate)acrylate (PGSA)

In 2007, Nijst *et al.* created a photocurable elastomeric polymer based on the previously made PGS. The group incorporated vinyl functional groups into the

polymer backbone by acrylating the available hydroxyl groups of the PGS prepolymer with acryloyl chloride. Due to the presence of these vinyl groups, the polymer achieved a 3D crosslinked network structure through an ultraviolet crosslinking mechanism, which eliminated the long and harsh post polymerization conditions used during the PGS synthesis. This increased polymer's potential to encapsulate cells or temperature–sensitive biomolecules.[36] It was also reported that the mechanical properties of polymer could be tuned according to the degree of acrylation (Table 3.2). Furthermore, co-polymerizing the polymer with PEG diacrylate was also shown to modulate the mechanical properties of the polymer.[36]

The prime interest of developing PGSA was to proliferate and differentiate stem cells into the desired tissue by encapsulating them in the porous matrix of the polymer. Interestingly, human embryonic stem cells (hESCs) encapsulated in the polymer matrix and allowed to grow for seven days showed a colonial organization expressing the Ki67 protein and all three germ layers.[41]

3.3.1.4 Poly(diol–citrate) (POC)

In 2004, Yang *et al.* designed a citric acid based polyester elastomer to avoid the long and harsh processing conditions seen in the synthesis of PGS. The group used citric acid as multifunctional monomer to react with different aliphatic diols ranging from 3–16 carbon chains in 1:1 molar ratios.[53] By controlling the post polymerization conditions, Yang and co-workers demonstrated the ability to tune the mechanical properties and degradation profiles of the elastomer, which allowed for customizable properties for a specific application.

As seen in Table 3.2, a wide range of mechanical properties was reported in order to meet the specific needs for engineering tissues such as cartilage, small

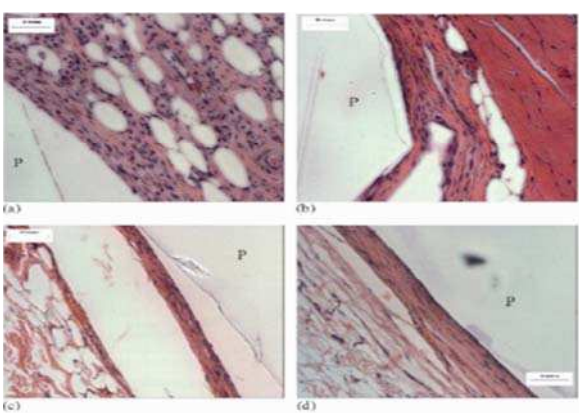

Figure 3.2. Foreign body response of POC (120°C, 2 Pa, 3 d) implanted subcutaneously in female Sprague–Dawley rats (scale bar = 50 μm). Implants and surrounding tissues were harvested after (a) 1 week; (b) 1 month; (c) 2 months; (d) 4 months implantation for H&E staining. "P" represents polymer section. Reprinted from Biomaterials, 27, Jian Yang *et al.*, Synthesis and evaluation of poly(diol citrate) biodegradable elastomers, 1889–1898, 2006, with permission from Elsevier.

diameter blood vessels, and tendons. The preliminarily biocompatibility tests confirm that the elastomer is a "cell friendly" material. Figure 3.2 shows H&E stained pictures of the foreign body response of POC subcutaneously implanted into female Sprague–Dawley rats[54] suggesting the biocompatibility of POC.

It was seen that POC supported the proliferation of human aortic smooth muscle cells and endothelial cells. Furthermore, the degraded products and the polymer are non-toxic in nature. Although the polymer is insoluble in water, the degraded products are soluble in water and can easily be eliminated from the body.[55] It has been shown that the 1,8–octanediol used in the reaction can be partially traded with N–methyldiethanolamine (MDEA) in order to increase the degradation rate, hydrophilicity, and mechanical properties of the elastomers.[54]

3.3.1.5 Poly(PEG–co–CA) (PEC)

Although Yang *et al.* already proposed the use of poly ethylene glycol as the diol to create poly(diol citrates), Ding *et al.* later published their work involving a similar synthesis procedure, but with a different monomer.[54] PEG200 was incorporated into the polymer to create a water-soluble elastomer with a rapid degradation profile. It has been shown that this polymer is highly elastic in nature (elongation of 1500%) with a tensile strength of $>$1.51 MPa. However, the primary objective of developing this material was to design drug-carrying devices. Its utility for tissue engineering applications is yet to be proved. Biocompatibility tests have not been reported for this elastomer, but the monomers (citric acid and PEG) have already been approved by the FDA for medical uses.[53]

3.3.1.6 Poly((1,2–propanediol–sebacate)–citrate) (PPSC)

After realizing the outstanding work of the previous groups, Lei *et al.* proposed synthesizing an oligomer terminated with an alcohol from the monomers sebacic acid and 1,2–propanediol. In addition to further linking these oligomers, citric acid was incorporated to fine tune the mechanical and degradation properties. The elastomer produced showed desirable mechanical properties with low water retention and a rapid degradation profile. However, biocompatibility tests and application oriented studies of this elastomer are not yet reported.[56]

3.3.1.7 Poly(1,8–octanediol malate) (POM)

A recent study by Wan *et al.*, proposed a thermoset elastomer based on 1,8–octanediol and malic acid utilizing a polycondensation reaction between the carboxylic acid and alcohol. This elastomer displayed a tensile strength of 7.32 ± 0.63 to 25.6 ± 1.42 Pa, a compressive Young's modulus of 0.12 ± 0.02 to 0.25 ± 0.01 KPa, and an elongation of $<$15%. However, no permanent deformation was reported after 500 press loading and release cycles with 30% maximum strain. The POM elastomer showed a linear degradation profile in PBS at 37°C, where the majority of mass loss (up to 90 %) was reported by week 13. The study was targeted for the

regeneration Annulus Fibrosus (AF). POM not only shows good AF cell growth, but also cell penetration into the scaffold. However, in order to support significant cell growth, POM required more than 2 days crosslinking at 120°C under vacuum (2 Pa).[57]

3.3.1.8 *Poly(alkylene maleate citrates) (PAMCs)*[58]

In our lab, we have created a new family of elastomers named poly(alkylene maleate citrates) (PAMCs). The synthesis of PMAC pre-polymers can be achieved within 2–6 hours without the use of any harsh processing conditions. A random polycondensation reaction between 1,8–octanediol, citric acid, and maleic acid has been carried out to form a pre-polymer with a vinyl functional group incorporated into the polymer backbone. Furthermore, C3–C16 diols can be utilized as the alcohol, and different vinyl–containing monomers such as fumaric acid, maleic acid, and maleic anhydride can be used to incorporate the vinyl group.

For the first time, a family of elastomers has been created to utilize a dual crosslinking mechanism. The dual crosslinking mechanism allows the polymer to be C–C crosslinked through redox or ultraviolet mechanisms, and further crosslinked through a polycondensation reaction with the addition of heat. The dual crosslinking mechanism is highly advantageous in that the polymer can be quickly crosslinked through the vinyl group, and the mechanical properties and degradation profile of the polymer can be fine tuned through the post polymerization process to meet the requirements for a variety of soft tissue engineering applications.

The PAMC family of elastomers has a wide range of mechanical properties (Young's Modulus of 0.05–1.8 MPa) and elasticity (elongation of 45–450%) that can be modulated through different monomer concentrations, photoinitiator concentration, polymer concentration while crosslinking, and the dual crosslinking mechanism. This wide range of mechanical properties has a huge potential for the regeneration of various types of soft tissues.

A preliminary biocompatibility test confirms that PMACs support 3T3 fibroblast and smooth muscles cell proliferation and adhesion. Once inside the host, the degraded products of the elastomer are biocompatible as confirmed by *in vivo* biocompatibility tests. PAMC samples subcutaneously implanted for 1 week produced a slight acute inflammation response, which was expected and consistent with the introduction of foreign materials into body (Fig. 3.3). After 4 weeks of implantation, the number of infiltrating cells declined and a thin fibrous capsule was formed surrounding the implanted PAMC indicating that the degradation products of PAMC are not toxic.

The pre-polymers of PMAC are in a liquid or gel state, which allows the polymer to be applied onto any contour of the skin as seen in Fig. 3.4. In addition, PMACs can be quickly polymerized within 3 minutes to seal any defects on the immediate surface. This PMAC property shows its potential in wound dressing and tissue sealant applications. Furthermore, pre-poly(octamethylene poly(ethylene

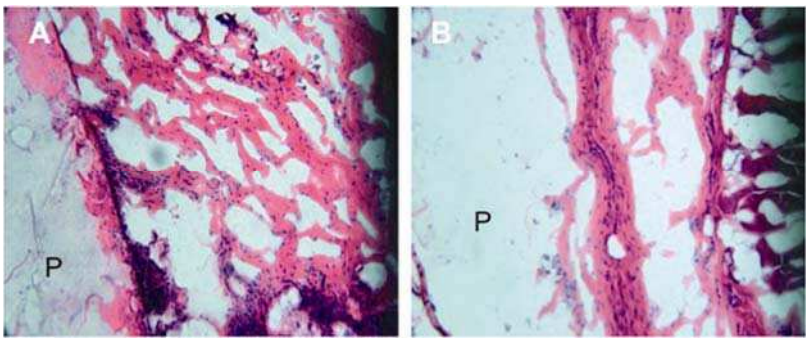

Figure 3.3. Foreign body response of PAMC (POMC 0.8, 20 mins. UV crosslinking) implanted subcutaneously in female Sprague–Dawley rats. Implants and surrounding tissues were harvested after (a) 1 week and (b) 4 weeks. "P" represents polymer section.

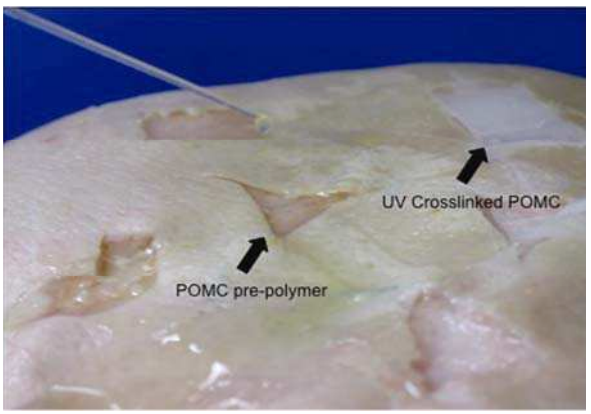

Figure 3.4. Pre-PAMC and ultraviolet crosslinked PAMC on porcine skin.

glycol) maleate citrate) (PPEGMC) and pre-poly(octamethylene poly(ethylene glycol) maleate (PPEGM) are two members of the PMAC family that are soluble in water, and can be used as a injectable crosslinkable polymers for *in situ* applications. Our lab has also been extensively investigating the fabrication of nanogels, microgels, and hydrogels with various PMAC members for the potential use as a carrier for temperature sensitive drugs and cells.

3.3.1.9 Crosslinked Urethane-Doped Polyesters[59,60]

The design and development of a soft, strong, and completely elastic (100% recovery from deformation) material for tissue engineering still remains a challenge. Our lab has also recently developed a new generation of elastomeric polyesters, named crosslinked urethane–doped polyesters (CUPEs). The rationale behind the synthesis of CUPE was to combine the totally elastic properties of crosslinked polyesters with the mechanical strength of polyurethanes to create a family of strong and elastic polymers. The tensile strength of CUPE was as high as

41.07±6.85 MPa with corresponding break strains of 222.66±27.84%. The Young's Modulus ranged from 4.14±1.71 MPa to 38.35±4.5 MPa.

Pre-CUPE polymers were synthesized with different feeding ratios of pre-POC:1,6–Hexamethyl diisocyanate (HDI) (1:0.6, 1:0.9, and 1:1.2, molar ratios). The varying ratios of the POC to HDI regulated the amount of urethane bonds inserted in the polyester backbone, which was shown to influence the thermal, mechanical, and degradation properties of the polymer. For example, the T_g was shown to increase with respect to the concentration of the diisocyanate used during synthesis. CUPE polymers containing higher ratios of diisocyanate were also found to be stronger than CUPEs with lower diisocyanate content for similar post polymerization conditions.

In addition to diisocyanate feed ratios, the post-polymerization conditions also affected the properties of CUPE polymers. With increasing post-polymerization time and temperature, CUPE polymers displayed increased strength and stiffness along with a reduced ultimate strain value, which was attributed to the increased degree of crosslinking between the polymer chains.

For *in vitro* cell culture on CUPE films, SEM results indicated that seeded 3T3 fibroblasts and smooth muscle cells (SMCs) maintained their phenotype while proliferating on the surface of CUPE films. From the Methylthiazoletetrazolium (MTT) assay, it could be seen that a larger number of cells initially attached to the CUPE films as compared to control PLLA films, and the rate of growth and proliferation was comparable to that on PLLA. Subcutaneous implantation of CUPE films and scaffolds in Sprague–Dawley rats was performed to evaluate the foreign body response to CUPEs. Tissue samples were explanted at 1 week and 4

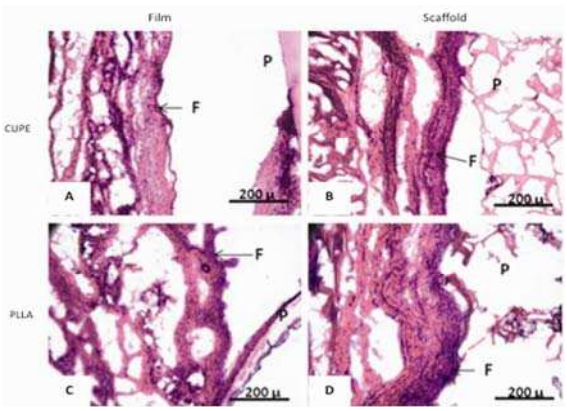

Figure 3.5. Histology of *in vivo* response to CUPE film (A) and scaffold discs (B). PLLA films (C) and scaffolds (D) served as control. P and F are used to indicate the regions of polymer and fibrous capsule respectively. All images were taken at 10x magnification. On the 1 week samples, although all samples were covered by a well defined fibrous capsule, CUPE implants were consistently surrounded by a thinner fibrous capsule as opposed to PLLA implants. In the case of the 4 week implants, overall, all the implants appear to trigger similar extent of tissue responses.

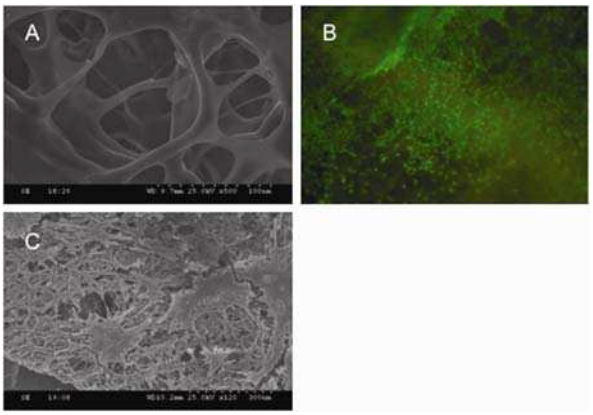

Figure 3.6. SEM images of scaffold surface indicate the presence of well defined pores (A) and even cell distribution of cells on the scaffold (B and C).

week time points, and examined histologically using H&E staining. At the 1 week time point, the fibrous capsules surrounding the CUPE implants were thinner than those surrounding the PLLA implants (Fig. 3.7). At 4 weeks, the fibrous capsule thickness was reduced for both the CUPE and PLLA implants, and was found to be comparable for both polymers. These results indicated that CUPE had a weaker acute inflammatory response and a similar chronic inflammatory response compared to PLLA.

We fabricated thin 3D porous soft and elastic scaffold sheets (150 μm thick) by a simple freeze–drying method (Fig. 3.6(A)). Based on a scaffold–sheet tissue engineering strategy, we proposed the use of CUPE scaffold sheets for tissue regeneration. The thin scaffold sheets allowed even cell seeding, growth, and distribution (Figs. 3.6(B) and 3.6(C)), as the cells did not have to penetrate too deep within the scaffold. Soft scaffolds would also facilitate scaffold–assembly into various shapes through folding, rolling, trimming, and bending. The mechanical strength of CUPE scaffolds would allow surgical handling and bioreactor training for the seeded scaffolds.

3.3.2 Polyurethanes

Polyurethanes are segmented block co-polymers, which consist of a soft and hard segment. The soft segment is composed of a macrodiol, and the hard segment is a combination of a diisocyanate and a chain extender. Typically, the macrodiol is usually a difunctional polyester or polyether segment, and a low molecular weight diol or diamine is used as a chain extender. The segmented architecture is responsible for the unique mechanical properties of polyurethanes. The partially crystallized hard segments act as virtual crosslinks to give polyurethanes their high tensile strength and elasticity.

Polyurethanes are a class of polymers which have been extensively used as biomedical materials since the 1960's.[61] In addition to good biocompatibility, their

controllable and diverse mechanical properties make them ideal biomaterials.[62] The typical applications of polyurethanes in medicine over the past years have included pacemaker leads, catheters, artificial heart prostheses, and coatings for silicone breast implants.[61,63] These applications require that the material remain stable inside the body for long periods of time. Subsequently, all traditional polyurethanes have been designed to be biostable and not degrade easily *in vivo*.

By using polyether soft segments, a more hydrolytically stable material was produced, which increased the stability of the polymer in an *in vivo* setting. However, the polyether soft segments proved to be more susceptible to oxidation. The oxidative effects lead to unwanted degradation of the material. Due to the toxic pre-cursors used in the polyurethane synthesis, the degradation of these biostable polyurethanes would cause the release of carcinogenic compounds inside the body. For example, toluene diisocyanate is one of the most commonly used diisocyanates in the synthesis of biostable polyurethanes. Upon degradation of the urethane bonds, it results in the formation of toluene diamine, which has been shown to be carcinogenic. The effect of oxidation and subsequent degradation of the polyether–urethanes led to the development of oxidation and hydrolysis resistant polycarbonate based polyurethanes.

Due to these complications, the interest in the hydrolytically unstable polyester based urethanes has increased over the last decade. Currently, the primary degradable polyurethanes used as a biomaterial in tissue engineering include polyester–urethanes, polyether–urethanes, and polyester–ether urethanes. Alternatively, hydrolytically labile bonds may be introduced in the hard segment to control the degradation rate of the polyurethane to suit a particular application.[64–66] Faster degradation rates can also be obtained by making the polyurethane degradable, both hydrolytically and enzymatically.[67] The different types of biodegradable polyurethanes are discussed in the following sections.

3.3.2.1 Polyester–urethanes

Polyester–urethane is a term used to describe a polyurethane comprising of a polyester based soft segment. Different polyesters such as poly(L–Lactide) (PLA), poly(ε–caprolactone) (PCL), poly(vinyl alcohol) (PVA), and poly(glycolic acid) (PGA) have been used by various researchers for the synthesis of polyester–urethanes with different properties.

3.3.2.2 PCL–based polyester urethanes

Poly(ε–caprolactone) (PCL)–diol has been used by various researchers to synthesize polyester–urethanes with a wide range of properties. Different polyester–urethanes can be obtained by varying the molecular weight of the PCL–diol, the ratio of hard segment and soft segment, and the properties of monomers used in the synthesis.[64] The low glass transition temperature of PCL (T_g -60°C) allows the polymer to be in an amorphous or semi-crystalline state at use temperature,

and is partially responsible for the strength and elasticity of the PCL based polyurethanes.

The molecular weight of the PCL used for synthesis affects the mechanical properties of the synthesized urethane. With all other parameters remaining the same, many researchers have noted a trend of increasing initial modulus and tensile strength when higher molecular weight PCL is used as the diol.[64,65,68] This phenomenon has been attributed to increasing phase separation leading to greater crystallinity of the higher molecular weight PCL soft segments. A wide range of mechanical properties were also obtained by replacing the PCL soft segment with a PCL–PLA co-polymer segment.[69] By varying the PLA to PCL content of the co-polymer used as the soft segment, the properties of the polyurethane could be varied from a very stiff, inelastic polymer to a soft, elastic elastomer.

Other factors that affect the mechanical properties of the materials include the choice of diisocyanate and chain extender. Skarja *et al.* synthesized two different PCL–urethanes using both HDI and lysine diisocyanate (LDI).[64,65] The greater reactivity of the HDI resulted in higher molecular weight polyurethanes compared to those synthesized with LDI. At low soft segment molecular weights, the HDI based polyurethanes displayed a greater degree of phase separation when compared to the polyurethanes synthesized using LDI. Although mechanical tests were not performed on the HDI based urethanes, the greater degree of phase separation displayed better tensile properties when compared to the LDI based polyurethanes which is due to the better packing of the hard segments. This phenomenon was illustrated in more recent work.[70] However, at higher soft segment lengths, the effect of the diisocyanate on the mechanical properties of the polyurethane was not significant.

The chain extender used during the synthesis is another means of modifying the mechanical properties of the polyurethane. Chain extenders are usually low molecular weight difunctional polyamines, polyfunctional polyamines, or polydiols that are used to increase the molecular weight of the polyurethane. In addition to incorporating ester bonds in the soft segment, the incorporation of the appropriate chain extenders containing hydrolytically labile ester linkages is a technique that has been exploited by researchers to increase polyurethane degradation rates.[64,65,70,71] Furthermore, researchers have also synthesized amino acid based chain extenders which are susceptible to enzymatic degradation.[29,67,72]

These amino acid based chain extenders in combination with non-toxic diisocyanates such as lysine diisocyanate are expected to result in non-toxic and biocompatible hard segment degradation products upon implantation. The effect of the chain extender on mechanical properties mainly depends on the structure and the reactivity of the chain extender used. Chain extenders with pendant side chains may impede hard segment packing as opposed to aliphatic chain extenders, thereby resulting in inferior mechanical properties.[68]

Tatai *et al.* demonstrated the effect of the reactivity of chain extender on the final properties of the polyurethane. Less reactive chain extenders resulted in polyurethanes with lower molecular weight and poorer mechanical properties

when compared to polyurethanes synthesized using more reactive chain extenders. Different chain extenders have also been used to specifically tune the mechanical properties of polyurethanes. Due to the use of aliphatic diisocyanates, the biodegradable polyurethanes lack the stiffness of those made with aromatic diisocyanates. In order to overcome this drawback, Hirt *et al.* introduced poly(hydroxybutyric acid)–co–(hydroxyvaleric acid) (PHB/PV) as a chain extender into a polyurethane with PCL–diethylene glycol–PCL triblocks and LDI as diisocyanate.[73] The PHB/PV chain extender crystallized quickly to form glassy domains thereby causing better aggregation of the hard domains. The hard segment aggregation increased the phase segregation, which resulted in a stiffer and stronger polyurethane.

3.3.2.3 *Poly(ester–ether) urethanes*

Although the incorporation of ester bonds in the soft segment made the polyurethane hydrolytically unstable, the rate of degradation was still found to be slow. This was primarily due to the fact that the soft segment aliphatic polyesters such as PGA, PLA, and PCL were inherently very hydrophobic. It was hypothesized that the incorporation of poly(ethylene glycol) (PEG) in the soft segment would increase the hydrophilicity of the polyurethanes, and accelerate the hydrolytic degradation.

Triblock co-polymers of PCL–PEG–PCL were first used by Cohn *et al.* for the synthesis of polyurethanes.[74] Further developments were made by Guan *et al.* who synthesized poly(ester–ether) urethanes using the triblock co-polymer as a soft segment, a hard segment comprising of 1,4–butane diisocyanate, and putrescine as the chain extender.[75] By varying the ratio of PCL and PEG in the soft segment, the studies showed that the mechanical properties of the polyurethane were improved. Reducing the lengths of the PEG segments, and increasing the length of the PCL segments increased the degree of crystallinity to improve the mechanical properties of the polyurethane.

Ciardelli *et al.* also observed a similar phenomenon. The higher molecular weight of the tri-block was able to increase the degree of soft segment crystallinity due to reduced interruption by the hard segments.[71] The effect of different ratios of the hydrophobic PCL and hydrophilic PEG on the overall hydrophilicity of the polyurethane has also been studied in detail.[76] A further increase in the degradation rates can be achieved by using hydrolytically and enzymatically labile chain extenders like phenylalanine diester in the hard segment of polyurethanes with PCL–PEG–PCL soft segments.[64]

Polyurethane materials have also been used for cardiac reconstruction for congenital heart defects. Guan *et al.* designed a polyester urethane (PEU) based on 1,4–butane diisocyanate, PCL, and putrescine which could be fabricated into highly porous scaffolds using a thermal induced phase separation technique.[77] These biodegradable PEU scaffolds were implanted in the heart of adult rats in which a surgical defect had been introduced. The PEU scaffolds were found

to permit greater cellular infiltration with minimal inflammation.[78] These PEU scaffolds were also fabricated into tubular constructs to evaluate their effectiveness as vascular grafts. The tubular scaffolds were evenly seeded with mouse derived smooth muscle cells using a rotational vacuum seeding technique. Tubular constructs prepared by this method had burst pressure and suture retention values which closely matched that of native arteries.[79]

In addition to vascular engineering, biodegradable polyurethanes based on methylene diphenyl diisocyanate (MDI) as the diisocyanate component have also been used as scaffold materials for ligament tissue engineering. PHB/PV based polyurethanes have also been investigated as bioresorbable nerve guide materials.[80] The nerve guides fabricated form these polyurethanes were degradable and supported nerve regeneration with reduced inflammatory response.

3.3.3 Polycarbonates

Polycarbonates are a family of block co-polymers, which are characterized by the presence of a carbonate bond in the backbone of the polymer chain. To date, the two major classes of biodegradable polycarbonates that have been extensively studied for biomedical applications are the co-polymers of poly(1,3–trimethylene carbonate), and tyrosine derived polycarbonates. The latter family of materials has a glass transition temperature ranging from 52–90°C, making it a rigid material at room temperature. Since the scope of this discussion is limited to elastomeric materials, tyrosine derived polycarbonates have been omitted.

Poly(1,3–trimethylene carbonate) (PTMC) is an amorphous polymer, which was first synthesized by Zhu *et al.* through a bulk ring open polymerization of 1,3–trimethylene carbonate in the presence of catalysts.[81] PTMCs were found to be rubbery materials at room temperature, and displayed low glass transition temperatures ranging from −26°C to −15°C. Further properties of PTMCs are covered in Table 3.3.

In addition to moderate elastomeric properties, the utility of homopolymeric PTMC as a temporary implant material was hampered by its slow degradation. Over a 30 week period, the PTMC samples suffered a mass loss of only 9%. However, subcutaneously implanted PTMC samples were rapidly degraded *in vivo*, and the implanted samples beyond a 3 week period could not be detected macroscopically.[82] This was attributed to hydrolytic resistance and enzymatic cleavage of the carbonate bonds. Co-polymerization with other polymers, primarily degradable polyesters like poly(D,L lactide) (PDLLA) and poly(ε–caprolactone) (PCL), have been employed to improve the degradability and the mechanical properties of polycarbonates.[82–84]

The potential of these co-polymers as scaffolds for heart tissue engineering and synthetic nerve guides for nerve regeneration have also been evaluated.[85–87] It was also found that high molecular weight PTMC was very flexible and tough due to the excellent ultimate stress and strain characteristics. These mechanical properties were attributed to strain induced crystallization of the polymeric network upon

Table 3.3 A comparison of the thermal and mechanical properties of PTMC and its co-polymers.

		Thermal Properties		Mechanical Properties		
Polymer Name	MW	$T_g(^\circ)$	$T_m(^\circ)$	Modulus (MPa)	Peak Stress (Mpa)	Break Strain (%)
Low MW PTMC	42,100	−15	–	2.94	0.49	160
High MW PTMC	324,000	−19	–	6	12	830
DLLA 21 TCM 79[a]	358,000	−9	–	5	2	270
DLLA 80 TCM 20[a]	718,000	33	–	1900	46	7
DLLA 50 TCM 50[a]	644,000	11	–	16	10	570
CL 23 TCM 77[a]	246,000	−25	–	3.9	0.8	103
CL 75 TCM 25[a]	183,000	−55	5.7	4.6	0.1	236
CL 90 TCM 10[a]	184,000	−22	49.9	252	40	906

[a]Denotes the mol % of each monomer in the co-polymer.

application of high deforming stresses. This phenomenon has also been studied extensively elsewhere.[88]

3.3.3.1 *Poly(D,L Lactide–co–1,3–trimethylene carbonate)*

Both high molecular weight and low molecular weight co-polymers of amorphous poly(D,L–lactide) and PTMC have been studied by different researchers.[84,89,90] In all cases, the co-polymer synthesis was carried out through a bulk ring open polymerization of the different monomer ratios with stannous octoate as the catalyst. High molecular weight poly(DLLA–co–TMC) co-polymers were obtained by varying the reaction conditions to reduce the degree of transesterification. The low molecular weight co-polymers were synthesized using 1,3–trimethylene carbonate with a T_g value of -26°C.[81,89]

As expected, the co-polymerization was able to produce co-polymers, which had properties that were intermediate to those of the PDLLA and PTMC homopolymers. The T_g temperatures of the co-polymers ranged from $-16 - 56^\circ$C depending on the percentage of lactide units in the chain. Co-polymers with a greater lactide percentage had higher T_g temperatures, and were generally stiffer. The mechanical properties of the different polymers ranged from weak elastomers to stiff and rigid materials. TMC–DLLA co-polymers with a higher TMC content exhibited high elongation at break (600–800%), but were weak (tensile strength $\leq$ 2 MPa) and underwent irreversible deformations upon extension. A higher DLLA content made the polymers strong (tensile strength 28–33 MPa), but brittle with low elongation at break (6–7%).[84]

Intermediate co-polymers containing similar molar percentages of both TMC and DLLA exhibited good elastomeric behavior. For example, a co-polymer containing equal molar percentages of DLLA and TMC (50%:50% molar ratio)

displayed a tensile strength of 10 MPa and an elongation up to 570%. The dependence of mechanical properties on the polymer molecular weight was also evident. The higher molecular weight co-polymers displayed better mechanical properties than their low molecular weight counterparts.[84,90] All the co-polymers were found to be hydrophobic because of the hydrophobicity of the starting monomers. The *in vitro* degradation was influenced by the percentage of the monomeric constituents. For example, the polymers with a higher TMC content underwent surface degradation, while the higher lactide containing polymers underwent bulk degradation.[89]

Pego *et al.* conducted a detailed investigation of the degradation behavior of these co-polymers over a 111–week period. During this study, the group examined the effect of the monomer composition on the *in vitro* degradation profiles. The effect of degradation on the thermal and mechanical properties of the various co-polymers was also examined.[91] Even though PDLLA had a higher number of ester bonds, the co-polymers showed faster degradation rates compared to PDLLA and PTMC. This phenomenon was attributed to the lower T_g of the co-polymers when compared to PDLLA, which made the chains more mobile. The added mobility increased water uptake, and allowed the ester bonds to be more accessible in the co-polymers. The loss of mechanical properties, mass loss, and water uptake could all be co-related to the loss of molecular weight of the polymers as the hydrolytic scission of their chains progressed over the evaluation period.

The co-polymers containing a high percentage of TMC were not resorbed over the evaluation period, and the high DLLA constituting co-polymers were resorbed within 11 months. An extensive yearlong study was also conducted by Pego *et al.* to evaluate the degradation profile of these polycarbonates *in vivo*.[82] It was determined that the polymers degraded faster under the influence of the physiological environment, as opposed to the long *in vitro* degradation times. This was caused by the catalytic degradation of the carbonate moieties similar to that reported earlier.[81] To further support the theory of enzymatic breakdown of the carbonate bond, poly TMC was totally resorbed within 3 weeks *in vivo*, while a negligible mass loss was shown over a 2 year period *in vitro*.

TMC–DLLA co-polymer films were seeded with rat cardiomyocytes to determine their suitability for cardiac applications. Seeded cardiomyocytes adhered and proliferated very well on the TMC–DLLA films.[86,87] Porous scaffolds made from TMC and DLLA co-polymers were prepared by a compression molding of the salt–polymer precipitate, and followed by salt leaching. Although the TMC–DLLA co-polymer films showed good cardiomyocytes growth, no cell culture results on TMC–DLLA co-polymer scaffolds have been reported.[86,92]

3.3.3.2 *Poly (ε–caprolactone–co–1,3–trimethylene carbonate)*

As mentioned previously, co-polymerizations were found to be a suitable method to modulate the degradation rates of elastomeric polymers based on PTMC. DLLA was employed as a co-monomer to increase the degradation rate of the produced

elastomers. However, for certain tissues repair applications such as synthetic nerve guides, it is desirable to use a material which is elastic and has a slower degradation rate.

Pego *et al.* hypothesized that the co-polymerization of ε–caprolactone and 1,3–trimethylene carbonate could yield a co-polymer, which could degrade slower than the TMC–DLLA co-polymers and retain their elasticity over a longer time period.[83] The rationale behind this idea was that poly (ε–caprolactone) is a semicrystalline polyester which degrades very slowly. High molecular weight (Mn>100,000) poly(caprolactone–co–trimethylene carbonate) co-polymers were synthesized through a ring open polymerization of the co-monomers in the presence of stannous octoate as a catalyst.

The glass transition temperatures of the co-polymers varied from -15°C to -60°C, depending on the molar percentage of each co-monomer in the melt. Co-polymers with higher caprolactone content had lower T_g values. As the caprolactone content increased, the co-polymers ranged from amorphous to semicrystalline in nature. As observed with the TMC–DLLA co-polymers, increasing the molar percentage of TMC in the melt produced weak polymers with low tolerance to deformation. Both *in vitro* and *in vivo* degradation studies were conducted to understand the mechanism of degradation of the co-polymers obtained.[82,91]

From the *in vitro* results, it was found that the TMC–CL based co-polymers degraded much slower than the TMC–DLLA based co-polymers.[91] The semicrystalline samples with a high CL content did not undergo any dimensional changes over a two–year period. In contrast, the amorphous samples with higher TMC content degraded and showed reduced dimensions. The hydrolysis rate *in vitro* was a function of the CL content in the co-polymer. Higher hydrolysis rates and subsequently higher water uptake and mass loss were detected in the polymers with higher ester content. Even in the *in vivo* study, the TMC–CL co-polymers degraded slower when compared to the TMC–DLLA co-polymers.[82] In contrast, the TMC–DLLA co-polymer degraded completely in 52 weeks, and the semicrystalline TMC–CL co-polymers suffered a mass loss below 7%. Apart from material characterization, the adhesion and proliferation of human Schwann cells on these co-polymers has been studied to determine their suitability as artificial nerve guides.[85,86]

Human Schwann cells (HSCs) were seeded on PTMC and poly(TMC–co–CL) co-polymers coated with fibronectin to evaluate the suitability of these elastomers as nerve guide materials. These materials are ideal for fabrication of nerve guides because of their long degradation rates, which are well suited to the long regeneration time of neural tissue. The number of primary HSCs which attached to the coated polymers was similar to the number of cells seeded on a control gelatin film.[85] In addition, *in vivo* studies have shown that poly(TMC–co–CL) co-polymers can be effective nerve guides in the regeneration of autonomous neural tissue.[93]

3.4 MEMS PRINCIPLES IN TISSUE ENGINEERING

In the past decade, microscale technologies have emerged as a powerful tool for biological and biomedical applications.[94] MEMS research and development has remained intense to solve complex problems at the cellular and molecular level.[2,95] Biological or Biomedical MEMS, BioMEMS, can be defined as the application of micro– and nanotechnology to develop devices or systems that are used for the processing, delivery, manipulation, analysis, or construction of biological and chemical modalities.[2,95] The advancement of BioMEMS technologies has progressed, and will have a broad and significant impact in the fields of biology and medicine if fully realized.[96]

Few other engineering techniques are able to closely match the micro to millimeter size dimension of tissues in the human body with the precision and accuracy of BioMEMS techniques.[95] Due to these advantages, BioMEMS holds great promise in addressing the challenges found in many disciplines such as diagnostic, therapeutic, sensing, detection, and tissue engineering applications.[2,97,98] The potential to mimic complex tissue architecture and *in vivo* conditions makes BioMEMS a powerful tool for tissue engineering.

3.5 MEMS APPLICATIONS IN TISSUE ENGINEERING

Although BioMEMS based tissue engineering is a rapidly advancing field, research involving the use of biodegradable elastomers coupled with microfabrication processes is new and fairly limited. Discussed in the following section are BioMEMS based techniques involving hydrogels and biodegradable elastomers to construct 3D structures, control cell adhesion, control cell morphology, and create microvasculature for 3D constructs.

The recent progress of MEMS based technologies has lead to new approaches to study *in vitro* cell culture environments. Many of these new techniques utilize a soft lithography approach to rapidly produce 3D microstructures. Leclerc *et al.* used a photosensitive caprolactone and lactide based polymer to fabricate biodegradable polymer microstructures down to 50 μm for tissue engineered liver constructs.[99]

As seen in Fig. 3.7, Leclerc *et al.* successfully created various single and multistepwise microstructures using a soft lithographic technique. In addition, the single stepwise microstructures supported the attachment, spreading, and growth of a variety of mammalian cell types. Other groups have also successfully created complex 3D polymer constructs for hepatic tissue engineering. In 2007, Tsang *et al.* created PEGDA hydrogel constructs for hepatic cell encapsulation. By combining a PEG based hydrogel with a multilayer fabrication method, Tsang and co-workers were able to fabricate highly cell–encapsulated scaffolds with architecture to facilitate nutrient delivery through convective flow.[100]

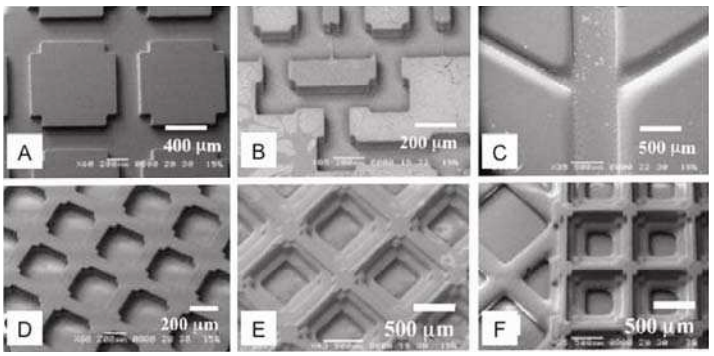

Figure 3.7. SEM photographs of fabricated microstructures. (A) microchambers and microchannels on pCLLA; (B) a microchannel network on pCLLA; (C) channels fabricated with direct UV exposure on pCLLA; (D) a single stepwise microstructure on pCLH fabricated by stamping; (E) a multistepwise microstructure on pCLLA fabricated by stamping; and (F) a multistepwise microstructure on pCLH fabricated by stamping. Reprinted from Biomaterials, 25(19), Leclerc E. *et al.*, Fabrication of microstructures in photosensitive biodegradable polymers for tissue engineering applications, 4683–4690, 2004, with permission from Elsevier.

In addition to creating 3D constructs, many research groups have incorporated micro scale technologies to promote and discourage cell adhesion. Mizutani *et al.* showed the ability to control cell adhesion on PLA films using photocured co-polymers.[101] Coating a PLA surface with a low molecular weight alcohol based co-polymer promoted endothelial cell adhesion, whereas the PLA surface coated with PEG–based co-polymer did not support cell adhesion. The different co-polymers coated on the PLA films were able to change the hydrophobicity of the surface to either encourage or deter endothelial cell adhesion.[101]

Another research group successfully proved to control tissue organization by immobilizing non-adhesive domains onto a surface. The group of Liu *et al.* used a photolithographic technique to immobilize a PEO–terminated triblock polymer onto various surfaces to deter cell adhesion for up to 4 weeks *in vitro*.[102] Expanding upon previous research by Neff *et al.*, the hydrophobic core of the polymer was modified with adhesive peptides to create non-adhesive domains.[103] This cell avoidance phenomenon can be explained by the polymer's ability to also deter proteins, which are necessary for cell attachment.[102]

The ability to control cell and protein behavior using mechanical cues in addition to chemical cues is critical in understanding tissue development.[102] While these mechanisms of cell behavior are not yet fully understood, research has shown that the extracellular matrix proteins of cells possess a 3D surface topography of sub-micron length scales.[104] The ability to control cellular structure and function by culturing cells on substrates modified with micron and sub-micron features is a field termed contact guidance.[105] Contact guidance has been shown to induce cellular responses in various cell types such as epithelial cells, fibroblasts, oligodendrocytes, and astrocytes.[106]

The use of poly (dimethyl siloxane) (PDMS) has been a major limitation of previous research involving contact guidance. PDMS, although elastic, is not biodegradable and has limited biocompatibility, which limits its use in tissue engineering applications. To overcome this limitation, Bettinger *et al.* is the first group to successfully use BioMEMS to introduce rounded sub-micron features onto an elastic, biodegradable substrate for contact guidance applications (Fig. 3.8). Using PGS, a novel biodegradable elastomer, the research group developed a photolithographic method to fabricate substrates with rounded features as small as 500 nm in scale.

Bovine aortic endothelial cells cultured on the microstructures exhibited a rounded and spindle–shaped morphology when compared to cells cultured on a flat substrate, which had a random orientation of cell projections and a flattened appearance. Thus, their results showed that filipodia of cells are able to detect regional gradients in substrate topography, which results in preferential cell adherence through cytoskeletal rearrangement.[106]

In addition to guiding the cell morphology, BioMEMS techniques have also been applied toward creating microvasculature for tissue engineered constructs. Creating tissue constructs on 3D scaffolds has been a heavily researched area.[18] However, creating constructs that provide adequate nutrient and oxygen transport

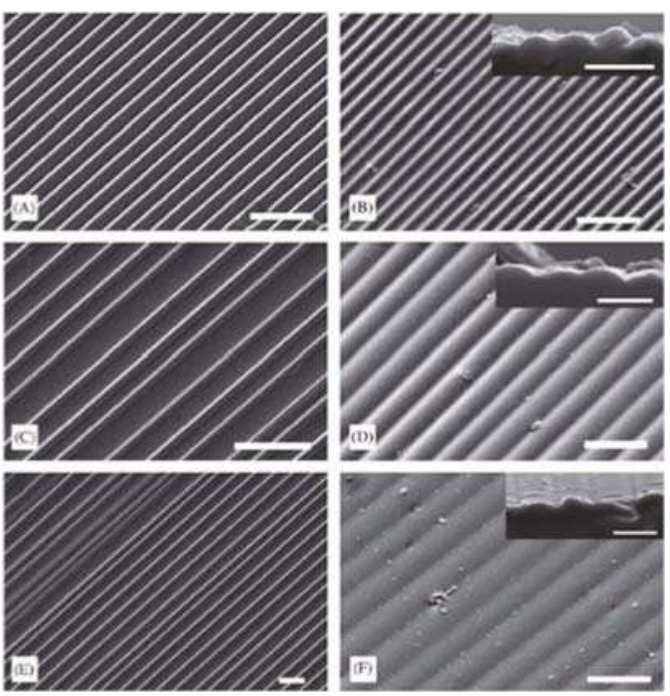

Figure 3.8. SEM photographs of silicon masters with cross–sections and their resulting PGS substrates. Reprinted from Biomaterials, 27(12), Bettinger CJ *et al.*, Microfabrication of poly (glycerol–sebacate) for contact guidance applications, 2558–2565, 2006, with permission from Elsevier.

to cells deeply embedded in the substrate has proven to be a formidable task.[100] The development of an established vasculature system to provide oxygen, nutrients, and waste removal is critical in the survival of tissue engineered organs.[107] From this limitation, current engineered tissue is limited to 150–200 micron thicknesses due to oxygen diffusion limitations.[108]

Fidkowski *et al.* have used BioMEMS to build capillary networks onto synthetic substrates. Using standard soft photolithography techniques, the research group patterned intricate capillary networks 10 microns in size onto PGS using silicon wafers as molds. Human umbilical vein endothelial cells (HUVECs) were seeded onto the PGS substrates and perfused under flow conditions to create confluent endothelialized two–dimensional cell layers. The HUVECs could be lifted from the PGS substrate and incorporated into other devices. Thus, this study showed the potential for using PGS in combination with BioMEMS techniques to create microvasculature *in vitro* towards the fabrication of vascularized organs.[107]

3.6 OUTLOOK

Many of the tissues in the body are soft and elastic. Much attention has been paid in using biodegradable soft and elastic scaffolds for tissue engineering soft tissues such as skin, blood vessel, tendon, ligament, cartilage, bladder etc. The roles of biodegradable elastomeric materials in tissue engineering have been increasingly emphasized as the evolving progress in understanding the cell/materials/host interactions. Soft and elastic scaffolds made of biodegradable elastomeric scaffolds not only provide a substrate for cells to adhere and proliferation, but also minimize the compliance mismatch with surround tissues and provide cues and signals to promote tissue development and functional integration with the host.

The design and synthesis of biodegradable elastomers will continuously evolve owing to the more stringent material requirements in personalized tissue regeneration. Despite the recognized importance of the mechanical properties of tissue engineering scaffolds on the tissue development, there has been a dearth on fundamental understanding on how the soft and elastic scaffolds affect the inflammatory response of the host and the tissue/graft integration.

The application of BioMEMS in tissue engineering has resulted in more understanding on how cells respond to micro/nano structure created by BioMEMS. Constructing vasculature with the aid of BioMEMS on biodegradable elastomeric scaffolds for tissue engineering is still in its infancy. The current studies lie on fabricating channels on two-dimensional films, and then stacking them into 3D channels on elastomers, mostly on PDMS. More studies should be focused on using biodegradable elastomeric substrates. More importantly, the vasculature should be built up within 3D porous scaffolds instead of just in between two-dimensional solid films. Our recent studies have resulted in 3D scaffolds with vasculature-like channels built using our recently developed CUPE polymers via the scaffold-sheet tissue engineering strategy combined with BioMEMS technology.

References

[1] Webb A. R., Yang J. and Ameer G. A., Biodegradable polyester elastomers in tissue engineering. *Expert Opin. Biol. Ther.*, **4**(6), 801–812 (2004).

[2] Bashir R., BioMEMS: state-of-the-art in detection, opportunities and prospects. *Adv. Drug. Deliv. Rev.*, **56**(11), 1565–1586, (2004).

[3] Pomfret E. A., Sung R. S., Allan J, Kinkhabwala M, Melancon J. K. and Roberts J. P., Solving the organ shortage crisis: the 7^{th} annual American Society of Transplant Surgeons' State-of-the-Art Winter Symposium. *Am J. Transplant*, **8**(4), 745–752 (2008).

[4] Kulig K. M. and Vacanti J. P. Hepatic tissue engineering. *Transpl Immunol*, **12**(3–4), 303–310 (2004).

[5] Chan C., Berthiaume F., Nath B. D., Tilles A. W., Toner M. and Yarmush M. L., Hepatic tissue engineering for adjunct and temporary liver support: critical technologies. *Liver Transpl*, **10**(11), 1331–1342 (2004).

[6] Ikada Y. Challenges in tissue engineering.*J. R. Soc. Interface*, **3**(10), 589–601 (2006).

[7] Evans G. R., Approaches to tissue engineered peripheral nerve. *Clin. Plast Surg.* **30**(4), 559–563, viii (2003).

[8] Selvam S., Thomas P. B. and Yiu S. C., Tissue engineering: Current and future approaches to ocular surface reconstruction. *Ocul. Surf.*, **4**(3), 120–136 (2006).

[9] Metcalfe A. D.and Ferguson M. W. Tissue engineering of replacement skin: the crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration. *J. R. Soc. Interface*, **4**(14), 413–437 (2007).

[10] Nahmias Y., Berthiaume F., Yarmush M. L., Integration of technologies for hepatic tissue engineering. **Adv. Biochem. Eng. Biotechnol, 103** 309–329 (2007).

[11] Beck J., Angus R., Madsen B., Britt D., Vernon B, Nguyen K. T., Islet encapsulation: strategies to enhance islet cell functions. *Tissue Eng.*, **13**(3), 589–599 (2007).

[12] Lee S. H, Shin H. Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. *Adv. Drug. Deliv. Rev.*, bf 59(4–5), 339–359 (2007).

[13] Stern-Straeter J., Riedel F., Bran G., Hormann K. and Goessler U. R. Advances in skeletal muscle tissue engineering. *In Vivo* **21**(3), 435–444 (2007).

[14] Sievert K. D, Amend B and Stenzl A. Tissue engineering for the lower urinary tract: a review of a state of the art approach. *Eur. Urol.*, **52**(6), 1580–1589 (2007).

[15] Zhang W. J, Liu W., Cui L. and Cao Y., Tissue engineering of blood vessel.*J Cell Mol. Med.*, **11**(5), 945–957 (2007).

[16] Hiles M. and Hodde J. Tissue engineering a clinically useful extracellular matrix biomaterial. Int Urogynecol *J. Pelvic Floor Dysfunct*, **Suppl 1**, S39–43 (2006).

[17] Rabkin E. and Schoen F. J., Cardiovascular tissue engineering. *Cardiovasc Pathol.*, **11**(6), 305–317 (2002).

[18] Vacanti J. P. and Langer R. Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. Lancet 1999 Jul;354 Suppl 1:SI32–34.

[19] Lee J., Cuddihy M. J. and Kotov N. A., Three-Dimensional Cell Culture Matrices: State of the Art. *Tissue Engineering Part B: Reviews*, 14(1), 61–86 (2008).

[20] Yang J., Webb A. R. and Ameer G. A., Novel Citric Acid-Based Biodegradable Elastomers for Tissue Engineering Adv. Mater **16**(6):511–516 (2004).

[21] Ratner B. D., Hoffman A. S., Schoen F. J. and Lemmons J. E., Biomaterial Science: An Introduction to Materials in Medicine. San Diego: Elsevier Academic Press, (2004).

[22] Levental I., Georges P. C. and Janmey P. A., Soft biological materials and their impact on cell function. *Soft Matter.,* **3**, 299–306 (2007).

[23] Hodde *J. Naturally occurring scaffolds for soft tissue repair and regeneration. Tissue Eng.,* **8**(2):295–308 (2002).

[24] Wang Y., Ameer G. A., Sheppard B. J. and Langer R. A tough biodegradable elastomer. *Nat Biotechnol*, **20**(6), 602–606 (2002).

[25] Younes H. M., Bravo-Grimaldo E. and Amsden B. G., Synthesis, characterization and *in vitro* degradation of a biodegradable elastomer. *Biomaterials,* **25**(22), 5261–5269 (2004).

[26] Stegemann J. P. and Nerem R. M., Phenotype modulation in vascular tissue engineering using biochemical and mechanical stimulation. *Ann. Biomed. Eng.,* **31**(4), 391–402 (2003).

[27] Niklason L. E., Gao J., Abbott W. M., Hirschi K. K., Houser S., Marini R. and Langer R. Functional arteries grown *in vitro. Science,* **284**(5413), 489–493 (1999).

[28] Lee J. H., Kisiday J. and Grodzinsky A. J,. Tissue-engineered versus native cartilage: linkage between cellular mechano-transduction and biomechanical properties. *Novartis Found Symp,* **249**, 52–64 discussion 64–59, 170–174, 239–141.

[29] Sarkar D., Yang J. C. and Lopina S. T. Structure-Property Relationship of L-Tyrosine-Based Polyurethanes for Biomedical Applications.*Journal of Applied Polymer Science,* **108**, 2345–2355 (2008).

[30] Wright E. R. and Conticello V. P., Self-assembly of block copolymers derived from elastin-mimetic polypeptide sequences. *Adv. Drug Deliv. Rev. Oct.,* **54(8)**, 1057–1073 (2002).

[31] Wang Y-Q. and Cai J-Y. Enhanced cell affinity of poly(L-lactic acid) modified by base hydrolysis: Wettability and surface roughness at nanometer scale. *Current Applied Physics,* **7S1**, e108–e111 (2007).

[32] Wang S, Cui W, Bei J. Bulk and surface modifications of polylactide. Anal. Bioanal. Chem., **381**(3), 547–556 (2005).

[33] Amsden B. Curable, biodegradable elastomers: emerging biomaterials for drug delivery and tissue engineering. *Soft Matter,* **3**, 1335–1348 (2007).

[34] Gallocher S. L, Aguirre A. F, Kasyanov V, Pinchuk L, Schoephoerster RT. A Novel Polymer for Potential Use in a Trileaflet Heart Valve. *Inc. J. Biomed. Mater Res Part B: Appl. Biomater.,* **79B**, 325–334 (2006).

[35] Kunioka M., Ninomiya F. and Funabashi M. Biodegradation of poly(lactic acid) powders proposed as the reference test materials for the international standard of biodegradation evaluation methods. *Polymer Degradation and Stability,* **91**, 1919–1928 (2006).

[36] Nijst C. L., Bruggeman J. P, Karp J. M, Ferreira L, Zumbuehl A, Bettinger C. J, Langer R. Synthesis and characterization of photocurable elastomers from poly(glycerol-co-sebacate). *Biomacromolecules,* **8**(10), 3067–3073 (2007).

[37] Yoda R. Elastomers for biomedical applications.*J. Biomater. Sci. Polym. Ed.,* **9**(6), 561–626 (1998).

[38] Gunatillake P. A. and Adhikari R. Biodegradable synthetic polymers for tissue engineering. *European cells & Materials* **5**, 1–16; discussion 16 (2003).

[39] Nair L. and Laurencin C. Biodegradable polymers as biomaterials. *Progress in Polymer Science*, **32**, 762–798 (2007).

[40] Lee S. Bacterial Polybhydroxyalkanoates. *Biotechnology and Bioengineering,* **49**, 1–14 (1996).

[41] Poirier Y., Nawrath C. and Somerville C. Production of polyhydroxyalkanoates, a family of biodegradable plastics and elastomers, in bacteria and plants. Bio/technology (Nature Publishing Company) **13**(2), 142–150 (1995).

[42] Hocking P. and Marchessault R. Syndiotactic poly[(R, S)-b-hydroxybutyrate] isolated from methylaluminoxane-catalyzed polymerization. *Polym Bull,* **30**, 163–170 (1993).

[43] Kemnitzer J., McCarthy S. and Gross R. Preparation of predominantly syndiotactic poly(b-hydroxybutyrate) by the tributyltin methoxide catalyzed ring-opening polymerization of racemic b-butyrolactone. *Macromolecules,* **26**, 1221–1229 (1993).

[44] Kemnitzer J., McCarthy S. and Gross R. Syndiospecific ringopening polymerization of b-butyrolactone to form predominantly syndiotactic poly(b-hydroxybutyrate) using tin(IV) catalysts. *Macromolecules,* **16**, 6143–6150 (1993).

[45] Hori Y. and Hagiwara T. Ring-opening polymerisation of beta-butyrolactone catalysed by distannoxane complexes: study of the mechanism. *International Journal of Biological Macromolecules*, **25**(1-3), 237–245 (1999).

[46] Williams S, Martin P, Horowitz D, Peoples O. PHA applications: addressing the price performance issue. *International Journal of Biological Macromolecules*, **25**, 111–121 (1999).

[47] Akhtar S. Physiochemical properties of bacterial P(HB-HV) polyesters and their uses in drug delivery.: University of Bath; 1990.

[48] Stock U. A, Nagashima M, Khalil P. N, Nollert G. D, Herden T, Sperling J. S, Moran A., Lien J., Martin D. P., Schoen F. J., Vacanti J. P. and Mayer J. E., Jr., Tissue-engineered valved conduits in the pulmonary circulation. *The Journal of Thoracic and Cardiovascular Surgery*, **119**(4 Pt 1), 732–740 (2000).

[49] Stock U. A and Mayer J. E., Jr. Tissue engineering of cardiac valves on the basis of PGA/PLA Co-polymers. *Journal of Long-term Effects of Medical Implants,* **11**(3–4), 249–260 (2001).

[50] Nagata M., Kiyotsukuri T., Ibuki H., Tsutsumi N. and Sakai W. Synthesis and Enzymatic Degradation of Regular Network Aliphatic Polyesters. *Reactive Functional Polymers,* **30**, 165–171 (1996).

[51] Sundback C. A., Shyu J. Y., Wang Y., Faquin W. C., Langer R. S., Vacanti J. P. and Hadlock T. A. Biocompatibility analysis of poly(glycerol sebacate) as a nerve guide material. *Biomaterials,* **26**(27), 5454–5464 (2005).

[52] Chen Q. Z., Bismarck A., Hansen U., Junaid S., Tran M. Q., Harding S. E., Ali N. N. and Boccaccini A. R. Characterisation of a soft elastomer poly(glycerol sebacate) designed to match the mechanical properties of myocardial tissue. *Biomaterials,* **29**(1), 47–57 (2008).

[53] Ding T., Liu Q., Shi R., Tian M., Yan J. and Zhang L. Synthesis, characterization and in vitro degradation study of a novel and rapidly degradable elastomer. *Polym Degrad Stab,* **91**, 733–739 (2006).

[54] Yang J., Webb A. R., Pickerill S. J., Hageman G. and Ameer G. A. Synthesis and evaluation of poly(diol citrate) biodegradable elastomers. *Biomaterials,* **27**(9), 1889–1898 (2006).

[55] Yang J., Webb A. R., Hageman G., Ameer G. A. Biodegradable elastomeric polymer for tissue engineering. In: Mallapragada S. NB, editor. Biodegradable polymer materials and their application: *American Scientific Publishers*, p. 191–232 (2006).

[56] Lei L., Ding T., R RS, Liu Q., Zhang L., Chen D. and Tian W. Synthesis, characterization and in vitro degradation of a novel degradable poly((1,2-propanediol-sebacate)-citrate) bioelastomer. *Polymer Degradation and Stability,* **92**, 389–396 (2007).

[57] Wan Y., Feng G., Shen F. H., Balian G., Laurencin C. T. and Li X. Novel biodegradable poly(1,8-octanediol malate) for annulus fibrosus regeneration. *Macromol Biosci*, **7**(11), 1217–1224 (2007).

[58] Yang J. and Gywali D., Tran M-TR, inventors. Composition and method of biodegradable elastic polymers, (2008).

[59] Yang J. and Dey J., inventors. Biopolymer and scaffold-sheet method for tissue engineering. USA, (2007).

[60] Dey J., Xu H. Shen J., Gondi S. R., Nguyen K. T., Sumerlin B. S., Tang L. and Yang J. Development of biodegradable crosslinked urethane-doped polyester elastomers. *Submitted*, (2008).

[61] Boretos J. and Pierce W. Segmented Polyurethane: A New Elastomer for Biomedical Applications. *Science,* **158**(3807), 1481 (1967).

[62] Guelcher SA. Biodegradable Polyurethanes: Synthesis and Applications in Regenerative Medicine. *Tissue Engineering: Part B*, **14**(1), 3–16 (2008).

[63] Zdrahala R. and Zdrahala I. *Biomedical Applications of Polyurethanes: A Review of Past Promises, Present Realities, and a Vibrant Future Journal of Biomaterials Applications,* **14**(1), 67–90 (1999).

[64] Skarja G. and Woodhouse K. Synthesis and Characterization of degradable polyurethane elastomers containing and amino-acid based chain extender. *Journal of Biomaterial Science,* Polymer Edition **9**(3), 271–295 (1998).

[65] Skarja G., Woodhouse K. Structure-Property Relationships of Degradable Polyurethane Elastomers Containing and Amino Acid based Chain Extender. *Journal of Applied Polymer Science,* **75**, 1522–1534 (2000).

[66] Skarja G., Woodhouse K. In vitro Degradation and Erosion of degradable, segmented polyurethanes containing and amino acid based chain extender. *Journal of Biomaterial Science,* Polymer Edition **12**(8), 851–873 (2001).

[67] Guan J., Wagner W. R. Synthesis, Characterization and Cytocompatibility of Polyurethaneurea Elastomers with Designed Elastase Sensitivity. *Biomacromolecules, 6*, 2833–2842 (2005).

[68] Guan J., Sacks M., Beckman E. and Wagner W. R. Synthesis, characterization and cytocompatibility of elastomeric, biodegradable poly(ester urethane)ureas based on poly(caprolactone) and putrescine. *Journal of Biomedical Materials Research,* **61**, 493 (2002).

[69] Kylma J. and Seppala J. V. Synthesis and Characterization of a Biodegradable Thermoplastic Poly(ester-urethane) Elastomer. *Macromolecules,* **30**, 2876–2882 (1997).

[70] Tatai L., Moore T. G., Adhikari R., Malherbe F., Jayasekara R., Griffiths I. and Gunatillake P. A. Thermoplastic biodegradable polyurethanes: The effect of chain extender structure on properties and *in-vitro* degradation. *Biomaterials,* **28**(36), 5407–5417 (2007).

[71] Ciardelli G., Rechichi A., Cerrai P., Tricoli M., Barbani N. and Giusti P. Segmented Polyurethanes for Medical Applications: Synthesis, Characterization and *in vitro* Enzymatic Degradation Studies. **Macromolecular Symposia, 218**, 261–271 (2004).

[72] Sarkar D, Yang J. C., Gupta A. S. and Lopina S. T. Synthesis and characterization of L-tyrosine based polyurethanes for biomaterial applications. *J. Biomed. Mater. Res., A* May 21 (2008).

[73] Hirt T. D., Neuenschwander P. and Suter U. W. Synthesis of degradable, biocompatible, and tough block-copolyesterurethanes. *Macromol Chem. Phys.,* **197**, 4253–4268 (1996).

[74] Cohn D., Stern T., Gonzalez M. F. and Epstein J. Biodegradable poly(ethylene oxide)/poly(caprolactone) multiblock co-polymers. *Journal of Biomedical Materials Research,* **59**, 273–281 (2002).

[75] Guan J., Sacks M., Beckman E. and Wagner W. R. Biodegradable poly(ether ester urethane)urea elastomers based on poly(ether ester) triblock copolymers and putrescine: Synthesis, characterization and cytocompatibility. *Biomaterials,* **25** 85 (2004).

[76] Abraham G. A., Marcos-Fernandez A. and Roman J. S. Bioresorbable poly(ester-ether urethane)s from L-lysine diisocyanate and triblock copolymers with different hydrophilic character. *J. Biomed. Mater. Res., A* **76**(4), 729–736 (2006).

[77] Guan J, Fujimoto K. L., Sacks M. S. and Wagner W. R. Preparation and characterization of highly porous, biodegradable polyurethane scaffolds for soft tissue applications. *Biomaterials,* **26**(18), 3961–3971 (2005).

[78] Fujimoto K. L., Guan J., Oshima H., Sakai T. and Wagner W. R. In vivo evaluation of a porous, elastic, biodegradable patch for reconstructive cardiac procedures. *Ann. Thorac Surg.,* **83**(2), 648–654 (2007).

[79] Nieponice A., Soletti L., Guan J., Deasy B. M., Huard J., Wagner W. R. and Vorp D. A. Development of a tissue-engineered vascular graft combining a biodegradable scaffold, muscle-derived stem cells and a rotational vacuum seeding technique. *Biomaterials,* **29**(7), 825–833 (2008).

[80] Borkenhagen M. , Stoll R. C., Neuenschwander P., Suter U. W. and Aebischer P. In vivo performance of a new biodegradable polyester urethane system used as a nerve guidance channel. *Biomaterials,* **19**(23), 2155–2165 (1998).

[81] Zhu K. J., Hendren R. W., Jensen K. and Pitt C. G. Synthesis, Properties and Biodegradation of Poly(1,3-trimethylene carbonate). *Macromolecules,* **24**, 1736–1740 (1991).

[82] Pego A. P., Van Luyn M. J., Brouwer L. A., van Wachem P. B., Poot A. A., Grijpma D. W. and Feijen J. *In vivo* behavior of poly(1,3-trimethylene carbonate) and copolymers of 1,3-trimethylene carbonate with D,L-lactide or epsilon-caprolactone: Degradation and tissue response. *J. Biomed Mater. Res., A* **67**(3), 1044–1054 (2003).

[83] Pego A. P., Poot A. A., Grijpma D. W. and Feijen J. Copolymers of trimethylene carbonate and epsilon-caprolactone for porous nerve guides: synthesis and properties. *J. Biomater. Sci. Polym. Ed.,* **12**(1), 35–53 (2001).

[84] Pego A. P, Poot A. A., Grijpma D. W. and Feijen J. Physical properties of high molecular weight 1,3-trimethylene carbonate and D,L-lactide copolymers. *J. Mater. Sci. Mater. Med., 14*(9), 767–773 (2003).

[85] Pego A. P., Vleggeert-Lankamp C. L., Deenen M., Lakke E. A., Grijpma D. W., Poot A. A., Marani E. and Feijen J. Adhesion and growth of human Schwann cells on trimethylene carbonate (co)polymers. *J. Biomed. Mater. Res., A* **67**(3), 876–885 (2003).

[86] Pego A. P., Poot A. A., Grijpma D. W. and Feijen J. Biodegradable elastomeric scaffolds for soft tissue engineering. *J. Control Release,* **87**(1–3), 69–79 (2003).

[87] Pego A. P., Siebum B., Van Luyn M. J., Gallego Y., Van Seijen X. J., Poot A. A., Grijpma D. W. and Feijen J. Preparation of degradable porous structures based on 1,3-trimethylene carbonate and D,L-lactide (co)polymers for heart tissue engineering. *Tissue Eng.,* **9**(5), 981–994 (2003).

[88] Pego A. P., Grijpma D. W. and Feijen J. Enhanced mechanical properties of 1,3-trimethylene carbonate polymers and networks. *Polymer,* **44**, 6495–6504 (2003).

[89] Jie C. and Zhu K. J. Preparation, Characterization and Biodegradable Characteristics of Poly(D, L-lactide-co-1,3-trimethylene carbonate). *Polymer International,* **42**, 373–379 (1997).

[90] Buchholz B. Analysis and Characterization of resorbable DL-lactide-trimethylene carbonate copolyesters. *Journal of Materials Science: Materials in Medicine,* **4**, 381–388 (1993).

[91] Pego A. P, Poot A. A, Grijpma D. W. and Feijen J. *In Vitro* Degradation of Trimethylene Carbonate Based (Co)polymers. *Macromolecular Bioscience*, **2**, 411–419 (2002).

[92] Chen Q., Harding S. E., Ali N. N., Lyon A. R. and Boccaccini A. R. Biomaterials in cardiac tissue engineering: Ten years of research survey. *Materials Science and Engineering R*, **59**, 1–37 (2008).

[93] Fabre T., Schappacher M., Bareille R., Dupuy B., Soum A., Bertrand-Barat J. and Baquey C. Study of a (trimethylenecarbonate-co-epsilon-caprolactone) polymer–part 2: in vitro cytocompatibility analysis and in vivo ED1 cell response of a new nerve guide. *Biomaterials,* **22**(22), 2951–2958 (2001).

[94] Khademhosseini A, Langer R, Borenstein J and Vacanti J. P. Microscale technologies for tissue engineering and biology. *Proc. Natl. Acad Sci.,* U S A **103**(8), 2480–2487 (2006).

[95] Desai T. A. Micro- and nanoscale structures for tissue engineering constructs. *Med. Eng. Phys., 22*(9), 595–606 (2000).

[96] Chatni M. R. The onset of a revolution in drug discovery. *Trends Biotechnol*, **25**(4), 142–144 (2007).

[97] Puleo C. M., Yeh H. C., Wang T. H. Applications of MEMS technologies in tissue engineering. *Tissue Eng.,* **13**(12), 2839–2854 (2007).

[98] Peppas N. A., Hilt J. Z., Khademhosseini A. and Langer R. Hydrogels in Biology and Medicine: From Molecular Principles to Bionanotechnology. *Advanced Materials,* **18**(11), 1345–1360 (2006).

[99] Leclerc E., Furukawa K. S., Miyata F., Sakai Y., Ushida T., Fujii T. Fabrication of microstructures in photosensitive biodegradable polymers for tissue engineering applications. *Biomaterials,* **25**(19):4683–4690 (2004).

[100] Tsang V. L. and Bhatia S. N. Fabrication of three-dimensional tissues. *Adv. Biochem. Eng. Biotechnol,* **103**, 189–205 (2007).

[101] Mizutani M., Arnold S. C. and Matsuda T. Liquid, phenylazide-end-capped copolymers of epsilon-caprolactone and trimethylene carbonate: preparation, photocuring characteristics, and surface layering. *Biomacromolecules,* **3**(4), 668–675 (2002).

[102] Liu V. A., Jastromb W. E. and Bhatia S. N. Engineering protein and cell adhesivity using PEO-terminated triblock polymers. *J. Biomed Mater. Res.,* **60**(1), 126–134 (2002).

[103] Neff J. A., Tresco P. A. and Caldwell K. D. Surface modification for controlled studies of cell-ligand interactions. *Biomaterials,* **20**(23–24), 2377–2393 (1999).

[104] Abrams G. A., Schaus S. S., Goodman S. L., Nealey P. F. and Murphy C. J. Nanoscale topography of the corneal epithelial basement membrane and Descemet's membrane of the human. *Cornea,* **19**(1), 57–64 (2000).

[105] Weiss P. Experiments on cell and axon orientation in vitro: The role of colloidal exudates in tissue organization. *Journal of Experimental Zoology,* **100**(3), 353–386 (1945).

[106] Bettinger C. J., Orrick B., Misra A., Langer R. and Borenstein J. T. Microfabrication of poly (glycerol-sebacate) for contact guidance applications. *Biomaterials,* **27**(12), 2558–2565 (2006).

[107] Fidkowski C., Kaazempur-Mofrad M. R., Borenstein J., Vacanti J. P., Langer R. and Wang Y. Endothelialized microvasculature based on a biodegradable elastomer. *Tissue Eng.* **11**(1–2), 302–309 (2005).

[108] Colton C. K. Implantable biohybrid artificial organs. *Cell Transplant* **4**(4), 415–436 (1995).