POLY(BETA-AMINO ESTERS) FOR CARDIOVASCULAR APPLICATIONS

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The Academic Faculty

by

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POLY(BETA-AMINO ESTERS) FOR CARDIOVASCULAR APPLICATIONS

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To L. W. Safranski,
For feeding me science every afternoon
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<thead>
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<th>Symbol</th>
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<tr>
<td>Tg</td>
<td>glass transition temperature</td>
</tr>
<tr>
<td>E</td>
<td>elastic modulus</td>
</tr>
<tr>
<td>E_r</td>
<td>rubbery modulus</td>
</tr>
<tr>
<td>MMA</td>
<td>methyl methacrylate</td>
</tr>
<tr>
<td>PEGDA</td>
<td>poly(ethylene glycol) diacrylate</td>
</tr>
<tr>
<td>HDDA</td>
<td>hexanediol diacrylate</td>
</tr>
<tr>
<td>3MOPA</td>
<td>3-methoxypropylamine</td>
</tr>
<tr>
<td>NDDA</td>
<td>nonanediol diacrylate</td>
</tr>
<tr>
<td>BDDA</td>
<td>butanediol diacrylate</td>
</tr>
<tr>
<td>I2959</td>
<td>Irgacure 2959</td>
</tr>
<tr>
<td>n</td>
<td>diffusional exponent</td>
</tr>
<tr>
<td>k_1</td>
<td>diffusional constant 1</td>
</tr>
<tr>
<td>k_2</td>
<td>diffusional constant 3</td>
</tr>
<tr>
<td>PLLA</td>
<td>poly(L-lactide)</td>
</tr>
<tr>
<td>PDLA</td>
<td>poly(D-lactide)</td>
</tr>
<tr>
<td>PDLLA</td>
<td>poly(D,L-lactide)</td>
</tr>
<tr>
<td>PGA</td>
<td>poly(glycolic acid)</td>
</tr>
<tr>
<td>PLGA</td>
<td>poly(lactic-co-glycolic acid)</td>
</tr>
<tr>
<td>PCL</td>
<td>poly(caprolactone)</td>
</tr>
<tr>
<td>AAA</td>
<td>abdominal aortic aneurysms</td>
</tr>
<tr>
<td>EVAR</td>
<td>endovascular repair</td>
</tr>
<tr>
<td>MMW</td>
<td>macromer molecular weight</td>
</tr>
<tr>
<td>Wt%</td>
<td>weight percent</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>DMPA</td>
<td>dimethoxyphenylacetophenone</td>
</tr>
<tr>
<td>CQ</td>
<td>camphorquinone</td>
</tr>
<tr>
<td>DSC</td>
<td>differential scanning calorimetry</td>
</tr>
<tr>
<td>DMA</td>
<td>dynamic mechanical analysis</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>M&lt;sub&gt;c&lt;/sub&gt;</td>
<td>molecular weight between crosslinks</td>
</tr>
<tr>
<td>ATR</td>
<td>attenuated total reflectance</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metalloproteinases</td>
</tr>
<tr>
<td>PBAE</td>
<td>poly(β-amino ester)</td>
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SUMMARY

Abdominal aortic aneurysms are a leading cause of death in the U.S. where 14,000 people die from aneurysm rupture and 178,000 are diagnosed each year. A novel alternative treatment for abdominal aortic aneurysms has been proposed, where a biodegradable polymer scaffold is photopolymerized in situ around the exterior of the aneurysm. This scaffold will mechanically constrain the aneurysm from further expansion, and will deliver a drug, doxycycline, to treat the underlying biological cause of the disease. In order for device development, a suitable polymer must be designed with appropriate mechanical properties, degradation rate, polymerization, and elution rate. Poly(β-amino ester) networks have been proposed as the material of choice; however, many of their structure-property relationships have yet to be determined.

Therefore, the overall goal of this work is to determine the structure-property relationships of the poly(β-amino ester) networks in order to advance the design of the treatment, and has been divided into three objectives: (1) understand the structure-property relationships of poly(β-amino ester) networks, specifically the polymerization, degradation rate, and thermo-mechanical properties, (2) determine the impact of doxycycline incorporation on degradation rate and mechanical properties, (3) evaluate the effect of simulated physiological conditions on degradation rate and mechanical properties.

In the initial chapters, the fundamental structure-property relationships are established between reactant chemical structure, step-growth polymerization, photopolymerization, thermo-mechanical properties, and degradation rate using a
systematic approach of two homologous series of reactants. Further tailoring of
degradation rate, water content, and modulus in vitro was performed by using a
copolymer network. Doxycycline inhibited photopolymerization due to overlapping
absorbance spectra with the photoinitiator, but full network formation occurred by
increasing the photoinitiator concentration. Networks displayed varying controlled
release rates, and the underlying release mechanism was determined for each network
using established methods.

In order to increase mechanical properties, a co-monomer, methyl methacrylate,
was added to the network to increase the glass transition temperature, toughness, and
deformation capacity. These co-networks displayed temporal-control of mechanical
properties in simulated physiological conditions, since degradation caused a shift in the
glass transition temperature, which changed the mechanical behavior of the network. The
temporal-control of mechanical properties was further investigated under degradation
conditions in vitro and in vivo. Due to the mechanically active loading environment in
vivo, networks displayed a decrease in toughness, yet maintained mechanical properties
similar to native biological tissues. These networks establish a multifunctional
biomaterials platform with materials that can be easily synthesized, photopolymerized
into various geometries, and sustain mechanical properties while undergoing degradation
and therapeutic agent release.
CHAPTER 1

INTRODUCTION

1.1 Motivation

Abdominal aortic aneurysms (AAA), dilations of the main artery leaving the heart, are the 13th leading cause of death in the U.S and the 10th leading cause of death for men over the age of 55. Nearly 178,000 people are diagnosed with AAA each year, and about 14,000 die from rupture of the aneurysm [1-2]. If rupture occurs, the mortality rate is 30-50% before even reaching a hospital, and 50-70% with surgery. The maximum diameter is the best indicator of rupture, where AAA at 4 cm have a annual rupture rate near 1%, but AAA near 7 cm or larger have an annual rupture rate at 30% or more [3].

The primary repair technique for AAA was open repair in the 20th century, which used a Dacron graft to replace the damaged area. Now endovascular aneurysm repair (EVAR) via small incisions in the groin has become the main choice of surgical procedure because of a lower mortality rate post-operation [4]. The open surgery option has a mortality rate of 4.6% compared to 1.6% of EVAR and longer recovery time. Additionally, the EVAR procedure is prone to repeat surgeries within four years [5]. The market price for an EVAR device is near $10,000, whereas a graft for open surgery is 10 times less.

Unfortunately, neither procedure works for all cases. High risk patients do not undergo open repair and patients with unsuitable anatomy, such as short or damaged segments for anchoring, cannot undergo EVAR [6-9]. There is no proven long term benefit to EVAR, and neither has been ruled as the optimal treatment option [10].

The proposed novel alternative therapy is a biodegradable polymer scaffold that will be photopolymerized in situ around the AAA. Not only would the scaffold provide
mechanical support, but it will be drug eluting to treat the underlying causes of AAA. A medical device of this caliber must meet strict criteria for further development and trials. The proposed scaffold must have suitable mechanical properties to constrain the AAA. The elastic modulus at body temperature must be high enough to contain the aneurysm, but not stiff to a degree to impair proper function. The material must have adequate properties to be pragmatic over a range of temperatures including implantation and \textit{in vivo} conditions. The glass transition temperature needs to be closely monitored if long term storage is to be at sub-ambient temperatures. The polymers’ degradation rate must match that of the required recovery time of the patient. The degradation profile must take into account the rate of drug elution and the mechanical loss of the device. The chemical structure must give both a biocompatible polymer once polymerized and biocompatible degradation products. The chemical structure of the material must allow for minimal swelling as not to put too much pressure on the adjacent anatomy. In addition, the incorporation of AAA-inhibiting drugs into the structure must not impair the mechanical properties or the polymerization time of the scaffold material. The balance of these requirements is necessary for continuation of testing and development.

1.2 Research Objectives

The overall objective of this research was to establish the fundamental structure-property relationships of poly(β-amino ester) networks in order to facilitate the future design of the proposed device. A systematic serial approach was undertaken to understand the key relationships between diacrylate chemical structure, polymerization, degradation rate, and thermo-mechanical properties. The innovation of this work results from the systematic approach to understanding structure-property relationships of poly(β-
amino ester) networks under multiple environments, which will allow rapid design of the desired biomedical device and will expand the scientific field of biodegradable photopolymerizable networks. The research was investigated through three aims:

1. **Understand the structure-property relationships of poly(β-amino ester) networks, specifically the polymerization, degradation rate, and thermo-mechanical properties.**

   Previous studies of poly(β-amino ester) networks established some structure-property relationships, but not all the aspects of the two-step polymerization, step-growth and free radical photopolymerization, were characterized and the network chemistries were not studied in a systematic manner. Therefore, two homologous diacrylate systems were used to investigate the effect of diacrylate chemistry and size on both polymerization steps. This allowed for the relationships between diacrylate chemical structure, polymerization, degradation, and thermo-mechanical properties to be firmly established.

2. **Determine the impact of doxycycline incorporation on degradation rate and mechanical properties.**

   Biodegradable polymers have been used widely as controlled release devices, where the chemical structure, crystallinity, hydrophilicity, network structure, and degradation mechanism influence the kinetics of drug release. Doxycycline is a widely accepted pharmaceutical agent, but has not been used in photopolymerizable biodegradable networks. Systemic administration is unwanted because of possible long-term side effects, thus targeted local delivery is desired through the degradation of the biodegradable polymer. Networks of varying degradation rate and varying doxycycline
concentration were characterized in order to establish relationships between doxycycline concentration, conversion during photopolymerization, mechanical properties, degradation rate, and release rate of the drug.

3. Evaluate the effect of simulated physiological conditions on degradation rate and mechanical properties.

Fundamental relationships between glass transition temperature and mechanical properties have been established for amorphous (meth)acrylate networks, where a maximum in mechanical properties exists when the glass transition temperature should be equal to or slightly above the operating temperature. Also, the effect of immersion in physiological conditions on the thermal and mechanical properties of nondegradable (meth)acrylate networks has been studied, where shifts in glass transition temperature may improve or reduce mechanical properties. The mechanical properties of poly(β-amino ester) networks were not sufficient because the glass transition temperature was far below ambient. The glass transition temperature was increased by adding a co-monomer, methyl methacrylate, during photopolymerization in order to increase the mechanical properties. Further structure-property relationships between methyl methacrylate concentration, poly(β-amino ester) size, glass transition temperature, rubbery modulus, bulk mechanical properties, and degradation were established.
The mechanical properties and elution rates of poly(β-amino ester) networks have not undergone systematic and thorough investigation. In order for biomedical application, devices need to be multifunctional, mechanically and biologically active. Poly(β-amino ester) networks can fill this niche because they can elute an agent to address the biological cause of diseases and be mechanically durable in vivo. Poly(β-amino ester) networks offer several advantages compared to current biodegradable polymers because they are able to undergo photopolymerization, which would allow for in situ polymerization into complex geometries. Also, current biodegradable polymers lose their mechanical properties within the first two to three months after implantation, where
The significance of this study comes from the establishment of structure-property relationships in a systematic manner in order to identify critical parameters for the design of the proposed cardiovascular device. Poly(β-amino ester) networks were investigated under multiple mechanical testing environments, where the chemical composition and glass transition temperature were key in developing the structure-property relationships. These relationships can be further applied to a myriad of other biomedical applications, such as multifunctional orthopaedic devices, *in situ* photopolymerizable drug delivery devices, or tough wound sealants for traumatic injuries. In the following chapters, a meticulous characterization of poly(β-amino ester) network properties is detailed. Discussions will focus on the structure-property relationships, comparisons to similar polymer networks, and the significance in the development of future biomedical devices.
CHAPTER 2

BACKGROUND

2.1 Biodegradable Polymers

During the latter half of the 20th century, biodegradable polymers underwent considerable research and development for biomedical applications. Previously, nondegradable materials were used in implants, thus revision surgeries were needed to extract the implant. The long-term biocompatibility was a concern for these nondegradable implants, thus ceramic and metallic implants are losing popularity because they are nondegradable, where polymeric biomaterials are replacing them and covering 88% of the biomedical materials market [11].

While commercial polymers have been in development since the early part of the 20th century, biodegradable polymers have an added constraint of biocompatibility. Biocompatibility is broadly defined as the ability of a material to elicit an appropriate host response in a given application, a property that must be sustained during the degradation of the implant. Biodegradable materials have further constraints: (1) they must be non-toxic and elicit a low inflammatory response during initial and long-term implantation, (2) degradation rates must match the given healing process, (3) appropriate changes in mechanical properties at implantation site, and (4) produce non-toxic degradation products [11].

Polymeric biomaterials are quite complex due to their range in chemistry, molecular weight, hydrophilicity, mechanical properties, implant shape, degree of swelling, and degradation, where current biodegradable polymers are shown in Table 2.1. This complexity also allows for a tremendous range in properties that are easily tailorable.
for specific applications. Future biodegradable polymers will not only replace the damaged biological function, but will augment healing and repair the damaged tissue.

Table 2.1 Structures of Current Biodegradable Polymers

<table>
<thead>
<tr>
<th>Name</th>
<th>Repeating Unit Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(glycolic acid)</td>
<td>![Poly(glycolic acid)]</td>
</tr>
<tr>
<td>Poly(lactic acid)</td>
<td>![Poly(lactic acid)]</td>
</tr>
<tr>
<td>Poly(caprolactone)</td>
<td>![Poly(caprolactone)]</td>
</tr>
<tr>
<td>Poly(orthoester)</td>
<td>![Poly(orthoester)]</td>
</tr>
<tr>
<td>Poly(anhydride)</td>
<td>![Poly(anhydride)]</td>
</tr>
<tr>
<td>Poly(β-amino ester)</td>
<td>![Poly(β-amino ester)]</td>
</tr>
</tbody>
</table>
2.1.1 Degradation Mechanisms

Biodegradation is the process of degrading bonds that are susceptible to hydrolytic or enzymatic cleavage. Typically, synthetic polymers undergo hydrolytic cleavage and natural polymers undergo enzymatic degradation. This degradation process can be further subdivided depending upon the polymer structure: (1) the biodegradable bond is in a linear polymer backbone, which cleaves into small water soluble products, (2) or a covalently crosslinked network with degradable linkages is degraded forming soluble products, but their size depends on the number of degradable linkages in the network, (3) or polymer solubilization occurs where the polymer swells and dissolves upon reaching a sufficient water content [12]. There are a number of bonds that are susceptible to hydrolytic cleavage: esters, orthoesters, anhydrides, and many others. While biodegradation describes the bond cleavage, erosion describes the complex process of mass loss, which is often simplified into two categories. If mass loss occurs homogeneously throughout the volume of the material, it is classified as bulk erosion. A number of polymers, such as PLLA, PGA, and PCL undergo bulk erosion. If mass loss occurs in a heterogeneous manner dependent on surface area losing mass from the outside to the inside, then it is classified as surface erosion. Polyanhydrides and poly(ortho esters) undergo surface erosion [12-13]. Ideal representations of the two erosion mechanisms are shown in Figure 2.1. Typically, surface eroding polymers display a linear mass loss profile, while bulk eroding polymers display a nonlinear mass loss profile [13-14].
2.1.2 Poly(α-ester)s

Poly(α-ester)s are thermoplastic polymers that have hydrolytically cleavable ester groups in their backbone. This class of biodegradable polymers is the most extensively investigated due to simple synthesis methods and suitable biocompatibility. Poly(glycolic acid) (PGA) is a semicrystalline polymer (40-55% crystallinity) with a high modulus, $T_g$ near 35°C, and $T_m$ near 200°C. It can be readily processed by extrusion, compression, and injection molding to form biomedical devices. PGA is considered one of the first synthetic biodegradable polymers to be used in biomedical applications, first gaining FDA approval as a suture material in 1969. Since it has fiber forming capabilities, it has been used as a non-woven scaffold for tissue regeneration, and as a PGA-fibrin composite as a dermal wound sealant [15]. Due to its high crystallinity, PGA has excellent mechanical properties with a bending strength near 218 MPa and a modulus near 7 GPa, making them suitable for orthopaedic fixation devices. PGA has ideal properties for initial tissue fixation, but within 4 weeks its loses 50% of its strength [16]. Unfortunately, it has a high rate of mass loss within 12 months, limiting its use to short term implant applications. Thus, other copolymers with slower degradation rates have been investigated.
Poly(lactide) has two optical isomers, poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA), which produces semicrystalline polymers (37% crystallinity). The racemic mixture poly(D,L-lactide) (PDLLA) is amorphous due to the random distribution of D-lactide or L-lactide. PLLA has a Tg range from 60 to 65°C and a Tm near 175°C, while PDLLA has a Tg closer to 55°C. Due to differences in crystallinity, PLLA has a modulus near 4 GPa, which is stiffer than PDLLA with a modulus near 1-2 GPa [11, 17]. Often used in orthopedics, PLLA shows more rapid degradation in vivo than in vitro, where most of the load carrying capacity is lost between 6 to 12 weeks of implantation, and only 3-4% load carrying capacity remains by 48 weeks for PLLA fibers [18]. Similarly, PLLA rods lost half their bending strength in 3 weeks and only a quarter remained by 6 weeks [19]. PDLLA has shorter degradation times similar to PGA near 12-16 months due to its amorphous structure. PLLA has extremely long degradation times over 5 years because the more crystalline sections do not completely degrade. This is a cause for concern, where these highly crystalline particles continue to produce a clinically identifiable swelling [20]. By mixing PLLA and PGA in ratios of 20-70% PLLA, the systems are more amorphous and the degradation rate increases, where the time for complete degradation can range from 1-6 months [11-12]. This tailorability enabled further production of biodegradable sutures, skin grafts, tissue scaffolds, and drug delivery devices.

Polycaprolactone (PCL) was originally produced by the Carothers group in the 1930’s [21]. While it was used in biomedical devices for a time, PLLA, PGA, and their copolymers became the biodegradable polymeric biomaterials of choice. PCL is a semicrystalline thermoplastic polyester that is easily processed because it is soluble in
many solvents like toluene, benzene, chloroform, has a low Tm near 60°C, and a Tg near -60°C. PCL has tensile strength near 23 MPa, but its failure strain is greater than 700% [22]. It undergoes hydrolytic cleavage of its aliphatic ester bond, but takes over 2 years to degrade completely depending on the molecular weight of the device. Degradation occurs in two phases where bulk hydrolytic cleavage of the polymer is followed by fragmentation. The fragments ultimately undergo phagocytosis from macrophages and giant cells for PCL under molecular weight 3000 [23-24]. This long degradation time is unappealing as well as the intracellular degradation mechanism compared to PGA and its copolymers. Also, PCL does not have the mechanical properties necessary for orthopaedic applications. However, tissue engineering has been a rapidly growing field and PCL has become a favorite candidate material for a number of scaffolds because of its ease in processing due to low Tm, high ductility, and biocompatibility [11, 21, 25-26].

2.1.3 Poly(ortho esters) and Poly(anhydrides)

Hydrophobic polymers with hydrolytically cleavable bonds that undergo surface erosion are further investigated in order to further control the release of drugs from delivery vehicles. Poly(ortho esters) were developed by ALZA to meet the demands of controlled release devices. These polymers are highly tailorable, where the degradation rate, pH sensitivity, and Tg can be controlled independently. While there are four classes of poly(ortho esters), class IV has the most potential for development and commercialization [11]. Initially, class I underwent autocatalytic hydrolysis due to acidic degradation products, but further modifications to the remaining classes addressed this degradation issue. Class IV has a very tailorable degradation rate range due to incorporation of glycolic or lactic acid linkages, which purposefully autocatalyze the
orthoester linkages [27]. Poly(ortho esters) have been used in a number of drug delivery devices, including the treatment of periodontal disease [27-28].

Polyanhydrides have been thoroughly characterized as surface eroding polymers, where the ester group is highly cleavable, but the polymer is quite hydrophobic. This allows near true surface erosion to take place. They were originally investigated by Langer in the 1980’s as drug delivery devices [29]. While polyanhydrides can be synthesized into homopolymers like poly(sebacic anhydride), they often have a hydrophobic aromatic comonomer, like poly((carboxy phenoxy propane)-(sebacic acid)) to tailor the degradation rate from days to years. The popularity of this polymer is due to its zero-order drug release profile and biocompatible degradation products [11, 30]. Anseth has further developed polyanhydrides into photopolymerizable crosslinked networks by adding methacrylate groups to anhydride oligomers [31-34]. These networks display tailorable degradation via altering ratio of methacrylated poly(carboxy phenoxy propane) and methacrylated poly(sebacic acid), tailorable mechanical properties by controlling crosslinking density, and in situ photopolymerization. Since surface erosion occurs, the loss in mechanical properties was reduced, where more than 70% of the modulus was maintained with 50% mass loss [34].

2.1.4 Poly(β-amino ester) Networks

Poly(β-amino ester)s are a relatively new class of biodegradable polymers, which have gained recent interest in the last decade. Originally developed for gene delivery, crosslinked poly(β-amino ester) networks are now being investigated as tissue scaffolds, load-bearing implants, and drug delivery devices.
2.1.4.1 Network Synthesis

Poly(β-amino ester) networks are synthesized through a two-step process of a step-growth polymerization to form macromers, then a free-radical photopolymerization of the macromers into a crosslinked network. The networks degrade via hydrolytic cleavage of the ester bond into bis(amo) acids, diols, and poly(acrylic acid) chains (Figure 2.2).

Figure 2.2 Two-step polymerization and degradation of poly(β-amino ester) networks.

A diacrylate and a primary amine or secondary amine are reacted together by a 1,4-conjugate addition, where the diacrylate is kept in molar excess in order to produce
macromers with acrylate endgroups. A large library of poly(β-amino ester) networks have been characterized through a semi-automated high-throughput screening method [35]. Further studies investigated the cytotoxicity, degradation, and efficiency in gene delivery [36-41]. As the diacrylate to amine ratio increases away from 1:1, the macromer molecular weight (MMW) decreases (Figure 2.3) [36] (used with permission). This allows macromers of varying size to be produced, but adds excess unreacted diacrylate to the sample as the diacrylate to amine ratio increases.

![Figure 2.3 Effect of reactant ratio on molecular weight for poly(β-amino ester)s.](image)

The free-radical photopolymerization is carried out in a manner like other acrylate networks, where a photoinitiator is cleaved by a UV light source and starts the initiation reaction of the chain-growth polymerization. Since all the molecules are theoretically difunctional, a heavily crosslinked network is formed. The rate of photopolymerization and degree of conversion increased as the diacrylate to amine ratio increased because of
the excess diacrylate groups and lower macromer molecular weight. The sol fraction decreases as diacrylate to amine ratio increases, thus supporting the lack of conversion at low diacrylate to amine ratios [42].

2.1.4.2 Network Properties

The semi-automated process was used to synthesize 120 diacrylate-terminated macromers at a diacrylate to amine ratio of 1.2:1. Degradation profiles showed a range of degradation times from less than 24 hours to over 90 days. The elastic modulus was evaluated by a rapid nanoindentation technique which produced values from 4 to 350 MPa for 79 of the 120 materials [35]. Select networks were studied to determine the influence of MMW on network properties. Since the MMW decreases as diacrylate to amine ratio increases, the crosslinking density increases, thus the modulus of the materials increases (Figure 2.4) [42] (used with permission).

![Figure 2.4 Tensile modulus as a function of diacrylate to amine ratio for three poly(β- amino ester) networks.](image-url)
The modulus decreases as the networks degrade, where the rate of modulus loss is composition dependent. The degradation rate decreases as MMW decreases due to a higher degree of crosslinking and lower number of hydrolytically cleavable bonds because excess diacrylate, which is nondegradable [42]. Tailoring of the modulus has been further investigated with the use of a triacrylate to increase the modulus by increasing the crosslinking density, which allowed enhancement of the mechanical properties of porous tissue scaffolds [43]. Poly(ethylene glycol) based poly(β-amino ester) networks underwent in vitro cyclic loading, which resulted in sample failure before significant mass loss could occur [44].

2.1.5 Controlled Release

The release characteristics of a drug from a polymer are dependent upon a complex interaction of factors: degradation, erosion, hydrophilicity, polymer-drug interaction, polymer structure, and device geometry. Delivery devices are often categorized as reservoir or monolithic matrix structures. Reservoir devices have a deposit of drug surrounded by a polymer coating, and monolithic matrix devices have the drug uniformly distributed throughout the volume of the polymer [12]. Common geometries for drug delivery vehicles include microspheres, cylindrical implants, or polymeric gels for topical application. The drug release from a polymer matrix can be controlled by varying mechanisms: diffusion through the polymer matrix, degradation of the polymer, or swelling-controlled where the diffusion of water transports the drug [12-13]. These classifications are useful for understanding the ideal scenarios, but in reality a complex combination of mechanisms occurs because the mechanism may change as the polymer degrades. The most commonly observed release profiles are shown in Figure 2.5 [12]
The zero-order release shows a constant rate of release over time, corresponding to a surface erosion mechanism. The first order release shows a decreasing release rate, which is consistent with a diffusion controlled device. The burst profile results from unincorporated drug or drug on the surface of the device. An initial burst effect is common for the previous release profiles as well, but to a lesser extent. The biphasic release, characteristic of PLGA microspheres, shows (1) an initial release due to drug diffusion from the surface and open channels, (2) a lag phase due to moderate release, and (3) then a secondary release due to further degradation of the polymer [45].

Figure 2.5 Common drug release profiles from biodegradable devices.
Peppas et al developed a number of simple mathematical expressions to describe the release mechanisms of biodegradable polymer systems [46-51]. The most widely used would be Equation 1, which is often referred to as the Ritger-Peppas equation and is based on the power law model. $M_t$ is the mass release at time $t$, $M_\infty$ is the mass release at infinite time, $k$ is a kinetic constant, $t$ the time, and $n$ is the diffusional exponent, which can range from 0.5 to 1.0 for slab geometries. If $n$ is near 0.5, then the release has classic Fickian diffusion behavior, where the transport of water through the network is faster than the relaxation of polymer chains. If $n$ is near 1.0, then the release has Case II transport behavior, where the release rate is independent of time and controlled by the relaxation of the polymer chains. If $n$ lies between 0.5 and 1.0, then it is termed non-Fickian anomalous transport, which is a mixture Fickian and Case II transport behavior when both diffusion and polymer relaxation contribute to the release. Equation 2.1 has been further modified to Equation 2.2 to account for the initial burst, which is seen in many polymer systems, where $b$ is the burst constant.

$$\frac{M_t}{M_\infty} = kt^n$$

Equation 2.1

$$\frac{M_t}{M_\infty} = kt^n + b$$

Equation 2.2

The contribution of Fickian and Case II transport to the release rates can be determined through Equation 2.3, the Peppas-Sahlin equation, where the first term on the right side is the Fickian component and the second term is the case II component. $m$ is a purely Fickian diffusional exponent, which is determined from sample aspect ratio and
diffusional exponent, \( t \) the time, \( M_t \) and \( M_\infty \) as previously described, \( k_1 \) and \( k_2 \) are kinetic constants for Fickian and case II transport, respectively [50, 52].

Equation 2.3. \( \frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m} \)

PCL, when chemically modified to enhance degradation, shows geometry-dependent drug release where rate increases in the following manner: film > plate > rod. The diffusional exponent was near 0.5 for slab geometry, thus Fickian diffusion was present for small molecules. Macromolecules with molecular weight near 4400 g/mol were degradation-limited as the elution profile had changed compared to the small molecules release [53]. Crosslinked polystyrene displayed anomalous transport characteristics (0.5 < \( n \) < 1.0), which was dependent on the degree of crosslinking and temperature [49]. Implants composed of calcium phosphates, PDLLA, and the antibiotic ciprofloxacin have been shown to exhibit release rates that decrease as mechanical load and PDLLA content increase. They exhibited anomalous release characteristics. If implants were made without PDLLA, then the erosion mechanism was dominant (\( k_2 > k_1 \)) and the contribution of the diffusion mechanism was negligible [54].

2.1.6 Applications of Biodegradable Polymers

The first biodegradable polymer used in a FDA approved device was the DEXON® suture in 1969, which was made of PGA. In 1971, PLLA was approved for use in sutures in order to improve upon the DEXON® suture [11]. Other sutures by Ethicon, the Vicryl family, are composed of a PLGA copolymers and Monocryl is composed of a PGA-PCL mixture. While sutures were the first devices developed, biodegradable polymers have had a tremendous impact on the orthopaedic market. PLA
and its copolymers have been the most popular due to their high crystallinity and high modulus. Arthrex produces several devices, such as the Bio-Tenodesis interference screw for tendon transfer, the Bio-Corkscrew suture anchor for rotator cuff repair, and the Full Thread Bio Interference Screws for ACL reconstruction.

Drug delivery vehicles have used a variety of biodegradable polymers for targeted release. CAPRONOR®, a PCL based device, delivers levonorgestrel as a long-term contraceptive [21]. The LUPRON DEPOT®, a PLGA based device, delivers a hormone therapy for treatment of prostate cancer or endometriosis. GLIADEL®, a polyanhydrides based device, delivers chemotherapy agents against brain cancer. While the orthopaedic market and drug delivery markets are established and filled with biodegradable polymer-based devices, the tissue engineering market is still emerging. Dermagraft skin replacements are using a Vicryl mesh as a scaffold structure and PLGA-co-collagen scaffold are used for treating periodontal defects (CYTOPLAST Resorbs®).

2.2 (Meth)acrylate Networks

(Meth)acrylate networks have been proposed for biomedical applications due to their ability to photopolymerize into complex shapes under UV or visible light, enabling their use in minimally invasive procedures. Their polymerization and thermo-mechanical properties have been well studied, where the main focus has been on tailoring network properties.

2.2.1 Synthesis

(Meth)acrylates undergo a free radical chain-growth polymerization, which is photoinitiated by UV or visible light. Multifunctional acrylates, called crosslinkers, have at least two acrylate groups and are used to create covalently bonded network points.
Monofunctional acrylates are sometimes referred to as linear chain builders because they create the linear chains between the crosslink points, and also decrease the crosslinking density. The most widely used photoinitiators are 2,2-dimethoxyphenylacetophenone (DMPA) Irgacure 2959 (I2959), and camphorquinone (CQ), where I2959 has been found to be the most cytocompatible [55]. These networks can range from very soft, low Tg hydrogels to highly crosslinked brittle networks for dental applications [56-60]. Photopolymerization of these materials has not only been tested on the benchtop, but explored in situ as well [31, 33, 61].

Kinetic models and experimental research have defined the structure of acrylate networks formed by free radical polymerization [62-66]. The three components that affect the rate of polymerization for multifunctional acrylates is shown in Figure 2.6 [63] (used with permission). At the start of the reaction, the rate is very rapid, but eventually slows. This rate change is referred to as autoacceleration, where the radical’s mobility is reduced because of limited diffusion, thus decreasing the termination rate. As the radical concentration increases, the polymerization rate increases. Finally, the polymerization rate obtains a maximum, and starts to decrease. This is called autodecceleration, where the crosslinked network limits the diffusion of the propagation reaction.
Difunctional monomers and monomers of greater functionality than two have different polymerization profile, where difunctional monomers are more reactive due to lower viscosities driven by lower individual molecular weight [64]. In particular, the average number of double bonds reacted per monomer has been shown to be one at maximum conversion for difunctional monomers [63].

Effects of light intensity, temperature, and concentration have been studied in thicker films where heat and mass transfer were taken into account as shown in Figure 2.7 [66] (used with permission). For thick samples, the light intensity decreases into the film, thus decreasing the polymerization rate. The polymerization reactions occur faster in the first stage before autoacceleration starts because of the exothermic reaction and heat from the light source. The thicker samples retained heat, thus allowing for greater conversion because of the increased molecular mobility and propagation kinetics.
2.2.2 Thermo-mechanical Properties

For crosslinked amorphous polymer networks, the glass transition temperature is the temperature at which the material transitions from a glassy state to a rubbery state. Below this temperature, the polymer chains have relatively limited mobility, mainly sidegroup motion. Above this temperature, the polymer chains can undergo long range segmental motion, which allows for large deformation capacity in rubbers. Tg has been explained in several phenomenological ways: kinetic effects due to changes in cooling rate and free volume or as second order thermodynamic transition [67-73].

Methacrylate copolymer networks represent a class of polymers that can exhibit a wide range of mechanical properties from rubbery gels to brittle glasses. Mostly amorphous, these networks’ thermomechanical properties are mainly governed by two parameters, the glass transition temperature (Tg) and the crosslinking density [35, 57, 74-76]. General relationships between chemical structure and the Tg exist where long,
flexible sidegroups decrease Tg and bulky, close sidegroups increase Tg by increasing steric hindrance. Also, the crosslinking density can influence the elastic modulus of the network and can be changed by altering the functionality and molecular weight of the crosslinker [35, 56].

The Tg for copolymers and networks has been thoroughly investigated [77-81]. The Tg of copolymer systems can be predicted using various methods, where DiMarzio and Gibbs used a simple rule of mixtures seen in Equation 2.4, where $w_1$ and $w_2$ are the weight percents and $T_{g1}$ and $T_{g2}$ are the glass transition temperatures of monomer 1 and monomer 2, respectively [81].

Equation 2.4 $T_g = w_1T_{g1} + w_2T_{g2}$

The Fox-Flory relationship has been amended by Fox and Loshaek to account for a wider range of molecular weight in Equation 2.5, where $T_{g\infty}$ is the glass transition temperature at infinite molecular weight, $K'$ and $K''$ are constants, and $M_n$ is number average molecular weight [79].

Equation 2.5 $T_g = T_{g\infty} - \frac{K'}{K'' + M_n}$

While these equations are applied to linear thermoplastics of large molecular weight, $M_n \approx 10^5$, crosslinking should also be taken into account. By adding another parameter, the increase in Tg due to crosslinking is taken into account in Equation 2.6, where $K$ and $K_x$ are constants and $\rho_c$ is the number of crosslinks per gram [79].

Equation 2.6 $T_g = T_{g\infty} - \frac{K}{M_n} + K_x\rho_c$

Two common methods for measuring Tg are differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA). The DSC measures changes in the heat flow of the sample compared to the changes in heat flow of an empty reference in order
to ascertain changes in the heat capacity of the material, where the differential heat flow profile is shown in Figure 2.8. The Tg is represented as an endothermic step, which is due to a change in the heat capacity of the material, a second order thermal transition. Melting and crystallization are recognized as first order transitions in heat flow with peaks, where melting is endothermic and crystallization is exothermic.

![Representative DSC profile showing Tg, crystallization, and melting of a polymer.](image)

In contrast to DSC, DMA takes a thermomechanical approach in measuring Tg. DMA can either be force controlled or strain controlled, where a strain controlled system was used for this study. A sinusoidal strain at a constant frequency is applied to a sample, and the load is measured while the sample is undergoing controlled heating or cooling. A representative DMA plot is shown in Figure 2.9, where the thermoset material exhibits a glassy region at low temperatures, a reduction in storage modulus during its glass transition in the viscoelastic regime, and a plateau in storage modulus in the rubbery regime.
Figure 2.9 Representative DMA plot of a thermoset with a rubbery plateau of the storage modulus ($E_r$) and a peak of the tan delta ($T_g$).

Viscoelastic properties are calculated from this oscillatory strain and the load measured in order to calculate storage modulus (elastic) and loss modulus (viscous). The tan delta is the ratio of the loss modulus to the storage modulus, a measurement of damping, or can be represented as the phase lag in the modulus [82]. $T_g$ is defined as the temperature when the tan delta reaches a maximum. In this regard, $T_g$ measured from DMA is now defined in a thermomechanical manner, and thus does not always correspond with that measured through DSC techniques, but in general a range of $T_g$ exists. Also, if the thermal transition is very broad and does not display a step in heat flow, then DSC may not have adequate sensitivity to detect the thermal transition, thus using both techniques in combination is recommended. DMA can also be used to infer the structure of the crosslinked network from the rubbery modulus. The effect of crosslinker concentration on rubbery modulus ($E_r$) has been previously determined in acrylate networks, where increasing the amount of crosslinker increases the $E_r$ as defined by the rubber elasticity theory and shown in Equation 2.7 [83-85]. The molecular weight between crosslinks can
be defined from Equation 2.7, where $E$, $\rho$, $R$, $T$, and $M_c$ is the modulus, density, gas constant, temperature in Kelvin, and the molecular weight between elastically effective chains, respectively.

Equation 2.7 \[ E = \frac{3\rho RT}{M_c} \]

A network’s heterogeneity should also be considered because multi-functional acrylates create highly crosslinked regions [76, 86-88]. These highly crosslinked regions are called “microgels”, but unreacted monomer areas can occur as well, thus leading to a wide distribution of mobilities. The distribution of mobilities or relaxation times can be revealed in dynamic mechanical behavior through the tan delta [76].

2.2.3 Mechanical Properties

The mechanical properties of crosslinked (meth)acrylate networks are highly temperature dependent because chain mobility and energy dissipation are temperature sensitive. Typically, three regimes of mechanical behavior are found in these polymers: glassy, viscoelastic, rubbery, as shown in Figure 2.10. In the glassy regime, the Tg is higher than the operating temperature, thus chain mobility is restricted. These polymers exhibit a high modulus with no yielding behavior since large chain movements are not possible. In the viscoelastic regime, the operating temperature is near the Tg, and the polymer has a moderate modulus with defined regions of elastic and plastic deformation. In the rubbery regime, the operating temperature is far above the Tg, and the polymer exhibits large strain capacity, low modulus, and mostly elastic deformation.
Figure 2.10 The three regimes of temperature dependent mechanical behavior of crosslinked networks.

Toughness is an important parameter that is not often considered in regards to developing polymer-based biomaterials that must undergo extensive mechanical loading and deformation in the in vivo environment. Intrinsic toughness is broadly defined as the energy required to induce failure of a material, which can be measured by the area under a stress-strain curve. While matching the modulus of the implant and native tissue is important, load-bearing biomaterials must be tough in order to survive the in vivo environment and not fail prematurely. Previous ways of improving mechanical properties, including toughness, of crosslinked networks have included using particle reinforcement, lowering crosslinking density, hyperbranching, or using double networks [89-92].

Understanding the intrinsic structure-property relationships that control mechanical properties is critical for developing biomedical materials based on (meth)acrylate networks. A number of studies have established relationships between
network structure, chemistry, failure strain, toughness, and hydrated conditions [93-98].
It is known that increasing the crosslinking density will increase the modulus, thus
increasing the strength of the material. These studies focused on other bulk mechanical
properties such as the failure strain (deformation capacity) and toughness of
(meth)acrylate networks. Ortega investigated a series of networks with a constant linear
chain builder and several dimethacrylates. The failure strain had an inverse relationship
with the crosslinker concentration (rubbery modulus). As the networks became stiffer,
they were unable to have large deformation capacity [93]. The networks investigated
showed the same trend due to the use of the constant linear chain builder. Another study
investigated the other possibility of network formation by using a constant crosslinker
and five linear chain builders [95]. Failure strain and rubbery modulus were still inversely
related to each other, but the type of linear chain builder had an influence on the failure
strain below a certain rubbery modulus. Two regimes were established where at high
crosslinking densities the linear chain builder’s chemical structure had no influence on
the failure strain, but at low crosslinking densities the linear chain builder’s chemical
structure had significant impact on the failure strain. Also, the mechanical properties
were examined as functions of temperature relative to the Tg. A peak in failure strain and
toughness was seen at a temperature below the Tg, which was also dependent on the
LCB’s chemical structure. This peak in failure strain was further investigated by Yakacki
et al [94]. The deformation peak decreased in strain capacity as the crosslinking density
increased, but did not change in temperature space as long as the Tg remained constant.

Smith et al has thoroughly investigated the relationship between toughness, Tg,
and hydrated conditions. Smith’s seminal work described relationships between
toughness and Tg, crosslinking density, network chemistry, and the short-term effects of phosphate buffered saline (PBS). In general, (meth)acrylate networks display a peak in toughness below Tg in air, where networks with low crosslinking densities typically display higher toughness. The absorption of water into the network structure decreases the Tg, thus changing the relative position of the toughness peak in relation to the operating temperature as shown in Figure 2.11 [97] (used with permission).

![Figure 2.11 Temperature-dependent toughness peak that shifts when hydrated.](image)

Further (meth)acrylate networks were designed to tailor Tg by using the copolymer effect and tailoring hydrophilicity. These networks displayed a toughness peak under hydrated conditions (1 week) when the operating temperature matched the Tg, thus it is necessary to match the Tg under wet conditions to the operating temperature to maximize toughness [96]. Recently, long term effects of immersion on (meth)acrylate networks displayed composition dependent trends. Glassy networks become more brittle over 9 months due to an increase in Tg, but networks that are initially viscoelastic maintain their properties due to a constant Tg after several months. Networks composed
of a diol dimethacrylate that was more hydrophobic displayed a toughness profile close to UHMWPE [98]. These studies have highlighted the importance of temporal control of toughness due to shifts in Tg for nondegradable (meth)acrylate networks under immersed conditions.

2.3 Abdominal Aortic Aneurysms

The aorta is the main blood vessel carrying blood away from the heart to the lower extremities. AAA often occurs below the kidneys, but above the iliac arteries, which carry blood to each leg as shown in Figure 2.12. The aorta wall is made of three layers: the intima, media, and adventitia. The intima is the innermost layer, which is made of one layer of endothelial cells. The media, the elastin rich middle layer, is surrounded by the adventitia, the collagen rich outermost layer as shown in Figure 2.12.

![Figure 2.12](image_url)

Figure 2.12 (top) AAA formation site. (bottom) structure of aorta.
Because 75% of patients with AAA are asymptomatic, routine examination is usually the best detection method. Ultrasound and CT scanning are methods commonly used to detect and monitor AAA. Once detection of the AAA has occurred, the AAA will be monitored to determine the size and rate of expansion. The main cause of AAA rupture is the decrease in wall strength, thus allowing for expansion and eventual rupture [3]. It is known that as the diameter increases, the risk for rupture increases. The relationship between expansion rate and rupture rate has not been fully understood and is still debated.

Factors that increase the risk of having AAA include smoking, male sex, older age, hypertension, and family history. Smoking has been shown to be a risk factor for developing AAA and expansion, but evidence has been conflicting on how it relates to AAA rupture. Whereas men are more than twice as likely to develop AAA at an earlier point in their lives than women, women are more prone to rupture and have a higher mortality rate if rupture occurs. Also, the criteria of critical diameter should not be the same for men and women. Another risk factor is hypertension, where the increased blood pressure will exacerbate the increased stress of the vessel wall.

The formation of AAA is caused by the remodeling of the connective tissue of the aortic wall [3]. Initially, there is an inflammatory response of monocytes and T-lymphocytes that releases pro-inflammatory cytokines, which in turn activates proteolytic enzymes [99]. The major enzymes are matrix metalloproteinases (MMP), which break down elastin and collagen. The degradation of elastin is combined with a decrease in elastin synthesis because the vascular smooth muscle cells are undergoing apoptosis. This combination leads to a drop in elastin concentration, thus allowing for expansion of the
blood vessel because elastin supports the load of physiological pressures. Due to the expansion and increased wall stress, the collagen synthesis and concentration increase [100]. If the collagen concentration remains stable, the AAA will remain the same size. If one of the above mentioned risk factors triggers a further increase in MMP concentration, the collagen synthesis and concentration will continue to decrease. When the degradation rate is greater than the collagen synthesis rate, the adventitia eventually fails, and the AAA ruptures[3].

Mouse models have been developed where apolipoprotein-E deficient mice are administered angiotensin II (AngII) and an atherogenic diet to induce hypertension and rapid atherosclerosis via subcutaneous osmotic mini-pumps [101-102]. These mice lack the gene to produce a protein called apolipoprotein-E (Apo-E), which processes fats and cholesterol from the bloodstream to the liver. This particular mouse model has seen some advantages because it is similar to humans where male mice develop AAA twice as often at female mice and AAA develops in a similar process. Within days there is a large inflammatory response in the media coupled with a degradation of the surrounding elastin. It is not clear whether the elastin degradation or the macrophage accumulation is the cause, but an increase in MMP activity is present. The broad spectrum antibiotic, doxycycline, has been shown to be a MMP inhibitor, thus decreasing the occurrence and severity of AAA formation [103]. The occurrence of AAA dropped from 86% to 35% when mice were administered doxycycline [104].

The biomechanical properties of healthy and aneurismal aorta have been extensively studied [105-109]. The aorta is not a mechanically uniform structure, where the circumferential modulus ranges from 50-200 kPa along the length of the aorta [107].
Aneurysms are mechanically different from healthy tissue, where stiffening occurs due to atherosclerosis and hypertension in Apo-E deficient mice that have been administered AngII. The blood vessels stiffen nine fold as compared to control even before aneurysm formation [109]. Marfan syndrome is a genetic mutation that promotes the development aneurysms in the aortas of mice. A four-fold increase in elastic modulus of the aorta was seen in the mice with aneurysms from 3 to $12.5 \times 10^6$ dyn/cm$^2$ [105]. Other means of inducing blood vessel damage have been explored. The effect of smoking on aorta mechanical properties was performed on mice, where an equivalent of 1 pack per day was the treatment. After 6 and 16 weeks, the circumferential elastic modulus had increased from 150 to 200 kPa for the control and treated mice, respectively. The increased stiffness of the aorta as it undergoes vascular disease is a pivotal characteristic to consider in the future design of biomaterials.
CHAPTER 3

EXPERIMENTAL

3.1 Materials

The diacrylates used in this study were: 1,4-butanediol diacrylate (BDDA) (Dajac Labs), 1,6-hexanediol diacrylate (HDDA), 1,9-nonanediol diacrylate (NDDA) (TCI America), and poly(ethylene glycol) diacrylate (PEGDA) of four varying number average molecular weight, $M_n$~258, 302, 575, and 700. The primary amine was 3-methoxypropylamine (3MOPA). Methyl methacrylate (MMA) was used in select networks. Doxycycline hyclate and rhodamine B were used in the release studies. The photoinitiator was 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone, Irgacure 2959 (I2959). All chemicals were used as received from Sigma, unless otherwise noted. These structures are given in Table 3.1.

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-butanediol diacrylate</td>
<td><img src="image1" alt="Structure" /></td>
</tr>
<tr>
<td>1,6-hexanediol diacrylate</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td><strong>Table 3.1 Continued</strong></td>
<td><img src="image1.png" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>1,9-nonanediol diacrylate</td>
<td><img src="image2.png" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Poly(ethylene glycol) diacrylate</td>
<td><img src="image3.png" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>n=3, 4, 10, 13</td>
<td></td>
</tr>
<tr>
<td>3-methoxypropylamine</td>
<td><img src="image4.png" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Doxycycline</td>
<td><img src="image5.png" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Rhodamine B</td>
<td><img src="image6.png" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>I2959</td>
<td><img src="image7.png" alt="Chemical Structure" /></td>
</tr>
</tbody>
</table>
3.2 Methods

3.2.1 Synthesis

In general, the step-growth polymerization followed this method: a diacrylate was mixed with 3MOPA at a desired molar ratio and the reaction occurred for 24 hours at 200 rpm at 90°C on a JKEM reaction block (RBC-20 with BTS-1500 shaker) to form the macromers. The range of diacrylate to amine ratio was 1.05:1 to 1.25:1. For specific systems, two diacrylates, PEGDA and HDDA, were mixed together before the addition of the amine, where molar ratios of 0:100, 10:90, or 25:75 PEGDA: HDDA were used. If a number is not stated after PEGDA, it is the 700 g/mol diacrylate.

For networks composed of difunctional macromers, 0.5 wt% I2959 was mixed prior to photopolymerization. The macromers were placed into a 5 cm x 6 cm x 0.1 cm teflon mold, sealed with glass slides, and polymerized for 30 minutes by a UVP Blakray lamp (≈ 10mW/cm²) to form chemically crosslinked networks. Due to the inherent heterogeneity, the materials were synthesized and all tested in triplicate, where mean ± standard deviation is reported.

For networks composed of macromer and MMA, macromers were mixed with varying wt% MMA, then 0.5wt% I2959 was mixed prior to photopolymerization. The solution was injected into a mold made of glass slides with 1 mm thick glass spacers and polymerized for 45 minutes by a UVP Blakray lamp (≈ 10mW/cm²) to form chemically crosslinked networks. Due to the inherent heterogeneity, the materials were synthesized and all tested in triplicate, where mean ± standard deviation is reported.

For networks with additional components, such as doxycycline or rhodamine B, the component was added after the photoinitiator, stirred and sonicated, where the
concentration of the components ranged from 0 mg/ml to 5.0 mg/ml. The polymerization time was dependent on the presence of MMA, where if MMA was present, 45 minutes was given and if MMA was not used, then 30 minutes was given.

3.2.2 Chemical Characterization

3.2.2.1 ATR-FTIR

A Nicolet Nexus 870 FTIR with attenuated total reflectance module (Pike Technologies MIRacle) was used to characterize the step-growth and free-radical polymerization. Macromer samples were taken at 2, 4, 8, 16, and 24 hours from the reaction block and the acrylate peak at 812 cm$^{-1}$ was monitored [110]. After 24 hours, the samples were mixed with 0.5 wt % I2959 and polymerized with the UVP Blakray lamp. The data collection was taken in real time for at least 5 minutes to ascertain the degree of conversion to a network from the peak at 812 cm$^{-1}$.

A FTIR (Bruker Vector 22) with ATR attachment (Pike Technologies MIRacle) with ZnSe crystal was used to characterize the chemical changes in the networks as degradation occurred. Solid samples were taken from in vitro or in vivo degradation conditions at 0, 2, and 8 weeks, then dried for 24 hours and measured from 1800 to 1000 cm$^{-1}$, specifically looking for changes in the ester, ether, and carboxyl bonds [111-112].

3.2.2.2 NMR

A Varian Mercury Vx 400 $^1$H NMR was used to verify the structures of the macromers after step-growth polymerization in deuterated chloroform. The spectrum was analyzed via MestRe-C software to determine the number of hydrogen as well as an estimate of the diacrylate to amine ratio, and therefore the molecular weight [42].
3.2.2.3 Sol Fraction

A sol fraction test was employed to determine the extent of conversion in network formation. Tert-butyl benzene was used, where 1 cm² squares cut from 1 mm thick sheets were soaked for 48 hours with a change in solvent at 24 hours. The samples were dried in an oven with dessicant to remove all traces of solvent and then allowed to equilibrate with the surrounding atmosphere for 3 days. The sol fraction is defined through Equation 3.1, where \( M_f \) is the final mass and \( M_i \) is the initial mass.

\[
\text{SolFraction} = 1 - \frac{M_f}{M_i}
\]

3.2.3 Thermo-Mechanical Characterization

3.2.3.1 Dynamic Mechanical Analysis

Dynamic mechanical analysis (DMA) of each network was performed via a TA Instruments DMA Q800. Rectangular samples were run in tension under strain control of 0.1% according to the following protocol: equilibrate at -100°C, isotherm for 2 minutes, ramp 3°C per minute to 100°C. For samples with MMA, the final temperature was extended 200°C. The glass transition temperature (Tg) was defined at the peak of the tan \( \delta \) curve. The rubbery modulus was defined as the modulus in the rubbery plateau, 75°C above the Tg. The mean ± standard deviation with \( n = 3 \) is given for each data point. The molecular weight between crosslinks was calculated from Equation 2.7 [113].

3.2.3.2 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) (TA Instruments Q100) was used to determine the glass transition temperature (Tg) of the networks under both degradation conditions after drying for 24 hours. The thermal sequence was to equilibrate at -90°C, isotherm for 2 minutes, then ramp at a rate of 3°C/min to 100°C. For samples with MMA,
the final temperature was extended 200°C. Tg was determined from the intersecting line method of the midpoint of the second order thermal transition. The mean ± standard deviation with n = 3 is given for each data point.

3.2.4 Bulk Mechanical Characterization

3.2.4.1 Standard Method

Strain to failure tensile tests were performed on a MTS Insight 2 with a 100N load cell using ASTM D-638 Type IV half-sized dog bone samples at a strain rate of $10^{-3}$ s$^{-1}$. A thermal chamber (Thermcraft, Inc., model LBO-14-8-5.25-1X-J8249_1A) was used to test samples in a dry atmosphere at 37°C. Toughness was calculated using the trapezoidal rule from the area under the stress strain curve and given in MJ/m$^3$. Modulus was determined from the slope of the initial linear region of the stress-strain curve. The mean ± standard deviation with n=4 is given for each data point.

3.2.4.2 Environmental Testing: Compression

Cylindrical samples, 1cm (d) x 2 cm (h), were soaked in phosphate buffered saline (PBS), pH 7.4, in an incubator at 37°C over 8 weeks. Bulk compression testing of each network was performed on a MTS Insight 2 with a 100N load cell and custom environmental chamber, which was filled with PBS and held at 37°C. Samples were compressed at a rate of $10^{-3}$ s$^{-1}$ until failure. Modulus was determined from the slope of the initial linear region of the stress-strain curve.

3.2.4.3 Environmental Testing: Tensile

Strain-to-failure tensile tests were performed on a MTS Insight 2 with a 100 N load cell. For samples degraded in vitro, ASTM D-638 Type IV half-sized dog bone samples were pulled at a strain rate of $10^{-3}$ s$^{-1}$ in a custom-built environmental chamber.
filled with PBS at 37°C. Dog bones were soaked in PBS and held at 37°C in an incubator for up to 8 weeks then tested. For *in vivo* degradation, samples followed ASTM D-638 Type IV, but were reduced to quarter-sized dog bone samples in order to fit subcutaneously in the mice. Dog bones were sterilized for 60 minutes under a UV light at 254 nm. Dog bones were tested at 2 and 8 weeks of implantation at a strain rate of $10^{-3}$ s$^{-1}$ in a thermal chamber at 37°C on the day of extraction from the host.

### 3.2.5 Water Content, Degradation, and Wettability

**3.2.5.1 Water Content and Degradation *in vitro***

In order to determine the degradation rate and water content of each material, each material was soaked for varying amounts of time. Each material was cut from a 1 mm thick sheet into a 1 cm$^2$ square and placed into a well plate with PBS, pH 7.4. The well plates remained in an incubator at 37ºC on a rotary shaker at 60 rpm. Samples were patted dry to remove excess water to obtain the wet sample mass. The samples were dried for 24 hours and the mass taken. The water content of each material will be defined by Equation 3.2, where $M_{wi}$ is the wet mass at time $i$ and $M_{di}$ is the mass at time $i$ after 24 hours of drying. The mass loss will be defined by Equation 3.3, where $M_{di}$ has been previously described and $M_i$ is the initial mass.

Equation 3.2  

\[
\text{WaterContent} = \frac{M_{wi}}{M_{di}} - 1
\]

Equation 3.3  

\[
\text{MassLoss} = 1 - \frac{M_{di}}{M_i}
\]

**3.2.5.2 Degradation *in vivo***

For *in vivo* degradation evaluation, ASTM D-638 type IV quarter-sized dog bone samples were massed prior to implantation for 2 or 8 weeks then underwent further
testing after extraction (mechanical). Subsequently, they were dried for 24 hours, massed again, and followed by further characterization (chemical and thermal). All animal experiments were conducted in accordance with the IACUC guidelines of Emory University. Eight week old immunocompetent male mice were used in the present study. The mice were each divided into 3 groups with networks of 0:100, 10:90, and 25:75 ratios of PEGDA: HDDA, where PEGDA:HDDA+ 55 wt% MMA was the ultimate composition. All the mice were on a standard chow diet (Purina, Certified Rodent Chow 5001). The different ratios of polymerized PEGDA: HDDA networks were delivered subcutaneously on the back of each mouse, n=3. Animals were euthanized by CO₂ inhalation at the prescribed time points, 2 weeks and 8 weeks. Implants were removed from the backs of each mouse and inspected for mechanical failure.

3.2.5.3 Surface Wettability

Contact angle was determined on a Ramé-hart goniometer (Mountain Lakes, NJ) using the sessile drop method. A 3 μL drop of deionized water was dropped onto the network surface, and the contact angle between the side of the drop and the surface was measured using a camera and DROPimage CA software. Measurements were taken from the average of the left and right contact angle on three separate 1 cm² squares of select compositions, where the mean ± standard deviation with n = 3 is given for each data point.

3.2.6 Drug Release

3.2.6.1 UV-Vis Spectroscopy

Select compositions were mixed with rhodamine B or doxycycline at a concentration of 1 mg/ml before photopolymerization. Three 1 cm x 1 cm x 1 mm
squares of each composition were soaked in 4 mL of PBS in an incubator at 37°C, where
the PBS was removed and refreshed daily for the first week, and once a week thereafter
for 4 weeks. The cumulative rhodamine B concentration was determined via the peak
absorbance at 556 nm and the cumulative doxycycline concentration was determined via
the peak absorbance at 350 nm from a multiplate-spectrophotometer (Molecular Devices
Spectra Max 384 Plus) [114-115]. The release kinetics of the model drug was determined
from Equation 2.1 [46]. The diffusional exponent, $n$, was determined by fitting linear
regression lines to log drug release vs log time plots of the experimental data. Constants
$k_1$ and $k_2$ were determined by fitting Equation 2.3 to the release profiles with a value of
$m= 0.475$ due to the aspect ratio of 10:1 from the sample geometry.

3.2.6.2 Mass Spectrometry

A Micromass Quattro LC mass spectrometer (Micromass, Beverly, MA)
connected to an Agilent 1100 Series LC (Agilent, USA) system was used to determine
doxycycline release. The mass spectrometer was operated in positive electrospray
ionization (ES+) mode. The desolvation temperature was 400°C and the source
temperature was 100°C. Full scans were taken from 100 to 1000 M/Z for degradation
products. The parameters for the multiple reaction monitoring of doxycycline: transition
from 445 to 428 (parent to daughter ion), collision energy 35 eV, cone energy 35V [116].
The chromatography was performed on a Ascentis Phenyl, 5cm x 2.1mm, 3 μm, column
(Supelco, USA). Solvent A was 95% water 5% acetonitrile and Solvent B was 5% water
95% acetonitrile, each with 0.1% HFPBA. A gradient was as follows: 0% B for 2 minutes
then to 100% B at 11 minutes, 100% B until 13.1 minutes then back to 0% B at 22.1
minutes, 0% B for another 2 minutes. The flowrate for 0.3 mL min$^{-1}$ and the injection
size was 10 μL. Two samples were run for each composition with at least two injections per sample.

3.2.7 Statistical Analysis

One-way ANOVA with Tukey’s HSD package in Igor Pro (WaveMetrics) was used to determine if any statistically significant difference was present ( p < 0.05). The mean ± standard deviation is given, where n =3, unless otherwise specified.
CHAPTER 4

THE EFFECT OF CHEMISTRY ON THE POLYMERIZATION,
THERMOMECHANICAL PROPERTIES, AND DEGRADATION RATE OF POLY(β-AMINO ESTER) NETWORKS

4.1 Introduction

Synthetic, biodegradable photopolymerizable networks have potential use in many biomedical applications due to the diverse chemistries available and broad range of mechanical properties. For example, porous poly(propylene fumarate) scaffolds have been proposed for bone applications because of their demonstrated compressive strengths and cytocompatible degradation products [117-120]. In addition, poly(anhydride) networks are currently being developed for injectable in situ polymerization for bone defects. These polymers degrade by surface erosion, thus maintaining their mechanical properties longer during the degradation cycle [31-34]. Poly(lactic acid) and its copolymer-based networks have also shown suitable osteoblast viability, controlled mechanical properties, and tailorable degradation [121-124]. Over the past decade, poly(β-amino ester)s (PBAEs) and their networks have gained attention as a biodegradable polymer with biomedical applications, such as non-viral gene delivery and tissue scaffolds [36-37, 42-43, 125]. It is important to understand the effects of changing the diacrylate to amine ratio and diacrylate molecular weight on the polymerization because this will subsequently affect the degradation rate and thermomechanical properties.

The purpose of this study is to determine the effect of the diacrylate molecular weight and chemistry on the polymerization, thermo-mechanical properties, and
degradation profiles of a model group of poly(β-amino esters). The effect of diacrylate molecular weight on polymerization is revealed through systematic variation of diacrylate molecular weight at constant molar ratio. Networks with varying molar ratio and diacrylate chemistry are studied in order to ascertain the influence of the diacrylate on the thermo-mechanical properties. The effect of diacrylate chemistry on degradation rate is studied by comparing poly(ethylene glycol) diacrylate based networks to diol diacrylate based networks.

4.2 Experimental

4.2.1 Materials

Poly(ethylene glycol) diacrylate (PEGDA) of four varying molecular weights, $M_n \sim 258, 302, 575, 700$, was used as one diacrylate system. The other diacrylate system, diol diacrylates (DDA), consisted of 1,4-butandiol diacrylate (BDDA or DDA198) (Dajac Labs), 1,6-hexanediol diacrylate (HDDA or DDA226), 1,9-nonanediol diacrylate (NDDA or DDA268) (TCI). The primary amine was 3-methoxypropylamine (3MOPA). 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959) was used as the photoinitiator. All chemicals were used as received from Sigma Aldrich unless otherwise noted.

4.2.2 Methods

The polymerization for the networks followed the method outlined in Chapter 3 for networks without MMA. DMA was run for each network in triplicate following the DMA method outline in Chapter 3 to a maximum temperature of 100°C. The molecular weight between crosslinks was calculated using Equation 2.7. DSC also followed the method from Chapter 3 with a maximum temperature of 100°C.
\(^1\)H NMR verified the structures of the macromers and the spectrum was analyzed via MestRe-C software to determine the number of hydrogen as well as an estimate of the diacrylate to amine ratio, and therefore the molecular weight [42]. ATR-FTIR was used to characterize the step-growth polymerization and free radical photopolymerization according to the method in Chapter 3. Sol fraction followed the method from Chapter 3 using Equation 3.1. Degradation and water content were determined using Equations 3.3 and 3.2, respectively.

4.3 Results

4.3.1 Thermo-mechanical Properties

Representative DMA and DSC curves are shown in Figure 4.1 for both PEGDA and DDA networks. The glass transition temperature is well below room temperature for all these materials, and thus the material is rubbery at ambient temperatures. PEGDA700-based networks have a hump in the modulus above Tg, which signifies crystallization and subsequent melting due to the high molecular weight of the PEGDA chain. The crystallization and melting can be seen in the DSC curves in Figure 4.1B for the PEGDA700-based network. The molecular weight of the PEGDA575-based network or the DDA226-based network is not high enough to promote crystallization, thus the lack of the hump in the DMA curves of Figure 4.1A and the lack of crystallization and melting peaks in the DSC curves of Figure 4.1B.
Networks formed from each diacylate and 3MOPA at varying molar ratios were tested on the DMA. The modulus in the rubbery regime at a temperature of Tg+75°C is given as a function of the molar ratio as shown in Figures 4.2A and 4.2B. In order to compare rubbery modulus between systems, the rubbery modulus was measured at the same relative temperature to Tg, Tg+75°C. The PEGDA 302 does not form a network at molar ratio 1.05:1 due to a lack of acrylate bonds. The modulus ranged from 0.14 to 5.36...
MPa for the PEGDA networks, and from 0.15 to 6.71 MPa for the DDA networks. The rubbery modulus increases as the molar ratio increases, as expected from a similar study [42]. There is a statistically significant difference in rubbery modulus as diacrylate to amine ratio is increased for all the DDA systems and the PEGDA575 and PEGDA700 systems. There was no statistically significant difference in the PEGDA258 and PEGDA302 systems as diacrylate to amine ratio increased. However, there is no obvious trend between the diacrylate molecular weight and the rubbery modulus. The Tg as a function of the molar ratio is shown in Figures 4.2C and 4.2D. The Tg ranged from -44.3°C to -31°C for the PEGDA networks, and from -50.9 to -35.6°C for the DDA networks. The Tg increases as the molar ratio increases and as the diacrylate molecular weight decreases for the DDA networks, but the Tg increases only as the diacrylate molecular weight decreases for the PEGDA networks. There was no statistically significant difference in the PEGDA systems as the diacrylate to amine ratio varied. There was a statistically significant difference in the DDA systems as the diacrylate to amine ratio increased. The molecular weight between crosslinks for PEGDA-based and DDA-based networks for each molar ratio is shown in Figures 4.2E and 4.2F. The molecular weight between crosslinks ranged from 1,500 to 115,000 g/mol for the PEGDA-based networks and from 1,200 to 59,000 g/mol for the DDA-based networks.
Figure 4.2. Rubbery modulus and Tg as a function of molar ratio for each network. Rubbery modulus of PEGDA-based networks (A) and DDA-based networks (B) at a temperature of Tg+75°C by DMA. Tg of PEGDA-based networks (C) and DDA-based networks (D). The molecular weight between crosslinks for PEGDA-based networks (E) and DDA-based networks (F).
Figure 4.2 Continued
4.3.2 Step-Growth Polymerization

A series of structural analyses were performed to help understand the trends in modulus presented in Figure 4.2. In order to understand the extent of conversion during step-growth polymerization, the acrylate bond was monitored via FTIR over 24 hours. Raw data of the 812 cm\(^{-1}\) peak is shown in Figure A.1 and Figure A.2. The molar ratio of 1.20:1 was examined for each diacrylate as shown in Figure 4.3. In Figure 4.3A, the PEGDA258 and PEGDA302 converted quicker and to a higher extent than the PEGDA575 and the PEGDA700 networks. In Figure 4.3B, the degree and rate of conversion increased as the DDA molecular weight decreased. PEGDA and DDA macromers formed from monomers of similar molecular weight have quite different step-growth conversions, where PEGDA monomers converted faster and to a higher degree. Due to the diacrylate to amine ratio being greater than 1, all of the diacrylate endgroups will not be completely consumed. When using a molar ratio of 1.20:1 of diacrylate to
amine and all of the amine endgroups react, there will be a theoretical diacrylate excess of 16.6%; thus, the expected amount of diacrylate endgroups consumed is 83.3%.

Figure 4.3. Conversion as a function of time for PEGDA macromers of 1.20:1 molar ratio (A) and DDA macromers of 1.20:1 molar ratio (B) as measured by FTIR.
After 24 hours of step-growth polymerization, NMR was used to verify the chemical structure of the macromers, especially the presence of acrylate end-groups, and the incorporation of the amine into the macromer as shown in Figure 4.4 by an absence of a peak near 1 ppm. From end-group calculations, an estimation of macromer molecular weight can be determined. The average molecular weight of the macromers from NMR for each backbone chemistry and molar ratio is shown in Figure 4.5. The average molecular weight of the macromers decreased as the molar ratio increased for both network chemistries. The macromer molecular weight ranged from 2200 to 37400 g/mol for the PEGDA-based macromers, and the macromer molecular weight of the DDA-based macromers ranged from 1700 to 11800 g/mol. The macromer molecular weight of the PEGDA-based macromers showed no direct relationship with diacrylate molecular weight, where the PEGDA575 and PEGDA258-based macromers had similar macromer molecular weights and the PEGDA700 and the PEGDA302-based macromers had similar macromer molecular weights. The PEGDA258 and PEGDA302-based macromers did reach a higher conversion during step-growth, and therefore have higher macromer molecular weight. The macromer molecular weight of DDA-based macromers converged as the molar ratio increased.
Figure 4.4. An exemplary NMR spectra of PEGDA575-based macromer, molar ratio 1.25:1. Corresponding letters (a-e) of the peaks to the structure are given, where a-c define the acrylate end-group, d defines the terminal methyl group of the amine, and e defines a bond that was previously unsaturated.

Figure 4.5. The molecular weight of macromers determined from NMR for PEGDA-based networks (A) and DDA-based networks (B).
4.3.3 Photopolymerization

From the FTIR-ATR photopolymerization, the conversion of remaining acrylate bonds was monitored for networks at a molar ratio of 1.20:1. Raw data of the photopolymerization is found in Figure A.3. The conversion of PEGDA networks and DDA networks is shown in Figure 4.6A and 4.6B, respectively. PEGDA575 and PEGDA700 networks reached high degrees of conversion, while PEGDA258 and PEGDA302 did not. The DDA226 and DDA268 reached higher degrees of conversion than the DDA198 network. In essence, the networks formed from macromers that had high degrees of conversion during step-growth did not reach high degrees of conversion during photopolymerization.
Figure 4.6. UV photopolymerization monitored by FTIR-ATR for PEGDA networks (A) and DDA networks (B).

To compare the conversion measured from FTIR-ATR photopolymerization, sol fraction testing was conducted across all molar ratios and diacrylate monomer chemistries and molecular weights as shown in Figure 4.7. PEGDA575 and PEGDA700 networks showed lower sol fractions, 0.03 to 0.06, respectively, compared to the PEGDA258 and PEGDA302 networks, 0.13 to 0.5, respectively. DDA networks showed similar levels of sol fraction to the PEGDA networks ranging from 0.04 to 0.38.
4.3.4 Degradation and Water Content

Degradation profiles for all diacrylate molecular weights and molar ratio of DDA are shown in Figure 4.8A. The degradation rates of the DDA226 and DDA268 are tightly grouped, while the DDA198 is distinct from the other DDA networks. The marked curves are the lowest molar ratio, 1.05:1, for each DDA molecular weight. The water content of the DDA226 and DDA268 does not exceed 1, while the water content of the DDA198 is
higher as shown in Figure 4.8B. The networks with rapid degradation match the networks with high water content. In the DDA system degradation rate is strongly influenced by molecular weight but not by ratio for this range of molecular weights.

Figure 4.8. Degradation profile for all diacrylate molecular weights of DDA and all molar ratios. The molar ratio of 1.05:1 is displayed by markers and the remaining curves represent the behavior of the other four molar ratios (8A) at each molecular weight and water content profile for the DDA networks at a molar ratio of 1.05:1 (8B).
The degradation profiles of PEGDA-based materials are presented in Figure 4.9A and 4.9B. The degradation profiles for PEGDA700 networks for the 5 different molar ratios are shown in Figure 4.9A. The lower PEGDA700 ratios degraded rapidly, while the higher ratios lasted for at least 24 hours. The degradation profiles for all PEGDA networks at a ratio of 1.10:1 and 1.25:1 are shown in Figure 4.9B. All PEGDA networks at a ratio of 1.10:1 degraded completely within 8 hours, while the networks at a ratio of 1.25:1 lasted 12 hours or more. The water contents for the four PEGDA networks at a ratio of 1.25:1 are shown in Figure 4.9C. The networks with high initial water contents match the networks with rapid degradation.

Figure 4.9. Degradation profiles for PEGDA700 at all molar ratios (9A). Degradation profiles for all PEGDA networks at molar ratios at 1.10:1, filled markers, and 1.25:1, open markers (9B). Water content profiles for PEGDA networks at a ratio of 1.25:1 (9C).
4.4 Discussion

In this study, the effect of diacrylate molecular weight, diacrylate to amine ratio, and diacrylate chemistry on PBAE network properties was explored. The results show that the degradation rate and thermomechanical properties are greatly influenced. The two systems, PEGDA-based and DDA-based, were selected for their different history of
use and their diverse chemical properties. From a biocompatibility standpoint, PEGDA-based polymers are known for their biocompatibility, and the degradation product containing 3MOPA has yet to be proven harmful. In addition, PEGDA-based systems were chosen due to previous testing of their mechanical properties under cyclical loading [44]. As a comparison, DDA-3MOPA systems were chosen to study the effect of changing the backbone chemistry from hydrophilic to hydrophobic on the thermo-mechanical properties and degradation. The molar ratio range was limited to 1.25:1 to prevent nondegradable crosslinks from forming from excess pure diacrylate. Prior work has demonstrated that the elastic modulus of the network is affected by changes in macromer molecular weight by varying the molar ratio. Previously, a combinatorial approach analyzed modulus and mass loss for several of the networks of molar ratio 1.20:1. For networks composed of PEGDA 258, 302, 700, DDA198 and DDA 226, the modulus as determined by a rapid indentation technique was higher than modulus reported here, which was determined by DMA. The modulus of the PEGDA575 network was comparable between the two techniques. The mass loss was equivalent for the PEGDA258 and PEGDA302, but not the PEGDA575 and PEGDA700 due to increased crosslinking density and nondegradable crosslinks. Also, the step-growth synthesis was only specified as overnight, thus the exact conversion is unknown. Here we explore the impact of diacrylate molecular weight and chemistry on step-growth polymerization, photopolymerization, and subsequent properties.

Dynamic mechanical analysis was used as a screening method in order to look at a range of networks of varying crosslinking density and chemistry. A commonality between all these materials is their low Tg, as shown by the large drop in storage
modulus in Figure 4.1, Figure 4.2C, and Figure 4.2D. The low Tg is due to the lack of steric hindrance usually created from bulky, rigid side groups, and the enhanced flexibility from the ethylene glycol, methylene, or amine groups incorporated into the backbone. Thus, by increasing the diacrylate molecular weight, more flexible groups are being incorporated resulting in the subsequent decrease in Tg. In addition, varying the molar ratio and diacrylate molecular weight and chemistry produced a broad range of rubbery moduli. The rubbery modulus increases as molar ratio increases, thus the crosslinking density increases as the macromer molecular weight decreases due to the increasing molar ratio as shown in Figure 4.2A and Figure 4.2B. However, the trends in elastic modulus with diacrylate molecular weight were less obvious and non-monotonic. Low molecular weight diacrylates may be expected to create denser networks, thus having a higher modulus. However, in Figure 4.2A, the low molecular weight diacrylates had lower rubbery moduli or in Figure 4.2B, the low and high molecular weight diacrylates had similar rubbery moduli. The crystallization and melting of PEGDA700-based networks was not expected, but is possible as the network structure has passed above its Tg in Figure 4.1. It also defines a molecular weight boundary, where PEGDA-based networks do not crystallize when PEGDA is below 700 g/mol. Because of these unexpected trends in modulus, a further understanding of both polymerization steps was explored to understand the relation between diacrylate molecular weight and network modulus.

The step-growth polymerization of PEGDA-based networks and DDA-based networks was studied by varying the diacrylate molecular weight and chemistry while maintaining a constant molar ratio of 1.20:1. The networks synthesized from lower
molecular weight diacrylates, such as PEGDA258 and PEGDA302, converted to a higher degree and at a faster rate than their higher molecular weight PEGDA counterparts. The increase of diacrylate molecular weight decreases the monomer’s mobility thus decreasing the rate and degree of conversion. With a molar ratio of 1.20:1, the conversion would have been expected to be equivalent for all PEGDA-based macromers. The higher degree of conversion for the PEGDA258 and PEGDA302-based macromers may have resulted from termination during the step-growth polymerization from monomers having only one acrylate endgroup instead of two or cyclization of the diacrylate to amine.

PEGDA monomers have near 14% impurities comprised of poly(ethylene glycol) chains and monofunctional poly(ethylene glycol) acrylate, where over 10% may be monofunctional poly(ethylene glycol) acrylate[126]. DDA monomers have near 10% impurities, where 3% comprises the monofunctional diol acrylate[127]. These monofunctional acrylate impurities will terminate the step-growth reaction early producing smaller molecules without any acrylate functionality or cause dangling ends which would not be elastically effective. The DDA-based networks’ rates and degrees of conversion decreased as the diacrylate molecular weight increased again due to decreased mobility. The effect of decreasing rate and degree of conversion as diacrylate molecular weight increases is in agreement with hyperbranched amine-acrylate systems[128]. The macromer molecular weight post-step-growth polymerization is a key determinant of the crosslinking density and thus the rubbery modulus. NMR can provide an estimate based upon the ratio of acrylate endgroups to amine groups and may count cyclization and dangling groups with only one acrylate endgroup. By using NMR, the macromer molecular weight can be compared to the elastically effective molecular weight between
crosslinks via DMA. Figure 4.2F and Figure 4.5B show similar molecular weights for the DDA-based networks, thus they are converting ideally and elastically effective chains are the majority of the chains present. PEGDA575 and PEGDA700-based networks also show similar molecular weights in Figure 4.2E and Figure 4.5A. The PEGDA258 and PEGDA302-based networks have a drastic difference, where the molecular weight via DMA is much higher. Thus, the step-growth polymerization is producing elastically ineffective chains, which would be comprised of a combination of dangling chains and cycles of low molecular weight as seen in the molecular weight via NMR. The low molecular weight diacrylates are more likely to form cycles as seen in kinetic models and other diacrylate systems, which lowers the formation of crosslinks, thus lowering their modulus [76, 129]. By comparing the repeating unit structure of DDA and PEGDA, PEGDA is the more flexible monomer based on its lower characteristic ratio, where poly(ethylene glycol) and polyethylene have characteristic ratios of 5.6 and 7.4, respectively [130]. This increase in flexibility at nearly the same molecular weight may contribute to the increased cyclization. Because the step-growth polymerization determines the macromer molecular weight and the degree of acrylate conversion, this step will also affect the subsequent polymerization, as will be further discussed.

The second polymerization, the UV-photopolymerization, is responsible for network formation. A critical population of acrylate endgroups is necessary for full network formation. The networks that reached high degrees of photopolymerization were the macromers that did not reach high levels of conversion during their step-growth polymerization. Thus because the PEGDA575 and PEGDA700-based networks reached high levels of photopolymerization conversion, they formed more complete networks and
obtained higher rubbery moduli. By examining the macromer molecular weight in combination with the degree of conversion, the arrangement of rubbery moduli in Figure 4.2A is made clear. The PEGDA258 and PEGDA575-based networks have nearly different degrees of photopolymerization, and thus possess differing rubbery moduli. Both DDA226 and DDA268-based networks converted to a higher degree during photopolymerization, but DDA226-based network has a lower macromer molecular weight than the DDA268, and thus formed networks with higher rubbery moduli compared with DDA268. The DDA198 converted to a lesser degree during photopolymerization, but still reached similar values of rubbery moduli as the DDA268. The results from the sol fraction, an alternative method of measuring network conversion, are in good agreement with the FTIR-ATR photopolymerization and the rubbery moduli. The networks suspected of having dangling endgroups and cycles, PEGDA258 and PEGDA302-based networks, had the highest sol fractions, thus this lack of network formation further decreased their modulus values.

The results of this study show that the effect of diacrylate molecular weight and chemistry on the polymerization and mechanical properties can only be fully understood only by considering structure after both the step-growth polymerization and photopolymerization. If the macromers do not reach high conversion during step-growth but obtain a high degree of conversion during photopolymerization, then the effect of diacrylate molecular weight on rubbery moduli can be understood from the macromer molecular weight. If two macromers have the same macromer molecular weight via NMR but convert differently during photopolymerization, then the degree of conversion during photopolymerization will dictate rubbery modulus. The step-growth
polymerization controls the degree of acrylate conversion necessary for network formation and the macromer molecular weight that influences crosslinking density. The photopolymerization controls network formation, but is greatly influenced by the amount of acrylate endgroups remaining from the step-growth polymerization.

The degradation profiles and water content of the networks are controlled by two different mechanisms. The degradability of DDA-based networks are affected by their diacrylate molecular weight as can be seen in Figure 4.8A. All five molar ratios of DDA226 and DDA268 have nearly the same degradation profile, and the five molar ratios of DDA198 are similar and distinct from the DDA226 and DDA268 networks. It is clearly seen that as the diacrylate molecular weight increases, the degradation rate decreases due to a decrease in water content as shown in Figure 4.8B. The water content follows the same trend as the degradation profiles, where the number of methylene units or the diacrylate molecular weight is the controlling factor. The independence of degradation rate from molar ratio, thus rubbery modulus is unexpected because increasing the crosslinking density typically alters the degradation rate.

Unlike the DDA-based networks, the PEGDA-based networks’ degradation profiles are controlled less by their diacrylate molecular weight, and more by their molar ratio. The low molar ratios networks are lightly crosslinked thus allowing for large amounts of water to enter the network, which leads to rapid degradation. The higher molar ratios of 1.20:1 and 1.25:1 plateau due to the formation of non-degradable crosslinks and a higher network density. All PEGDA-based networks follow the same trend, regardless of the diacrylate molecular weight. All PEGDA-based networks eventually had water content greater than 500% by the time of full degradation, which is
the main cause for their rapid degradation. The dual mechanisms illustrate the difference in backbone chemistry of the diacrylates. Degradation in the DDA-based networks is more controlled by diacrylate molecular weight while degradation in the PEGDA-based networks is dominated by molar ratio. This separation of degradation rate and modulus for the DDA-based networks is significant, where it will allow for enhanced tailoring of these networks for tissue scaffolds and drug release devices.

4.5. Conclusions

In this study, PBAEs networks were synthesized through step-growth and photopolymerization reactions. The rubbery modulus was affected by the macromer molecular weight and the acrylate end-group conversion during the step-growth polymerization, and by the degree of network formation during photopolymerization. The lack of high conversion during step-growth polymerization is essential for full conversion during photopolymerization. The diacrylate molecular weight influences the rate and degree of conversion during step-growth polymerization, thus affecting the subsequent polymerization. The degradation rate for DDA is dependent on the diacrylate molecular weight, and independent of the molar ratio, thus independent of the modulus. The degradation rate for PEGDA networks is dependent on the molar ratio, where as ratio increases the time to complete degradation increases. This understanding of how the two step polymerization of poly(β-amino ester) networks affects the mechanical properties and degradation will allow for the precise tailoring of poly(β-amino ester) networks for future biomedical applications.
CHAPTER 5

EFFECT OF POLY(ETHYLENE GLYCOL) DIACRYLATE CONCENTRATION ON NETWORK PROPERTIES OF POLY(β-AMINO ESTER) NETWORKS

5.1 Introduction

Degradable polymers such as poly(β-amino ester)s and their associated networks are showing promise at the crossroads where materials engineering meets biological and clinical applications. Poly(β-amino ester)s are currently being investigated for cancer, cardiovascular and orthopedic applications [43, 131-132]. This class of polymer is formed via the step-growth reaction of a primary amine and a diacrylate. By keeping the molar ratio of diacrylate to amine greater than one, macromers with acrylate end-groups are produced, which allows photopolymerization into networks. A current focus of polymer science has been on modulating monomer chemistry in various classes of polymers and copolymers in order to change the degradation rate and mechanical properties [23, 121, 123, 133-134]. Anderson et al. initially demonstrated using a combinatorial approach to determine a wide range of degradation rates and mechanical properties [35]. Further investigations determined the relationship between macromer molecular weight and mechanical properties, in which lower macromer molecular weight increases the rubbery modulus [42]. Compositions were doped with a triacrylate crosslinker in order to increase the crosslinking density to improve the mechanical properties for a porous tissue scaffold [43].

The objective of this study was to establish a novel method for tailoring the properties of poly(β-amino ester) networks by using two diacrylates of different
chemistries. This is the first known characterization of combining two diacrylates within poly(β-amino ester)s to adjust properties, especially water content and degradation. Networks with defined degradation and mechanical property profiles are created by varying molar ratios of two chemically distinct diacrylates, hexanediol diacrylate and poly(ethylene glycol) diacrylate, while maintaining a constant diacrylate to amine ratio. The synthesis was characterized using $^1$H Nuclear Magnetic Resonance Spectroscopy (NMR), Fourier Transform Infrared Spectroscopy (FTIR) and sol fraction tests. The networks were analyzed in vitro for their dynamic mechanical properties, bulk mechanical properties, degradation, and water content profiles. These subtle changes in chemistry that modulate degradation and mechanical properties can be used to develop customizable poly(β-amino ester) materials for various scientific and clinical applications.

5. 2 Experimental

5.2.1 Materials

Poly(ethylene glycol) diacrylate $M_n \sim 700$ (PEGDA) and 1,6-hexanediol diacrylate (HDDA) were the diacrylates used. The primary amine was 3-methoxypropylamine (3MOPA). 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959) was used as the photoinitiator. All chemicals were used as received from Sigma Aldrich.

5.2.2 Methods

Macromers were synthesized with PEGDA:HDDA ratios of 0:100, 10:90, and 25:75 at a diacrylate to amine ratio of 1.20:1, and photopolymerized according to the methods in Chapter 3. DMA was run for each network in triplicate following the DMA
method outline in Chapter 3 to a maximum temperature of 100°C. The molecular weight between crosslinks was calculated using Equation 2.7. Tg was defined as the peak of the tan delta curve. Environmental mechanical testing of cylinders over 8 weeks was performed in compression following the methods of Chapter 3.

$^1$H NMR was used to determine the structure of the copolymer after step-growth polymerization. ATR-FTIR was used to characterize both the step-growth polymerization and the free-radical photopolymerization according to Chapter 3. As an alternative method to determine network formation, sol fraction was determined for the three networks using Equation 3.1.

Degradation and swelling profiles were determined for the three networks for 12 weeks, where the mass loss was calculated by Equation 3.3 and the water content was calculated by Equation 3.2. Contact angle was performed on select networks of 0:100, 25:75 and 100:0 PEGDA:HDDA following the method of Chapter 3. A 100:0 network was used as a control since it is a nearly 100% PEGDA hydrogel.

5.3 Results

5.3.1 Copolymer Characterization

The diacrylate conversion during the step-growth reaction of the three macromers is shown in Figure 5. 1. As the PEGDA concentration increased, the rate of diacrylate conversion increased. The average diacrylate consumption during step-growth polymerization ranged from 85.5 to 87.6% as determined by peak area change from FTIR. Theoretically, if a molar ratio of 1.20:1 of diacrylate to amine is present and all of the amine endgroups react, there will be a diacrylate excess of 16.6%; thus, the expected amount of diacrylate endgroups consumed is 83.3%, which closely corresponds to the
FTIR measurement. The discrepancy in the step-growth polymerization can be attributed to monofunctional acrylate impurities, which can range from 3 to 10% for diacrylates [126-127].

![Conversion profile of PEGDA-HDDA-3MOPA macromers during step-growth polymerization as monitored by FTIR, given as mean± standard deviation, n=3.](image)

A representative NMR spectrum is shown in Figure 5.2A. The chemical structure inset highlights the unique hydrogen signals labeled a-g. The acrylate end-groups are confirmed by peaks “a” and “b” and the amine’s methyl is confirmed by “c”. Four unique signals, d-g, represent the methylene groups adjacent to the ester groups as shown in Figure 5.2B for each macromer. The methylene-ester groups are of four types: HDDA end-group (e), PEGDA endgroup (g), HDDA-3MOPA bonded (d), and PEGDA-3MOPA bonded (f). The 0:100 macromer contains only (d) and (e) as no PEGDA is present. The 10:90 has a minimal amount of PEGDA and with only (f) present, all the PEGDA groups have been incorporated to the inside of the macromers. The 25:75 has a mixture of internal HDDA and PEGDA chains and external HDDA and PEGDA chains.
Figure 5.2.(A) $^1$H NMR spectra of a PEGDA-HDDA-3MOPA polymer, where the acrylate endgroups are represented by (a) and (b), the terminal methyl on the amine (c). A lack of peak at ~1.1 ppm shows the amine was incorporated. (B). $^1$H NMR spectra inset of the three PEGDA:HDDA macromers. Hydrogen peaks (d–g) correspond to the methylene groups adjacent to the four types of ester bonds. (d) for the HDDA methylene adjacent to the ester bonded to the amine, (e) for the HDDA methylene adjacent to the ester bonded to a terminal acrylate group, (f) for the PEGDA methylene adjacent to the ester bonded to the amine, (g) for the PEGDA methylene adjacent to the ester bonded to a terminal acrylate group.
5.3.2 Network Characterization

The real time photopolymerization was monitored through FTIR-ATR. All three macromers reached acrylate conversions greater than 90% within 5 minutes as shown in Figure 5.3. Network properties are given in Table 5.1 for the PEGDA-HDDA-3MOPA networks. The average sol fraction after network formation ranged from 5.3 to 8.4%. The average rubbery modulus at a temperature of Tg+75°C ranged from 4.4 to 5.7 MPa. The temperature of Tg+75°C was chosen in order to have a relative measurement for each composition and to ensure the material was in the rubbery regime. The glass transition temperature remained near -40°C for all compositions. The molecular weight between crosslinks, as determined by Equation 2.7, ranged from 1602 to 1987 g/mol. The molecular weight values determined via DMA are more reflective of the network structure as they represent the elastically effective chains, which would have had diacrylate endgroups.

Figure 5.3. Exemplary real time FTIR-ATR UV photopolymerization curves of the 3 PEGDA-HDDA-3MOPA macromers.
Table 5.1. Properties of 3 PEGDA-HDDA-3MOPA Networks, given as mean± standard deviation, n=3.

<table>
<thead>
<tr>
<th>PEGDA: HDDA</th>
<th>Rubbery Modulus (MPa)</th>
<th>Glass Transition Temperature (°C)</th>
<th>Molecular Weight between Crosslinks (g/mol)</th>
<th>Sol Fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:100</td>
<td>5.4 ± 0.2</td>
<td>-40.6 ± 0.6</td>
<td>1809 ± 91</td>
<td>6.3 ± 0.9</td>
</tr>
<tr>
<td>10:90</td>
<td>4.4 ± 0.3</td>
<td>-41.8 ± 0.6</td>
<td>1987 ± 127</td>
<td>14.3 ± 1.5</td>
</tr>
<tr>
<td>25:75</td>
<td>5.7 ± 0.7</td>
<td>-40.7 ± 0.5</td>
<td>1602 ± 203</td>
<td>4.7 ± 1.2</td>
</tr>
</tbody>
</table>

The water content profile for 12 weeks is shown in Figure 5.4A for the PEGDA-HDDA-3MOPA networks. The average water content for the 0:100 network was always below 0.5. The 10:90 networks’ average water content ranged from 0.5 to near 1.0 and the 25:75 networks’ average water content ranged from 1.0 to 2.0. The degradation profile for 12 weeks is shown in Figure 5.4B for the PEGDA-HDDA-3MOPA networks. The rate of initial mass loss increases as the PEGDA concentration increases. There is an initial burst in the first week for the networks that contain PEGDA. After 4 weeks, the degradation continues, but at a slower rate for the networks containing PEGDA. The average mass loss of the 0:100 network shows minimal increase over 8 weeks, while the average mass loss of the 10:90 and 25:75 networks show dramatic increases between 24 hours and 8 weeks. The majority of the mass loss occurred between 24 hours and 2 weeks, but continued after the 2 week time-point. Contact angle measurements are found in Table 5.2, where the contact angle decreased as the PEGDA concentration increased.
Figure 5.4. (A) Water content as a function of time for the PEGDA-HDDA-3MOPA networks in PBS. (B) Normalized Mass Loss as a function of time for the PEGDA-HDDA-3MOPA networks in PBS, given as mean ± standard deviation, n=3.

Table 5.2 Contact angle measurements for three PEGDA-HDDA-3MOPA Networks, given as mean ± standard deviation, n=3.

<table>
<thead>
<tr>
<th>PEGDA:HDDA</th>
<th>Contact Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:100</td>
<td>66.7 ± 6.7</td>
</tr>
<tr>
<td>25:75</td>
<td>41.3 ± 12.9</td>
</tr>
<tr>
<td>100:0</td>
<td>~ 0</td>
</tr>
</tbody>
</table>
The modulus profile over 8 weeks under *in vitro* conditions is shown in Figure 5.5. As the PEGDA concentration increases, the loss in modulus increases. The 0:100 network’s modulus decreased from 5.7 to 3.6 MPa, and the 10:90 network’s modulus decreased from 2.59 to 0.82 MPa. The 25:75 shows the most drastic change in modulus, where it decreases two orders of magnitude over 8 weeks from 3.36 MPa to 17 kPa.

![Figure 5.5](image)

**Figure 5.5.** Modulus of the three PEGDA-HDDA-3MOPA networks over 8 weeks under *in vitro* conditions, given as mean± standard deviation, n≥4.

### 5.4 Discussion

From previous work, the diacrylate chemistry has tremendous influence on the properties of poly(β-amino ester) networks. The rubbery modulus is dependent upon both diacrylate to amine ratio and the diacrylate chemistry. The degradation profile of PEGDA-based networks is dependent on the diacrylate to amine ratio, while the degradation profile of diol diacrylate-based networks is dependent upon the diacrylate molecular weight from Chapter 4. The hydrophilic PEGDA networks had high water contents and rapid degradation profiles, while the hydrophobic HDDA networks had low
water contents (< 1 g/g) and slower degradation profiles over 16 weeks. Based on their disparate degradation profiles, these diacylates were copolymerized with 3MOPA during the step-growth polymerization to form macromers containing a random mixture of PEGDA and HDDA. The molar ratio of 1.20:1 was maintained for each composition in order to compare to previous work. The network with molar ratio 0:100 was chosen to serve as a baseline. A network with molar ratio 50:50 degraded *in vitro* within a week, thus networks at 10:90 and 25:75 were chosen for further testing. This allowed us to investigate the impact of PEGDA:HDDA ratio on network properties, especially degradation and modulus.

The step-growth polymerization of PEGDA:HDDA networks was studied by varying the PEGDA concentration while maintain a constant diacrylate to amine ratio. Even though the PEGDA is a larger monomer than HDDA, adding PEGDA increased the rate of polymerization in the initial hours as shown in Figure 5.1. Due to the lower characteristic ratio of PEGDA, it is more flexible, which may allow it to be more mobile during the reaction with the amine [130]. The faster reaction rate supports the NMR spectra. The incorporation of PEGDA was to the inside backbone chain of most of the macromers, since no PEGDA endgroups (g) are present in Figure 5.2B for the 10:90 macromer. While endgroups (g) is not detectable in the 10:90 macromer, minute quantities may be present. Due to the higher amount of PEGDA in the 25:75 macromer, both (f) and (g) are present, but (g) groups are not in high quantities. The PEGDA monomers are reacting quicker with the amines than the HDDA monomers, thus are becoming part of the inside backbone of the macromer instead of the endgroups. Due to the higher molecular weight of the PEGDA, they are not likely to form intramolecular
cycles with the amine. The endgroups are the last monomers to react with the amine, thus with HDDA monomers being slower than the PEGDA monomers, the HDDA monomers constitute a majority of the endgroups. The real-time photopolymerization curves from Figure 5.3 do not have any large differences between them in their photopolymerization, thus this agrees with the HDDA endgroups being the majority of the endgroups or that PEGDA and HDDA endgroups are equivalent during the photopolymerization.

The initial characterization of network properties showed that the three compositions had nearly equivalent dry mechanical properties (in terms of rubbery modulus), molecular weight between crosslinks, and amounts of diacrylate consumed during step-growth polymerization. This is expected since the diacrylate to amine ratio was held constant at 1.20:1. The diacrylate to amine ratio controls the macromer molecular weight, which dictates the crosslinking density and the rubbery modulus. The molecular weight between crosslinks, representative of the elastically effective macromers with acrylate groups on each end, varies slightly by a couple hundred g/mol, which is comparable to variations in macromer molecular weight of other poly(β-amino ester)s with a ratio of 1.20:1. Also, the sol fraction is comparable to other poly(β-amino ester) networks at the same molar ratio[42]. The sol fractions from Table 5.1 are indicative of high degrees of conversion for these networks. Similar photocrosslinked networks of poly(ethylene oxide) dimethacrylate underwent transdermal photopolymerization and reached high conversion levels within the same amount of time [61]. Thiol-acrylate systems convert quicker, reaching their final conversion value in 90 seconds, because of a mixed step-growth/chain growth polymerization [135]. Other poly(β-amino ester) networks have similar photopolymerization rates, reaching their final
conversion values in under 10 minutes [42]. Such rapid conversion times may allow for
in situ polymerization of tissue scaffolds.

Previously, a range of degradation times was produced by varying the chemical
structures of the diacylate and/or amine [35]. In this study, we tailored the degradation
and modulus profiles by mixing monomers with contrasting degrees of hydrophilicity,
thus allowing the profiles to be controlled without large changes in the crosslinking
density, only the relative composition. Contact angle measurements verified the
assumption that adding PEGDA would increase the hydrophilicity of the network. The
100:0 PEGDA:HDDA networks were hydrophilic to such an extent that the water
droplets were absorbed immediately into the bulk of the hydrogel. The control of
degradation rate was accomplished by starting with HDDA based networks, which absorb
low amounts of water. By mixing HDDA with PEGDA at the initial polymerization step,
macromers were formed that would absorb water between HDDA and PEGDA. The
water content of the three compositions can be tuned as shown in Figure 5.4A. The water
content of the 25:75 network initially increases then decreases at 4 weeks because the
network has degraded near 60% and cannot hold as much water. This decrease of water
content could be attributed to a change in the hydrophilicity of the network structure. The
increased mass loss is due to the more hydrophilic PEGDA components of the network,
thus they are degrading out of the network before the HDDA components. The drop in
water content would mark a point where the hydrophilicity of the network changes due to
the increased HDDA content, which is more hydrophobic. It was shown that when the
PEGDA concentration was increased by only 10% (0:100 to 10:90), the water content in
the network doubles. Because of the tailorability of water content, degradation can also
be controlled as shown in Figure 5.4B. Such slight amounts of PEGDA can be added to
the system with remarkable increases in mass loss, for example, the mass of 0:100
network was 0.2 at 12 weeks, while the mass loss of the 10:90 network was near 0.6 at 12
weeks. Thus, doubling the water content can more than triple the amount of mass loss for
these networks, thus the water content and mass loss are not monotonic. Also, the mass
loss profiles include the sol fraction, which would leach out of the network at early
timepoints. The sol fraction is below 10% of the mass, thus the remainder can be
attributed to hydrolytic degradation of the network. Bulk erosion is more likely in the
networks with PEGDA because of the nonlinearity of the mass loss profile as well as the
high water contents. A low water content does not necessarily indicate surface erosion for
the HDDA network, but more linear mass loss profile is an indicator of surface erosion
behavior. By adding PEGDA to the HDDA network, the water content increases
dramatically, which allows for a more bulk erosion behavior. Similar to the
PEGDA:HDDA networks, biodegradable thiol-acrylate networks had their network
structure controlled by varying the amount of poly(ethylene glycol) to tailor their
degradation profile [136]. Previous poly(β-amino ester) networks had their degradation
rate varied by changing the macromer molecular weight (crosslinking density); however,
their initial mechanical properties were not equivalent [42]. The addition of PEGDA to
HDDA networks severely decreases the modulus as seen in Figure 5.5. The increase in
PEGDA concentration increases the mass loss, thus reducing the modulus. This modulus
decrease is more characteristic of bulk degradation of the networks; however, the slow
decrease in the modulus of HDDA networks indicates a more surface degradation
behavior [133]. For the PEGDA:HDDA networks, in vitro degradation testing
demonstrated the tuning of the degradation rate, water content, and modulus of these networks, which would ultimately be necessary for a tailored tissue scaffold.

5.5 Conclusions

Poly(β-amino ester) networks were synthesized with varying concentrations of PEGDA and HDDA in order to control the degradation rate and mechanical loss profile. The macromers formed had random units of HDDA and PEGDA units, where HDDA was the dominant endgroup. By increasing the PEGDA concentration of the network, the water content increased, which increased the rate of mass loss and furthered the decrease in modulus, thus these networks show future promise in biomedical applications where precise degradation rates and mechanical properties are vital.
CHAPTER 6
THE EFFECT OF DOXYCYCLINE ON POLY(β-AMINO ESTER) NETWORK PROPERTIES AND CONTROLLED RELEASE BEHAVIOR

6.1 Introduction

Biodegradable polymers have been used drug delivery devices for many years to treat infection, deliver contraceptives, and to delivery chemotherapy agents. Doxycycline is a broad spectrum antibiotic used to treat a number of diseases, such as dental, periodontal, and bone infections [137]. Several doxycycline-controlled release systems have been developed to treat periodontal disease, such as biodegradable ceramic systems and biodegradable PLGA-PCL microspheres [114, 137-138]. Doxycycline is also a MMP-inhibitor, where MMPs are known to promote AAA formation [103]. Oral systemic administration of doxycycline has been shown to reduce occurrence and the severity of AAA in mice [104]. While drug delivery devices are often made from biodegradable thermoplastics, fewer have been made with biodegradable photopolymerizable networks. In one study, a photocrosslinkable PEG based hydrogel’s release properties were examined with varying molecular weight model drugs, where the release rate decreased as the molecular weight increased [139].

Therapeutic release from a polymer device is dependent on polymer chemical structure, network structure, degradation rate, hydrophilicity, and drug chemistry [12]. The release rate can elute the drug in a matter or days to years depending on the degradation rate of the polymer, where surface eroding polymers are often preferred because their elution rates are more predictable. Peppas has established a number of equations to describe the release kinetics from polymers [51-52, 140]. These equations
and resulting calculations categorize the release mechanisms as Fickian, non-Fickian anomalous transport, and Case II transport, where drug release rate is independent of time.

The purpose of this study is to determine the effect of doxycycline on the network formation, mechanical properties, and degradation of poly(β-amino ester) networks. Also, the release behavior of doxycycline and a model drug, rhodamine B, will be explored. The effect of doxycycline on network properties is established through systematic variation of doxycycline concentration while varying both network composition and photoinitiator concentration. Networks with varying PEGDA:HDDA ratio were used to understand the influence of network composition on release behavior.

6.2 Experimental

6.2.1 Materials

PEGDA:HDDA networks of four ratios, 0:100, 5:95, 10:90, and 25:75 were synthesized according to the step-growth synthesis in Chapter 3, using two concentrations, 0.5% and 5.0%, of photoinitiator I2959. Doxycycline was added after the step-growth reaction in concentrations varying from 0 to 5 mg/mL. The photopolymerization took place for 30 minutes for all networks.

6.2.2 Methods

Sol fraction, elastic modulus from standard tensile testing at 37°C, and mass loss from Equation 3.3 were determined according to the methods in Chapter 3. Statistical analysis was run according to the methods in Chapter 3, where three samples were run for each datapoint. UV-VIS spectra were taken for I2959, doxycycline, rhodamine B, and degradation products from 200 to 600 nm (Shimadzu UV-VIS 1610). Release profiles
were determined from the methods of Chapter 3. The diffusional exponents and constants for doxycycline and rhodamine B were determined from fitting Equation 2.1 and Equation 2.3 to the experimental data.

Mass spectrometry was carried out according to the method in Chapter 3 for the release profile and characterization of the degradation products. The doxycycline-degradation product interaction study used two methods. Method 1: Add a 1 cm x 1cm x 1mm slab of 25:75 PEGDA:HDDA network to a solution of 5 μg/mL doxycycline in distilled water. Solution was removed at 1 day and 1 week and mass spectrometry was run on degradation products, a control doxycycline solution, and the doxycycline-degradation product mixture. Method 2: A 1 cm x 1cm x 1mm slab of 25:75 PEGDA:HDDA network was allowed to degrade for 1 day and 1 week. At those timepoints, a 5 μg/mL solution of doxycycline was added to the degradation products, and mass spectrometry was run on the degradation products, a control doxycycline solution, and the doxycycline-degradation product mixture. ^1H NMR was run according to the method in Chapter 3, except the solvent was D₂O. The 25:75 PEGDA:HDDA network was degraded in D₂O for 1 week prior to analysis.

6.3 Results

6.3.1 Effect of Doxycycline on Network Properties

The sol fraction for four networks at two photoinitiator concentrations of varying doxycycline concentration is shown in Figure 6.1. The sol fraction increased as the doxycycline concentration increased for each network at 0.5% PI, where network formation did not occur for the 5mg/mL doxycycline concentration by 30 minutes. For all four networks at 0.5% PI, there was a statistically significant difference between the sol
fraction at 3 mg/mL and at 0 mg/mL. Also, there was a statistically significant difference between the two PI concentrations for each network at 3mg/mL doxycycline. For the 0:100, 5:95, and 25:75 PEGDA: HDDA networks at 5.0% PI, there was no appreciable change in sol fraction as the doxycycline concentration was varied; however, the 10:90 PEGDA: HDDA network increased in sol fraction as doxycycline concentration increased.

Figure 6.1 Sol Fraction as a function of doxycycline concentration for four networks of varying PEGDA:HDDA ratio (A-D) and two photoinitiator concentrations (0.5% and 5.0%). Points given are mean ± standard deviation, n=3. * statistically significant difference (p <0.05) between PI concentrations at constant doxycycline concentration. # statistically significant difference (p <0.05) between doxycycline concentration and at 0 mg/mL concentration at 0.5% PI concentration.
Figure 6.1 Continued
The elastic modulus of each network at both PI concentrations from 0 to 3 mg/mL doxycycline is shown in Figure 6.2. The modulus decreased as the doxycycline concentration increased for most networks at 0.5% PI. For the majority of the networks, there was a statistically significant decrease in the modulus between the 3 mg/mL sample and the 0 mg/mL sample at 0.5% PI. For the 0:100, 5:95, and 25:75 PEGDA: HDDA networks at 5.0% PI, there was no appreciable change in modulus as the doxycycline concentration was varied; however, the 10:90 PEGDA: HDDA network decreased in modulus as doxycycline concentration increased.
Figure 6.2 Modulus as a function of doxycycline concentration for four networks of varying PEGDA: HDDA ratio (A-D) at two photoinitiator concentrations (0.5% and 5.0%). Points given are mean ± standard deviation, n=3. * statistically significant difference (p <0.05) between PI concentrations at constant doxycycline concentration. # statistically significant difference (p <0.05) between doxycycline concentration and at 0 mg/mL concentration at 0.5% PI concentration.
The degradation profiles for the four networks at varying doxycycline concentrations at 0.5%PI are shown in Figure 6.3. The amount of mass loss increased as the PEGDA: HDDA ratio increased, as expected from previous chapter. The effect of doxycycline concentration on mass loss was composition dependent. There was no noticeable trend for the 0:100 PEGDA: HDDA network. For the 5:95 PEGDA:HDDA
network, the mass loss increased by 28 days for the 3mg/mL concentration compared to the 0 and 1 mg/mL. There was no difference for the 10:90 PEGDA:HDDA network after 2 weeks. The 25:75 PEGDA:HDDA network displayed increased levels of mass loss when doxycycline was added. No distinct trends were found between mass loss and PI concentration at a constant PEGDA:HDDA ratio and doxycycline concentration.

Figure 6.3 Degradation profiles for four networks of varying PEGDA: HDDA ratio (A-D) at three concentrations of doxycycline (0, 1, 3 mg/mL). Points given are mean ± standard deviation, n=3.
6.3.2 Release Characterization

UV-Vis spectra for the chemicals and degradation products in distilled water are found in Figure 6.4, where each spectrum was normalized by its maximum peak. The photoinitiator, I2959, has a large peak centered at 280nm. Doxycycline has two distinct peaks at 270 and 350 nm. Rhodamine B has a very distinct peak from the other chemicals around 550 nm. The degradation products from a 25:75 PEGDA:HDDA network after 1
week show peaks below 300 nm that match with I2959, but at a lower intensity. The distinct peaks of doxycycline at 350 nm and rhodamine B near 550 nm were used to determine release profiles.

![UV-Vis spectra of I2959, doxycycline, rhodamine B, and degradation products of 25:75 PEGDA:HDDA network.](image)

Figure 6.4 UV-Vis spectra of I2959, doxycycline, rhodamine B, and degradation products of 25:75 PEGDA:HDDA network.

The release profile for doxycycline from the four networks over 28 days is shown in Figure 6.5. In general, doxycycline was released in greater amounts as the PEGDA:HDDA ratio increased; however, there was no discernable difference in the amount released from the 0:100 and 5:95 PEGDA:HDDA networks. The 0:100 and 5:95 PEGDA:HDDA networks showed a more linear release profile, while the 10:90 and 25:75 PEGDA:HDDA network showed nonlinear release behavior that reached a plateau within 4 and 7 days, respectively. The 0:100, 5:95, and 10:90 PEGDA:HDDA networks released 15-20% of their theoretical amount, while the 25:75 PEGDA:HDDA network released near 50%. The diffusional exponents and constants calculated from Equations 2.1 and 2.3 are given in Table 6.1 for doxycycline release. The diffusional exponent
decreased as the PEGDA:HDDA ratio increased from 1.035 to 0.426. As PEGDA:HDDA ratio increased, $k_1$ increased from 0.0 to 0.246 and $k_2$ decreased from 0.005 to 0.0.

![Graph showing release profile for doxycycline](image)

Figure 6.5 Release profile of doxycycline from four networks of varying PEGDA:HDDA ratio. Points given are mean ± standard deviation, n=3.

<table>
<thead>
<tr>
<th>PEGDA:HDDA</th>
<th>$n$</th>
<th>$k_1$</th>
<th>$k_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:100</td>
<td>1.035 ± 0.249</td>
<td>0.0 ± 0.0</td>
<td>0.005 ± 0.003</td>
</tr>
<tr>
<td>5:95</td>
<td>0.793 ± 0.07</td>
<td>0.01 ± 0.004</td>
<td>0.003 ± 0.001</td>
</tr>
<tr>
<td>10:90</td>
<td>0.469 ± 0.119</td>
<td>0.099 ± 0.033</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>25:75</td>
<td>0.426 ± 0.051</td>
<td>0.246 ± 0.05</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Table 6.1 Diffusional Exponents and Constants for Doxycycline.

The release profile for rhodamine B from the four networks over 28 days is shown in Figure 6.6. In general, rhodamine B was released in greater amounts as the PEGDA:HDDA ratio increased; however, the 0:100 released slightly more than the 5:95 PEGDA:HDDA networks and the 10:90 network released over 30% more than the 25:75 network. All the networks showed a nonlinear release profile, where they started to approach a plateau by day 7. The 0:100, 5:95, and 25:75 PEGDA:HDDA networks released 33, 28, 39% of their theoretical amount, respectively, while the 10:90 PEGDA:HDDA network released near 73%. The diffusional exponents and constants calculated from Equations 2.1 and 2.3 are given in Table 6.2 for rhodamine B release.
The diffusional exponent showed no trend for the 0:100, 5:95 and 10:90 PEGDA:HDDA networks, but decreased at the 25:75 PEGDA:HDDA network. As PEGDA:HDDA ratio increased, $k_1$ showed a general increase, but $k_2$ showed no discernable trend.

![Figure 6.6 Release profile of rhodamine B from four networks of varying PEGDA:HDDA ratio. Points given are mean ± standard deviation, n=3.](image)

Table 6.2 Diffusional Exponents and Constants for Rhodamine B.

<table>
<thead>
<tr>
<th>PEGDA:HDDA</th>
<th>$n$</th>
<th>$k_1$</th>
<th>$k_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:100</td>
<td>0.769 ± 0.016</td>
<td>0.038 ± 0.005</td>
<td>0.025 ± 0.002</td>
</tr>
<tr>
<td>5:95</td>
<td>0.825 ± 0.013</td>
<td>0.022 ± 0.003</td>
<td>0.025 ± 0.001</td>
</tr>
<tr>
<td>10:90</td>
<td>0.794 ± 0.016</td>
<td>0.079 ± 0.013</td>
<td>0.063 ± 0.006</td>
</tr>
<tr>
<td>25:75</td>
<td>0.705 ± 0.025</td>
<td>0.072 ± 0.01</td>
<td>0.023 ± 0.004</td>
</tr>
</tbody>
</table>

6.3.3 Mass Spectrometry

As a secondary method, mass spectrometry was used to evaluate the release of doxycycline, and to evaluate the doxycycline-degradation product interaction. An exemplary raw MRM scan is shown in Figure 6.7, where the peak near 8 minutes was integrated to determine the concentration from a standard curve. The release profile of 25:75 PEGDA:HDDA network over 2 weeks is shown in Figure 6.8. A nonlinear release profile is displayed here, similar to the profile in Figure 6.5. The amount released as measured by mass spectrometry was different from the amount measured by UV-Vis, so
the interaction between doxycycline and the degradation products was further studied as shown in Table 6.3. Again, Method 1 doped doxycycline into the sample before the network degraded, and Method 2 doped doxycycline into the sample after the network degraded. There was a greater reduction in doxycycline at both 1 day and 1 week for method 1; however, a 64% reduction in doxycycline signal was present for Method 2 after 1 day of degradation byproducts had accumulated.

Figure 6.7 Exemplary MRM mass spectrometry data of doxycycline release from 25:75 PEGDA:HDDA network at 1 week.
Figure 6.8 Doxycycline release profile determined by mass spectrometry for 25:75 PEGDA:HDDA network with 1mg/mL doxycycline. Points given are mean ± standard deviation, n=4.

Table 6.3 Doxycycline interaction with degradation products of 25:75 PEGDA:HDDA network. Percent reduction of doxycycline signal between 5μg/mL and 5μg/mL with degradation products.

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Doped before Degradation</th>
<th>Doped after Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>79.9</td>
<td>64.7</td>
</tr>
<tr>
<td>7</td>
<td>85.5</td>
<td>78.7</td>
</tr>
</tbody>
</table>

The degradation products were further analyzed to determine their chemical structure and molecular weight. The $^1$H NMR spectrum of the degradation products from the 25:75 PEGDA:HDDA network after 1 week of degradation is shown in Figure 6.9. Ethylene glycol groups are present at 3.5 ppm, and hexanediol groups at 1.5 ppm are also present, but in smaller quantities. The hydroxyl group from these small molecules is presented near 3.6 ppm, while unpolymerized acrylate groups are found near 6 ppm. Full mass spectrometry scans were performed to determine the range in molecular weights of the degradation products, where an exemplary profile is shown in Figure 6.10. The molecular weight of the degradations products ranged from 115 to 994 m/z. Peaks for hexanediol at
118, the bis-amino acid at 234, ethylene glycol chains near 600, and poly(acrylic acid) chains above 700 were found.

Figure 6.9 NMR spectrum of 25:75 PEGDA:HDDA degradation products in D$_2$O.
Figure 6.10 Exemplary full scan mass spectrometry data of degradation products from 25:75 PEGDA:HDDA network at 1 week.

6.4 Discussion

In this study, the effect of doxycycline concentration on the sol fraction, modulus, degradation profile, and drug release rate of poly(β-amino ester) networks was explored. The PEGDA:HDDA ratio was varied from 0:100 to 25:75 in order to have networks of varying water content where PEGDA concentration controls water content (Chapter 5), and to determine whether doxycycline or network chemical structure had a greater influence on network properties. Two concentrations of I2959 were used to determine the effect of photoinitiator concentration on the network properties. The results show that
doxycycline concentration can significantly change the sol fraction and modulus of select networks. The release studies examined the effect of PEGDA:HDDA ratio on networks containing doxycycline or rhodamine B. While doxycycline is the therapeutic agent of interest, rhodamine B was also used in the release studies because of its distinctive peak that is shifted away from the degradation products. Diffusional exponents and constants were calculated to give insight into the release mechanisms from these biodegradable networks. The impact of doxycycline concentration, PEGDA:HDDA ratio, and photoinitiator concentration on poly(β-amino ester) network properties and release rates will be discussed.

Doxycycline is the chosen therapeutic agent because of its clinical acceptance and previously demonstrated ability to hinder formation and reduce severity of AAA in mice [104]. While doxycycline was used previously for controlled release in periodontal applications, it was released from materials that were not photopolymerized [138]. Doxycycline absorbs in the UV range and overlaps with the absorbance spectrum of I2959, thus UV light necessary for the initiation reaction to cleave I2959 is being absorbed by the doxycycline (Figure 6.4). For a constant amount of photopolymerization time of 30 minutes, the sol fraction increases as the doxycycline concentration increases due the inhibition of the photoinitiator at low concentrations of I2959 (Figure 6.1). When high concentrations of I2959 were used to increase absorbance of I2959, sol fraction remained constant, thus network formation occurred even at 5 mg/mL doxycycline. An increase in sol fraction reduces network formation, thus leaving the network with fewer elastically effective chains and reducing the modulus (Figure 6.2). The degradation profiles are more dependent on PEGDA:HDDA ratio, where increased PEGDA content
increases the water content and allows for increased mass loss. Several of the networks displayed increased mass loss with doxycycline. This may be due to the decreased crosslinking density from a lower modulus that would allow for increased water penetration into the network.

Polymerization issues arise because the chosen photoinitiator and the chosen therapeutic agent have overlapping absorbance ranges. Two possible solutions were considered to address this problem: (1) change the photoinitiator or (2) encapsulate the drug to change its absorbance via microspheres. While the first possibility may seem like the easiest approach, it is not a fruitful approach. Various photoinitiators have been studied in the dental materials field, where they are categorized into Type I and Type II initiators, where Type I have a unimolecular reaction and Type II have a bimolecular reaction, thus needing a co-initiator [141-142]. The Type II initiators react at higher wavelengths away from the absorbance spectrum of doxycycline; however, it is the use of their co-initiator that makes them unproductive. Typically, an additional tertiary amine is used, where the nitrogen donates a free-electron, which is followed by a secondary transfer of a hydrogen from a carbon atom neighboring the nitrogen to form the radical on the amine [143]. This presents its own problem because a tertiary amine structure is incorporated into the backbone of the macromer due to the 3-methoxypropyl amine bonded to the diacrylates. This method of photoinitiation would generate free radicals from the backbones, while propagating and terminating as well. Thus, incorporating doxycycline-loaded microspheres with a distribution of degradation times into the network would be a likely solution if the absorbance of doxycycline is altered by encapsulation.
The release behavior of doxycycline from the networks exhibited a predictable trend, where increasing PEGDA:HDDA ratio increased the release rate and amount (Figure 6.5). PEGDA:HDDA ratio not only controls degradation, but also the water content of the network (Chapter 5), thus allowing for faster release of doxycycline from the network. The 0:100 PEGDA:HDDA network showed zero-order release, where the release rate is independent of time, which was confirmed by a negative $k_1$ and positive $k_2$. The networks without PEGDA are more hydrophobic, uptake less water, and exhibit a more linear degradation profile, which may make them more ideal candidates for drug elution. The 5:95 network displayed anomalous transport characteristics by having a value of $n$ between 0.5 and 1.0, and having values of $k_1$ and $k_2$ that were within one order of magnitude, thus the network has a mixture of Fickian and Case II transport. The values of $n$ near 0.5 for the 25:75 PEGDA:HDDA networks makes them similar to other hydrogel acrylate networks, which often display pure Fickian transport due to the increased water transport [50].

Similar to doxycycline, the release amounts of rhodamine B seemed to be dependent on the PEGDA:HDDA ratio, where increased PEGDA:HDDA ratio increased the amount of rhodamine B released. The effect of PEGDA:HDDA ratio on the diffusional exponents and constants was not as great, where all networks exhibited anomalous transport. Specifically, the 0:100, 5:95, and 10:90 PEGDA:HDDA networks had similar values for $k_1$ and $k_2$. The 25:75 PEGDA:HDDA network showed a lower value of $n$ due to the greater $k_1$ value, which seems appropriate since doxycycline had pure Fickian transport at that composition. Rhodamine B is slightly larger than doxycycline, 479 g/mol compared to 445 g/mol, respectively, which may change the
release characteristics. Also, doxycycline may be more hydrophilic than rhodamine B due to the greater number of hydroxyl and ketone groups on doxycycline, thus the more Fickian behavior as PEGDA concentration increased.

Mass spectrometry was used as a secondary method to analyze the release rate of doxycycline; however, due to cost and equipment time, only one network was analyzed, the 25:75 PEGDA:HDDA with 1 mg/mL doxycycline. While the network displayed a nonlinear release profile similar to the profile in Figure 6.5, the release amounts were never greater than 5 μg, when it was expected to release near 100 μg theoretically. In a previous study, doxycycline was found to have no chemical interaction with PLGA when encapsulated in PLGA microspheres; however, the drug-degradation product interactions were not studied [144]. Further studies on the interaction of doxycycline and the degradation product were undertaken in order to understand the apparent low release (Table 6.3). The degradation products interact with doxycycline enough to mask more than 60% of the drug from MRM mass spectrometry with only 1 day of degradation occurring. While the drug is eluting, the interaction is forming a drug-degradation product complex with a different mass that is not being detected. While the exact complex is not known, the acidic degradation products could be bonding to doxycycline at its primary amine functionality or one of its hydroxyl groups.

The degradation products from the cleavage of the ester bonds are: hexanediol, oligo(ethylene glycol), a bisamino acid based on 3-methoxypropylamine, and poly(acrylic acid). Degradation is occurring as seen by the mass loss in Figure 6.3, but also due to the presence of the hydroxyl group near 3.6 ppm in Figure 6.9. The ethylene glycol groups and hexanediol groups indicate those degradation products. From the full
scan mass spectrometry results, the distribution of peaks suggests a heterogeneous size distribution of the oligo(ethylene glycol) groups and poly(acrylic acid) chains, and the presence of the bisamino acid and hexanediol. The oligo(ethylene glycol) products would have m/z near 700 and less, thus the greater peaks are due to the poly(acrylic acid) chains. Because mass spectrometry cannot be used in conjunction with PBS due to the accumulation of salts in the instrument, the reduced signal from degradation product interaction, and cost, UV-Vis is the recommended method for doxycycline characterization for these networks.

6.5 Conclusions

In this study, the influence of doxycycline on poly(β-amino ester) network properties was examined. Doxycycline inhibits the initiation reaction of the photoinitiator, thus limiting the network formation, which decreases the modulus. Degradation is still mainly governed by PEGDA concentration, but doxycycline increases degradation in select networks. Increasing PEGDA concentration changes the release mechanism from Case II to anomalous to Fickian for doxycycline release and rhodamine B showed a similar trend at high PEGDA concentrations. These networks allow for controlled drug delivery since the release mechanism and rate can be readily tailored by changing the network’s chemical structure.
CHAPTER 7
THERMO-MECHANICAL PROPERTIES OF POLY(β-AMINO ESTER)-CO-METHYL METHACRYLATE NETWORKS UNDER SIMULATED PHYSIOLOGICAL CONDITIONS

7.1 Introduction

Methacrylate copolymer networks represent a class of materials that display a wide range of mechanical properties. These networks’ thermo-mechanical properties are mainly controlled by two parameters, the glass transition temperature (Tg) and the crosslinking density [35, 57, 74-76]. These networks can be further altered with additional functional groups that allow for partial or full degradation of the polymer networks making them suitable candidates for tissue scaffolds [59, 121, 124, 145]. This added dimension of functionality allows for a broad range of mechanical properties and degradation processes. Understanding the intrinsic structure-property relationships that control mechanical properties is critical for developing biomedical materials. A number of relationships have been established between crosslinking density, glass transition temperature, bulk mechanical properties, and environmental conditions for methacrylate networks (Chapter 2). Poly(β-amino ester) networks have been proposed as a family of synthetic biodegradable networks that undergo hydrolytic cleavage. The glass transition temperature of these networks is often sub-ambient due to the high molecular weight of the crosslinkers and the lack of steric hindrance due to a lack of rigid sidegroups. There has been limited evaluation of the mechanical properties of these networks to date, as they do not possess the inherent toughness for use as load bearing materials. Profiles of modulus under immersed conditions have been studied, where the modulus is shown to
decrease due to the degradation of the crosslinks [42]. Typically, the testing has been done in compression; however, the modulus decrease has been similarly observed in tension as well [146]. Specifically, the failure strain and toughness have yet to be systematically reported for these polymer networks.

The current poly(β-amino ester) networks have low Tg and high crosslinking density, thus may be lacking in sufficient toughness to survive implantation in load bearing applications. In order to increase their mechanical properties, additional monomers can be added to the network to increase Tg and decrease crosslinking density. Therefore, the purpose of this work was to determine the effect of varying methyl methacrylate concentration on the thermo-mechanical properties of poly(β-amino ester)-co-MMA networks under physiological conditions. Crosslinkers of varying diacrylate to amine ratio were used to determine the effect of varying the crosslinking density on these poly(β-amino ester)-co-MMA networks. The effect of MMA concentration on glass transition temperature, rubbery modulus, failure strain, toughness, and degradation is studied through systematic variation of MMA concentration. Select networks were tested in a simulated physiological environment in order to understand the effect of degradation on the mechanical properties. The use of MMA to increase the Tg and decrease the crosslinking density should enhance the toughness of the networks during degradation.

7.2 Experimental

7.2.1 Materials

Networks were composed of HDDA, 3MOPA, and MMA in this study. I2959 was the photoinitiator. Macromers were formed from reacting HDDA and 3MOPA in molar ratios ranging from 1.05:1 to 1.20:1 according to the step-growth polymerization method.
in Chapter 3. MMA was added in varying wt% before photopolymerization, where 45 minutes was given according to the method in Chapter 3.

### 7.2.2 Methods

DMA was run according to Chapter 3 to a maximum temperature of 200°C. The Tg was defined as the peak of the tan delta, and the rubbery modulus was defined as the modulus in the rubbery plateau at a temperature of Tg+75°C. The mean ± standard deviation with n=3 is given for each data point. DSC was run according to Chapter 3 to a maximum temperature of 200°C. Samples were degraded in PBS for varying timepoints, up to 8 weeks, dried in an oven for 24 hours to remove any water, then analyzed by DSC to determine any changes in Tg due to degradation. The Tg was determined from the intersecting line method of the second order thermal transition. The mean ± standard deviation with n=3 is given for each data point.

Bulk mechanical tests were performed according to the methods in Chapter 3 for both standard testing at 37°C and environmental tensile testing *in vitro* at 37°C over 8 weeks of immersion. Toughness was calculated using the trapezoidal rule from the area under the stress strain curve and given in MJ/m$^3$. Modulus was determined from the slope of the initial linear region of the stress-strain curve. The mean ± standard deviation with n=4 is given for each data point.

Degradation profiles and water content experiments were run according to the method in Chapter 3 for 8 weeks. Water content and mass loss and were calculated using Equations 3.2 and 3.3, respectively.
7.3 Results

7.3.1 Thermo-mechanical Properties

The Tg and storage modulus at different temperatures were characterized via DMA. The Tg as a function of wt% MMA is shown in Figure 7.1. By adding MMA to the degradable crosslinkers, the Tg increased from -44°C to 80°C in a manner independent of the diacrylate to amine ratio.

![Figure 7.1](image.png)

Figure 7.1. The glass transition temperature as determined by the tan delta from DMA. Biodegradable crosslinkers formed from four varying HDDA:3MOPA ratios were mixed with varying amounts of MMA.

The storage modulus at 37°C varied over two orders of magnitude as MMA concentration increased as shown in Figure 7.2A. The networks transitioned from a rubbery state to a glassy state as the wt% MMA increased. At MMA concentrations under 45%, the crosslinkers were influenced by their diacrylate to amine ratio due to the low Tg of the materials. The modulus increased as the ratio increased in this lower MMA concentration region, but as the MMA concentration increased above 45%, the diacrylate
to amine ratio did not influence the storage modulus at 37°C because the material was now glassy at this temperature. From the same DMA scans, the storage modulus at a temperature of Tg+75°C decreased as wt % MMA increased, as shown in Figure 7.2B. While Figure 7.2A is at a constant temperature, Figure 7.2B accounts for the variation in the Tg of each composition, and the material is in its rubbery state at this temperature. At a given MMA concentration, the modulus decreased due to the decrease in crosslinking density as the diacrylate to amine ratio decreased. Similarly, at a constant diacrylate to amine ratio, the modulus decreased as the crosslinking density decreased due to higher percentages of MMA. There was a statistically significant difference in the storage modulus in Figure 7.2B as the wt% MMA increased.

Figure 7.2. (A) The storage modulus as a function of MMA concentration at 37°C. (B) The storage modulus at a temperature of Tg+75°C as a function of MMA concentration. Four crosslinkers composed of HDDA:3MOPA at varying diacrylate to amine ratio were mixed with MMA.
7.3.2 Mechanical Properties

The initial mechanical properties of these networks were characterized using tensile tests at 37°C under dry conditions. Representative stress-strain curves of the HDDA 1.15 crosslinker with varying wt% MMA are shown in Figure 7.3. As wt% MMA increased, the failure strain increased, but there was little variation in the modulus of the material until large concentrations of MMA were used (>75%), where the material acted in an elastic-plastic manner as demonstrated by a high modulus, yield point, and reasonable strain to failure.
A toughness vs. elastic modulus plot of the four crosslinkers at varying %MMA is shown in Figure 7.4A. As the wt% MMA increased, the toughness increased by nearly three orders of magnitude. The diacrylate to amine ratio showed no appreciable effect on toughness with only small changes in elastic modulus. Eventually, as the wt% MMA increased, the modulus increased due to the transition from a rubbery to a glassy state. The toughness as a function of the temperature difference between the glass transition temperature and testing temperature (37°C) is shown in Figure 7.4B. As the wt% MMA increased, the toughness increased due to the shift in the glass transition temperature. As the Tg shifted closer to and beyond 37°C, the toughness increased at least one order of magnitude. Again, the diacrylate to amine ratio showed no significant effect on toughness.
Figure 7.4. (A) Toughness as a function of elastic modulus for four different crosslinkers of varying diacrylate to amine ratios of varying %MMA. (B) Toughness as a function of temperature relative to the glass transition temperature, where the testing temperature was 37°C.

7.3.3 Degradation and Mechanical Properties under Physiological Conditions

The degradation profile of networks composed of HDDA:3MOPA 1.15 with varying wt% MMA is shown in Figure 7.5A. The mass loss decreased as the wt% MMA increased. HDDA 1.15 without MMA degrades at a slow rate, where 20% mass loss was observed over 12 weeks (Chapter 4). The amount of water uptake, as shown in Figure
7.5B, was 30% of the original weight of the network, where the 55%MMA and 75%MMA networks have decreased water contents. The Tg as measured by DSC over 8 weeks of immersion is shown in Figure 7.6, and exemplary raw DSC curves are shown in Figure A.4. The extent of change and direction of change in Tg was dependent on composition. The 0% MMA networks had no noticeable change in Tg over the 8 weeks. The 35, 45, and 55%MMA networks showed an upward shift by 10 to 20°C. The 75%MMA showed a decrease in Tg by 7°C after 8 weeks. There were statistically significant differences in Tg for each %MMA concentration over the 8 weeks.

Figure 7.5. (A) Degradation profile over 8 weeks for 5 networks of varying %MMA. (B) The water content profile over 8 weeks for 5 networks of varying %MMA.
Figure 7.6. The glass transition temperature as determined by DSC for the 5 networks over 8 weeks of immersion in saline.

Exemplary stress-strain curves for 5 HDDA 1.15-co-MMA networks of varying wt% MMA over 8 weeks of immersion under physiological conditions are shown in Figure 7.7. HDDA 1.15 has low stress and failure strain under immersed conditions (Figure 7.7A). As time increased, its failure strain increased and modulus decreased allowing for greater deformation, but eventually the failure strain and failure strength
decreased by 8 weeks. The 35% and 45% MMA networks behave in a similar manner, where the modulus decreased and the failure strain increased up to 6 weeks, but by 8 weeks, the modulus increased again. The 55% MMA network showed minimal change in mechanical properties over time, maintaining high failure strains for all 8 weeks. The 75% MMA network shows initial softening as the modulus decreases and the failure strain increases during the initial weeks. At 8 weeks, the material returns to a more glassy state, absent of yielding and plastic deformation.

Figure 7.7. (A-E) Stress-strain curves of HDDA 1.15-co-MMA networks over 8 weeks. The wt% MMA varied from 0 to 75%.
Figure 7.7 Continued
Figure 7.7 Continued

Failure strain and toughness profiles of the HDDA-co-MMA networks are shown in Figure 7.8. As wt% MMA increased, failure strain increased by two orders of magnitude. As immersion time increased, the failure strain behavior was composition dependent. The 0%MMA network saw an increase in failure strain at 4 weeks, but then a decline by 8 weeks. The 35% and 45%MMA networks show an overall increase in failure strain during the 8 weeks. The failure strain of the 55%MMA network remained
unchanged during the 8 weeks. The failure strain of the 75%MMA network had an increase from 4-6 weeks, but then a decrease by an order of magnitude at 8 weeks. As the %MMA increased, the toughness increased for all networks. The effect of immersion time on toughness varied depending on the wt% MMA. For example, the toughness of the 35% and 45% MMA networks increased over 8 weeks. The 0%MMA network showed an increase in toughness at 4 weeks then a decrease by 8 weeks, similar to its failure strain profile. The 75%MMA network had the highest toughness for 6 weeks, but decreased at 8 weeks leaving the 55%MMA network with sustained toughness for the 8 weeks. The toughness of the 75% MMA network approached values close to pure PMMA [96]. The 35%MMA network showed a statistically significant difference between 0 weeks and 6 weeks. The 75%MMA network showed a statistically significant difference between the 8 week time point and all other timepoints.

![Graph](image)

Figure 7.8. (A) Failure strain profile over 8 weeks for HDDA-co-MMA networks. (B) Toughness profile over 8 weeks for HDDA-co-MMA networks.
In this study, the effect of varying the MMA concentration on the mechanical properties of poly(β-amino ester) networks was explored. Biodegradable crosslinkers of varying molecular weight were used to determine if the crosslinking density had an effect on the mechanical properties. The results show that wt% MMA can significantly change the thermo-mechanical properties of the networks, particularly in the presence of saline. The poly(β-amino ester) system of HDDA:3MOPA was chosen because its degradation rate and elastic modulus can be tailored independently of its modulus and has previously demonstrated promise for biocompatibility (Chapter 4) and [146]. While other linear chain builders of different Tgs could have been used, MMA was chosen because of its wide clinical use in bone cement. While, pure MMA is nondegradable, mass loss was expected due the biodegradable nature of the HDDA:3MOPA crosslinker. The impact of wt% MMA on poly(β-amino ester) networks’ mechanical and thermal properties, especially under simulated physiological conditions, will be discussed below.
DMA was used as a screening method to evaluate thermo-mechanical properties. Since a rule of mixtures often applies to Tg for amorphous acrylates, adding MMA is expected to increase the Tg, thus allowing the networks to transition from a rubbery to glassy state, as seen in Figure 7.1 and Figure 7.2A. Each network, at a constant wt% MMA had the same Tg regardless of the molecular weight of the HDDA:3MOPA crosslinker. The crosslinking density change was not sufficient to alter Tg of the networks; however, the molecular weight of the crosslinkers did influence the rubbery modulus, as seen in Figure 7.2B. This is expected since the molecular weight between crosslinks is inversely proportional to the modulus for rubbery materials [147]. At a constant diacrylate to amine ratio, increasing the wt% MMA not only increases Tg, but decreases crosslinking density, which was seen in the decrease of the rubbery modulus, as seen in Figure 7.2B. Lowering the crosslinking density can enhance the mechanical properties by allowing larger strain capacity [90].

After exploring the changes in Tg and rubbery modulus, further bulk mechanical properties were examined to determine the relationship between wt% MMA and toughness. First, adding MMA to the networks increased the failure strain, thus increasing the toughness, but did not significantly alter the modulus. Thus, the addition of MMA allows for unique tailoring of mechanical properties, where toughness can be maximized in a specific range of moduli. Figure 7.4A further demonstrates the tailorability of this system, where the molecular weight (diacrylate to amine ratio) controls the modulus and the wt% MMA controls the toughness. Further tailoring can be achieved by considering the testing or environmental temperature. The toughness is dependent upon the Tg of the network as seen in Figure 7.4B. With a set operating
temperature and varying Tg, the networks with Tg above the operating temperature had the highest toughness. If Tg continued to shift above the operating temperature, eventually the material becomes brittle and has a lower toughness, such as PMMA which has a toughness near 1 MJ/m$^3$ [96]. Using a pure PMMA network or lightly crosslinked network would not maximize toughness at 37°C, since PMMA has maximized toughness near 100°C. Therefore, it is critical to tailor both the Tg and the crosslinking density in order to tailor toughness for the desired application.

For the networks under immersed conditions, the starting HDDA:3MOPA 1.15:1 composition has limited degradation over 8 weeks, where it previously degraded near 20% over 12 weeks of immersion, thus the low mass loss observed in Figure 7.5A. Networks of 35% and 45% MMA show similar trends in degradation rate, thus full degradation may be expected over the long term. Theoretically, if enough crosslinks degraded, the network would fall apart; however, if the number of crosslinks were not sufficient as in the 55% and 75% MMA networks, the networks may not cleave enough to enable complete degradation with MMA backbones present. The steady increase in water content in the networks suggests full degradation is possible as the increased water content further promotes the hydrolytic cleavage of the crosslinkers. Further long term testing may be necessary to reveal the structural changes over extended periods of time.

While many biomedical devices are grouped into two categories, degradable or nondegradable, these HDDA-co-MMA networks represent a semi-degradable option, which would allow for some degradation for drug release and still maintain sufficient mechanical properties. Another semi-degradable system was formed from a composite of a nondegradable poly(vinyl alcohol) scaffold with degradable PLGA microspheres for
articul cartilage repair [148]. This approach combined nondegradable and degradable components at the molecular-scale, where the current approach creates a semi-degradable polymer network at the network-scale. Processing with a porogen would no longer be an issue for incorporating microspheres, since the matrix itself would have degradable linkages to allow tissue incorporation and release of therapeutic agents.

Since the Tg of the networks is intrinsically related to both the chemical structure and the mechanical properties, it was necessary to understand Tg during the course of degradation. The Tg increased in several networks due to the degradation of the low Tg component, the biodegradable crosslinker, thus leaving more MMA in place. The 75% MMA network represented a different case, where due to its lack of degradation and low water content, the Tg decreased due to the infiltration of water, which has been previously observed in nondegradable methacrylate based networks [96]. In the other networks, the upward shift in Tg verifies the loss of the degradable crosslinker, but this shift in Tg also has effects on the mechanical properties.

Interestingly, the mechanical properties, namely failure strain and toughness, changed in a manner opposite of what would be expected for degradable networks. Typically, a decrease in modulus and toughness occurs for degradable networks. However, the presence of MMA to maintain a high Tg and the loss of the low Tg component allowed for the shift in Tg to enhance the mechanical properties over time for several networks in a composition dependent manner. As immersion time increased, the failure strain of the 0%MMA network increased due to the decrease in crosslinking density, but eventually further degradation decreased the failure strength and failure strain by 8 weeks. The 35% and 45% MMA networks behave in a similar manner, where
the modulus decreased and the failure strain increased due to the degradation of the crosslinks up to 6 weeks, but by 8 weeks, the modulus increased again due the increase in Tg. The 45% MMA network showed an increase in both Tg and toughness during the 8 weeks, indicating that an increase in Tg under physiological conditions can enhance and sustain toughness of these networks. The 55% MMA network showed minimal change in mechanical properties over time due to the lack of degradation and low water content. This composition would represent a balance point, where degradation would cause a decrease in modulus, but the increase in Tg would increase the modulus. While networks with moderate concentrations of MMA (35-55%) displayed sustained toughness, the 75% network became brittle by 8 weeks. The loss of the low Tg component and increasing water uptake would have made the structure more similar to pure PMMA, thus the decrease in failure strain and toughness.

Poly(β-amino ester)-co-MMA networks may be used to independently tailor the modulus and toughness, and the choice of linear chain builder and the resulting shifts in Tg can enhance mechanical properties over sustained degradation. Several modifications to these networks could allow for further tailorability. The Tg of the linear chain builder could range from subambient to well over 100°C. This would allow further control of the shift in Tg as the biodegradable component leaves the network. Other linear chain builders could augment the hydrophilicity and water content, thus increasing the degradation rate, the shift in Tg, and the resulting change in mechanical properties. This method of combining a low Tg degradable crosslinker with a nondegradable linear chain builder establishes a platform for design of a multitude of semi-degradable materials. The biodegradable crosslinkers can be produced in a facile manner and readily mixed with
any number of methacrylates to produce semi-degradable networks that would allow possible therapeutic release and sustained mechanical properties in the \textit{in vivo} environment.

\textbf{7.5 Conclusions}

In this study, poly(\( \beta \)-amino ester)-co-MMA networks were polymerized to create semi-degradable tough networks. The influence of the molecular weight of the biodegradable crosslinker is limited to the modulus when the material is in its rubbery state. MMA concentration has a dual effect of increasing Tg and decreasing crosslinking density, which toughens the networks by several orders of magnitude. Under more physiological conditions, the mechanical properties change in a composition dependent manner, where select networks increase in toughness or sustain toughness while undergoing degradation. These networks establish a platform for designing semi-degradable networks with improved mechanical properties during degradation, which would allow for therapeutic release from tough implants.
CHAPTER 8

EFFECT OF DIACRYLATE CHEMISTRY ON MECHANICAL PROPERTIES OF SEMI-DEGRADABLE POLY(B-AMINO ESTER) NETWORKS IN VITRO AND IN VIVO

8.1 Introduction

Biodegradable polymers have seen broad application in the biomedical field due to their range of mechanical properties and suitable biocompatibility. Currently, they are used in applications, such as orthopedic implants, drug delivery devices, and tissue scaffolds [11, 149-150]. They are widely accepted because of their ability to degrade under physiological conditions, but the degradation leads to a loss in mechanical properties, a pertinent characteristic for load-bearing implants. The loss of mechanical properties will always occur for these biodegradable polymers, but the rate is dependent upon the erosion mechanism and the rate of degradation. Often used in orthopedics, poly(L-lactic acid) (PLLA) shows more rapid degradation in vivo than in vitro, where most of the load carrying capacity is lost between 6 to 12 weeks of implantation, and only 3-4% load carrying capacity remains by 48 weeks for PLLA fibers [18]. Similarly, PLLA rods lost half their bending strength in 3 weeks and only a quarter remained by 6 weeks [19]. Bulk-degrading poly(glycolic acid) (PGA) loses more than 50% of its strength within 4 weeks, where slower surface eroding poly(anhydrides) lost 30% tensile modulus at 50% mass loss [11, 16, 34].

Biodegradable polymers are used as controlled release devices that can deliver drugs locally instead of systemically. Degradation occurs by hydrolytic or enzymatic cleavage of specific bonds, where cleavage can occur along a linear polymer backbone or
at multiple sites within a polymer network to produce low molecular weight water soluble products [12-13]. The erosion of the biodegradable polymers occurs by a bulk mechanism with homogeneous hydrolysis, a surface mechanism with hydrolysis limited to the outside of the device, or a complex mixture of the two. Besides these mechanisms, the polymer degradation and release kinetics of the drug are influenced by several factors: crystallinity, polymer molecular weight, hydrophilicity, swelling, and crosslinking density [45, 47, 53, 151]. Glassy hydrophilic acrylate networks composed of poly(HEMA-co-MMA) showed composition dependent release rates due to increased hydrophobicity of the MMA comonomer [48]. A photocrosslinkable biodegradable PEG-PLA hydrogel showed rapid release of low molecular weight molecules, but slowed the release of large molecules because diffusion was limited by the hydrogel mesh size [152].

Poly(β-amino ester)s and their networks are a class of biodegradable polymers that have a wide range of chemistries and properties [35]. From the point of view of tailoring mechanical properties, poly(β-amino ester) networks are a subclass of acrylate networks with a degradable linkage inserted into the polymer backbone. The exploration of the poly(β-amino ester)s networks’ mechanical properties has been limited, where a previous study has looked at controlling crosslinking density with triacrylates for tissue scaffolds [43]. While the crosslinking density dictates the modulus in the rubbery regime, the toughness is primarily controlled by the glass transition temperature (Tg) for (meth)acrylate networks [96]. The Tg can be increased by adding monomers, like methyl methacrylate, which have a higher Tg than the macromers (Chapter 7). By varying the crosslinking density and Tg independently, the modulus and toughness exhibit large ranges that can more closely match the properties of biological tissues.
Current biodegradable polymers have the appropriate modulus range, but do not possess sustained toughness to provide mechanical stability *in vivo*. Therefore, the purpose of this study was to determine the effect of varying diacrylate chemistry on the mechanical and thermal properties, and degradation of poly(β-amino ester)-co-MMA networks while degrading *in vitro* and *in vivo*. In order to control toughness during degradation, poly(β-amino ester) networks will be tailored with varying diacrylates and a comonomer of a different Tg. Initially, networks were toughened by adding a set concentration of MMA. The effect of diacrylate chemistry on the degradation rate, mechanical properties, glass transition temperature, and network structure is revealed by systematically varying the ratio of two diacrylates, while holding constant the diacrylate to amine ratio and the concentration of MMA. Select networks were immersed in simulated physiological conditions or implanted subcutaneously, then characterized with multiple techniques. The use of two diacrylates to control the degradation rate and resulting shift in glass transition temperature should allow for temporal control of toughness.

8.2 Experimental

8.2.1 Materials

HDDA, PEGDA, 3MOPA, MMA, I2959, and rhodamine B were the materials used in this chapter. PEGDA and HDDA were mixed in molar ratios of 0:100, 10:90, and 25:75 before reacting with 3MOPA at a molar ratio of 1.15:1. The step-growth polymerization followed the method from Chapter 3. The macromers were mixed with 55 wt% MMA and polymerized for 45 minutes following the photopolymerization method
for MMA in Chapter 3. Rhodamine B was added at a concentration of 1 mg/mL before photopolymerization for the networks for the release study.

8.2.2 Methods

The *in vitro* degradation method was followed to determine mass loss from Chapter 3. The *in vivo* degradation method from Chapter 3 was followed for implantation of dog bone samples for mechanical testing and further characterization.

Strain-to-failure tensile tests were performed on samples degraded *in vitro* and *in vivo* according to the environmental tensile testing method in Chapter 3. ATR-FTIR was run on *in vitro* and *in vivo* degraded samples according to the method of Chapter 3 at 0, 2, and 8 weeks.

DSC was run on samples that were degraded *in vitro* and *in vivo* according to the methods in Chapter 3 to a temperature of 200°C. The mean ± standard deviation with n = 3 is given for each data point. DMA was used to characterize the rubbery modulus and Tg of the *in vitro* degraded samples following the methods in Chapter 3 to a temperature of 200°C. The mean ± standard deviation with n = 3 is given for each data point.

Each of the three compositions was mixed with a model drug, rhodamine B, at a concentration of 1 mg/ml before photopolymerization. The drug release protocol was followed from the method in Chapter 3 using 556 nm on the UV-Vis instrument. Equation 2.1 and Equation 2.3 were used to determine the diffusional exponent and constants. Statistical analysis of mass loss, toughness, and Tg via DSC followed the method of Chapter 3.
8.3 Results

8.3.1 Degradation of Networks

Degradation was characterized by mass loss over 8 weeks for the three networks under both \textit{in vitro} and \textit{in vivo} conditions as shown in Figure 8.1. The mass loss increased as the PEGDA:HDDA ratio increased under both conditions. The 25:75 PEGDA:HDDA network showed a rapid mass loss within the first two weeks, but the 10:90 and 0:100 PEGDA:HDDA networks showed lower amounts of mass loss by 8 weeks. There was no statistically significant difference in mass loss for each material at 2 weeks and 8 weeks between \textit{in vitro} and \textit{in vivo} degradation conditions.

![Figure 8.1. Mass loss profiles of PEGDA:HDDA 1.15 +55\%MMA networks of varying PEGDA:HDDA ratio over 8 weeks (A) \textit{in vitro} and (B) \textit{in vivo}. Each point is the mean ± standard deviation, n=3.](image)

Figure 8.1. Mass loss profiles of PEGDA:HDDA 1.15 +55\%MMA networks of varying PEGDA:HDDA ratio over 8 weeks (A) \textit{in vitro} and (B) \textit{in vivo}. Each point is the mean ± standard deviation, n=3.
ATR-FTIR was used to determine the changes in chemical structure of the network for both degradation conditions. Figure 8.2 shows exemplary spectra for the 10:90 PEGDA:HDDA network under both degradation conditions. Characteristic peaks for carbonyl, carboxyl, and ether bonds were analyzed at 1730, 1590, and 1147 cm\(^{-1}\), respectively [111-112]. For *in vitro* degradation shown in Figure 8.2A, the carbonyl bonds and ether bonds decrease at both 2 weeks and 8 weeks; however, the carboxyl bonds are not prominent until 8 weeks of degradation. For *in vivo* degradation shown in Figure 8.2B, the carbonyl bonds and ether bonds also decrease at both 2 weeks and 8 weeks, but the carboxyl bonds became prominent by 2 weeks of degradation.
8.3.2 Mechanical Properties

Exemplary stress-strain curves of the three networks under \textit{in vitro} degradation conditions are shown in Figure 8.3. The 0:100 PEGDA:HDDA network maintains the same mechanical behavior over 8 weeks without any significant changes in failure strain or modulus. The 10:90 PEGDA:HDDA network maintained similar properties during the first 4 weeks with increases in average ultimate strength from 2.0 to 2.8 MPa. From 4 to 6 weeks, the 10:90 PEGDA:HDDA network had an increase in average failure strain from 71\% to 182\%. By 8 weeks, the 10:90 PEGDA:HDDA network had reduced average
failure strain, 123%, and increased average modulus from 6.1 to 18.2 MPa. The 25:75 PEGDA:HDDA network undergoes the largest changes in mechanical properties, where initially it acts as a highly crosslinked rubber then shifts to a more glassy material with both elastic and plastic deformation. From 0 to 2 weeks, the average modulus and average failure strength increased from 3.7 to 115 MPa and 2.1 to 7.6 MPa, respectively, but the failure strain remained equivalent. From 2 to 4 weeks, the 25:75 PEGDA:HDDA network increased in average failure strain from 80% to 148% and further increased in average failure strength from 7.6 to 9.3 MPa. At 6 weeks, the 25:75 PEGDA:HDDA network still continued to increase in strength, but underwent less plastic deformation and failed at a lower average failure strain of 66%. By 8 weeks, the 25:75 PEGDA:HDDA network had lost much of its ductility, where after yielding it underwent less plastic deformation and failed at an average failure strain of 9%.

Figure 8.3. Exemplary stress-strain curves of PEGDA:HDDA 1.15 +55%MMA networks of varying PEGDA:HDDA ratio over 8 weeks in vitro. Ratio (A) 0:100, (B) 10:90, (C) 25:75.
Exemplary stress-strain curves of the three networks degraded in vivo are shown in Figure 8.4. The 0:100 PEGDA:HDDA network maintains its properties and behavior over 8 weeks, where a 1-2 MPa loss in ultimate strength occurs over 8 weeks. The 10:90 PEGDA:HDDA network exhibits rubber-like behavior initially, and continues at 2 weeks with an increase in modulus and decrease in average failure strain from 139% to 58%. At 8 weeks, the 10:90 PEGDA:HDDA network exhibits an initial elastic region followed by
plastic deformation and ultimately failure, where the average modulus increased from 5.9 to 86.0 MPa. Again, the 25:75 PEGDA:HDDA network showed large changes in mechanical behavior over the 8 weeks. Initially, it had a rubber-like behavior with high average failure strains of 198% and an average modulus of 2.7 MPa. At 2 weeks, it had transitioned to a hard, ductile polymer with an initial elastic region with a modulus of 99.3 MPa followed by plastic deformation and average failure strain at 103%. At 8 weeks, it had transitioned into brittle behavior with a loss of ductility, where no noticeable plastic region was observed. Only one sample of the 25:75 PEGDA:HDDA network could be tested at the 8 week time period as the other two samples had failed prior to removal from the animal.

Figure 8.4. Exemplary stress-strain curves of PEGDA:HDDA 1.15 +55%MMA networks of varying PEGDA:HDDA ratio over 8 weeks in vivo. Ratio (A) 0:100, (B) 10:90, (C) 25:75.
Figure 8.4 Continued

Toughness profiles over 8 weeks for both degradation conditions are shown in Figure 8.5. The networks degraded in vitro have profiles that are composition dependent. The 0:100 PEGDA: HDDA network remains between 2-3 MJ/m$^3$ for the entire 8 weeks with no statistically significant changes in toughness. The 10:90 PEGDA:HDDA network increases in toughness at 6 weeks from 1.18 to 3.37 MJ/m$^3$, which is statistically significant and maintains toughness through 8 weeks with no further statistically
significant changes. The 25:75 PEGDA:HDDA network increases in toughness to a maximum at 4 weeks with an average toughness of 10.12 MJ/m$^3$, which is a statistically significant increase from all other time points. At 8 weeks, the 25:75 PEGDA:HDDA network’s average toughness is less than its initial toughness, but not a statistically significant difference. The networks degraded in vivo exhibit similar toughness profiles between compositions. The 0:100 PEGDA: HDDA network decreases by 1 MJ/m$^3$ from 0 to 2 weeks, which is statistically significant. The 0:100 PEGDA: HDDA network maintains toughness for the remaining 6 weeks without any statistically significant changes. Initially near 4 MJ/m$^3$, the 10:90 PEGDA:HDDA network and 25:75 PEGDA:HDDA network both decrease by about 3 MJ/m$^3$ from 0 to 2 weeks, which is statistically significant. The 10:90 PEGDA:HDDA network then increases by 1 MJ/m$^3$ from 2 to 8 weeks to a toughness equivalent to the 0:100 PEGDA:HDDA network, which is a statistically significant increase. The 25:75 PEGDA:HDDA network’s toughness increases from 2 to 8 weeks; however, the 8 week timepoint consists of only one tensile test.
8.3.3 Thermo-mechanical Properties

DSC was used to determine the Tg of the three networks under both degradation conditions as shown in Figure 8.6A and 8.6B. DMA was used as a comparative technique to determine Tg as shown in Figure 8.6C for in vitro degraded networks. All three networks showed an increase in Tg after in vitro degradation in Figure 8.6A from near 20°C to near 50°C; however, Tg increased more quickly as PEGDA:HDDA ratio
increased. The 25:75 PEGDA:HDDA network showed an increase by 30°C within the 2 weeks, and the Tg held at that temperature for the remaining 6 weeks. The 10:90 PEGDA:HDDA network showed a steady increase in Tg over the 8 weeks with an overall increase near 30°C. The 0:100 PEGDA:HDDA network showed an increase in Tg at 2 weeks, then it was constant for 4 weeks. Eventually, a large shift in Tg occurred between 6 to 8 weeks. The networks degraded in vivo showed similar increases in Tg, where at the 8 week timepoint there is no statistically significant difference between the in vitro Tg and in vivo Tg for each network. As the PEGDA:HDDA ratio increased, Tg increased more quickly in the first two weeks. Similarly, the 25:75 PEGDA:HDDA network showed the largest increase in Tg in the first 2 weeks, which was maintained. The 10:90 PEGDA:HDDA network and the 0:100 PEGDA:HDDA network showed smaller increases in the first 2 weeks, but their Tgs had increased by 8 weeks to 40-45°C. From the DMA measurements, the increase in Tg followed the same pattern as the DSC, but not as large increases for the 0:100 and 10:90 PEGDA:HDDA network. The 25:75 PEGDA:HDDA network showed the largest increase in Tg by increasing 40°C from its initial Tg. The Tg of the 10:90 PEGDA:HDDA network increased by 20°C, but the Tg of the 0:100 PEGDA:HDDA network increased by only 5°C during the 8 weeks. Again, DSC is measuring a thermal transition to determine Tg, and DMA is examining the thermo-mechanical transition at tan delta to define Tg. When a heterogeneous network is examined, its Tg may be very broad, thus the difference between the two methods, where a broad thermal transition is more difficult to detect on DSC.
Figure 8.6. Glass transition temperature profiles for PEGDA:HDDA 1.15 +55% MMA networks of varying PEGDA:HDDA ratio over 8 weeks (A) *in vitro* and (B) *in vivo* determined by DSC. (C) *in vitro* determined by DMA. Each point is the mean ± standard deviation, n=3.
In order to characterize the network structure during degradation, the modulus was measured in the rubbery regime at a temperature of Tg+75°C for the three networks that degraded in vitro. Figure 8.7 shows the change in rubbery modulus over 8 weeks. As the PEGDA:HDDA ratio increased, there was a greater decrease in the rubbery modulus. The 0:100 PEGDA:HDDA network had a constant rubbery modulus during the 8 weeks with no statistically significant differences. The rubbery modulus of the 10:90 PEGDA:HDDA network decreased by 0.84 MPa by 8 weeks, which was a statistically significant difference. The rubbery modulus of the 25:75 PEGDA:HDDA network decreased by 1.17 MPa by 8 weeks, which was a statistically significant difference.
8.3.4 Model Drug Release

In order to demonstrate the feasibility of eluting a model drug from the networks, rhodamine B was added to the networks and monitored by UV-Vis during degradation. The release profile of the model drug from the three networks is shown in Figure 8.8. The amount of rhodamine B released increased as the PEGDA: HDDA ratio increased. For example, the 25:75 PEGDA: HDDA network released nearly 25% of its theoretical loaded value within 1 week, whereas the 10:90 and 0:100 PEGDA:HDDA networks released approximately 5% within the first week. The diffusional exponents and constants calculated from Equation 2.1 and Equation 2.3 are found in Table 8.1. The values of n are greater than 0.5 and less than 1.0, thus non-Fickian anomalous transport is present. Also, the values of $k_1$ and $k_2$ are similar to value, thus a mixture of Fickian and Case II transport is present.
Figure 8.8. Cumulative release of rhodamine B for PEGDA: HDDA 1.15 +55\% MMA networks of varying PEGDA:HDDA ratio over 4 weeks. Each point is the mean ± standard deviation, n=3.

Table 8.1. Diffusional exponents and constants of rhodamine B released from PEGDA: HDDA networks in vitro.

<table>
<thead>
<tr>
<th>PEGDA: HDDA</th>
<th>Diffusional exponent, $n$</th>
<th>$k_1$</th>
<th>$k_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:100</td>
<td>0.786 ± 0.006</td>
<td>0.006 ± 0.0</td>
<td>0.006 ± 0.0</td>
</tr>
<tr>
<td>10:90</td>
<td>0.647 ± 0.003</td>
<td>0.015 ± 0.0</td>
<td>0.004 ± 0.0</td>
</tr>
<tr>
<td>25:75</td>
<td>0.893 ± 0.019</td>
<td>0.015 ± 0.005</td>
<td>0.034 ± 0.002</td>
</tr>
</tbody>
</table>

8.4 Discussion

In this study, the effect of varying PEGDA:HDDA ratio on the thermal and mechanical properties, and degradation rate of poly(β-amino ester) networks was explored in vitro and in vivo. The diacrylate to amine ratio was held constant in order to have crosslinkers of similar molecular weight, and the wt\% MMA was held constant in order to have initially tough networks with similar Tg. Also, MMA acts as a linear chain builder, which decreases the crosslinking density of the network and increases deformation capacity. The results show that changes in PEGDA: HDDA ratio can significantly change the degradation rate, the Tg, and resulting mechanical behavior both
in vitro and in vivo. Poly(β-amino ester)-co-MMA networks with only HDDA as the diacrylate had previously been explored, but their degradation rate and amount of mass loss had been minimal, so PEGDA was added to increase the degradation rate. The 0:100 PEGDA: HDDA network was chosen as a baseline because it had previously displayed a low amount of mass loss and near constant toughness over 8 weeks of degradation in vitro. The impact of PEGDA: HDDA ratio and degradation environment on poly(β-amino ester) networks’ thermal and mechanical properties will be discussed.

Biodegradable polymers often have different degradation rates depending upon environment; however, these poly(β-amino ester)-co-MMA networks displayed no difference in mass loss between in vitro and in vivo conditions over the 8 weeks of study, and both conditions displayed increases in mass loss as PEGDA: HDDA ratio increased. While they may have equivalent mass loss over time, the network’s chemical structure is changing in slightly different manners. The degradation of the PEGDA: HDDA crosslinkers is causing the decrease in ester and ether bonds in vitro and in vivo. Carboxyl groups form because of hydrolysis of the ester bond adjacent to the carbon-carbon backbone of poly(acrylic acid), but this occurs at 2 weeks in vivo and later at 8 weeks in vitro. This suggests that hydrolysis may be occurring earlier in vivo, but the rate of erosion and mass loss is equivalent.

The in vitro mechanical behavior showed composition dependent changes over 8 weeks of degradation. The PEGDA: HDDA ratio controlled the amount of mass loss to occur, where the degradable crosslinkers with low Tg leave the network, so the overall Tg of the network will increase towards the remaining high Tg MMA component. The 0:100 PEGDA: HDDA showed the smallest changes in mechanical behavior because its
Tg shifted the least under both degradation conditions, where it maintained more rubbery behavior at all timepoints. The 10:90 PEGDA: HDDA network has a faster degradation rate, thus a faster increase in Tg. The network displays a more elastic-plastic behavior with a defined linear elastic region and plastic region. The 25:75 PEGDA: HDDA, the fastest degrading network, had a rapid increase in Tg, thus it quickly increased in stiffness and eventually became brittle. Typically, a biodegradable polymer’s mechanical properties decrease over time. By using network components of low Tg and high Tg, the network’s Tg changes while it degrades, which enhances the mechanical properties of the networks. Nondegradable (meth)acrylate networks undergo shifts in Tg due to absorption of water, where the in vitro mechanical properties may increase or decrease depending upon relative position of the networks’ Tg to operating temperature [96]. The in vitro toughness of these networks shows very interesting behavior, where the 0:100 PEGDA: HDDA network maintains toughness due to the small amount of degradation, smaller shift in Tg, and consistency of its crosslinking density (proportional to rubbery modulus in Figure 8.7). The 10:90 PEGDA: HDDA network eventually increases in toughness at 6 and 8 weeks, but longer tests would be necessary to see its final mechanical behavior. Perhaps the 25:75 PEGDA: HDDA network shows the future outcome of the 10:90 PEGDA: HDDA network, where the 10:90 PEGDA: HDDA may have a maximum in toughness and decline as it transitions to a more brittle material. The 25:75 PEGDA: HDDA network was the other extreme, where it rapidly increased in toughness at 4 weeks because all mechanical properties increased, but the continued shift in Tg and degradation left a brittle material by 8 weeks. Although the Tg profile indicates that it has reached a plateau near 2 weeks, the rubbery modulus profile suggests continued
degradation of the low Tg crosslinkers due to its continued decrease over 8 weeks. These networks represent three possible ways to control toughness in a temporal manner: (1) Very slow degradation and small shifts in Tg result in a near constant toughness, (2) moderate degradation and shift in Tg results in the eventual toughening of the material, and (3) rapid degradation and shift in Tg can bring both large increases and decreases in toughness. The *in vitro* trends in mechanical properties are relevant only for non-load bearing conditions, thus the networks were degraded *in vivo* to ascertain the influence of a mechanically active environment.

Since the mass loss and shifts in Tg are similar *in vivo* and *in vitro*, one may expect the mechanical behavior and properties to be similar under both conditions, but there is an overall decrease in toughness *in vivo*. The *in vivo* 0:100 PEGDA: HDDA network had similar rubbery behavior as *in vitro* over the 8 weeks. The 10:90 PEGDA:HDDA network *in vivo* showed a decrease in stress and strain at 2 weeks before an increase in stiffness at 8 weeks, which contributed to a drop in toughness at 2 weeks. The 25:75 PEGDA: HDDA network *in vivo* showed a similar decrease in failure stress and strain at 2 weeks before transitioning to brittle behavior at 8 weeks. The mass loss of the low Tg component increases the Tg of the network *in vivo*, thus transitioning the network to a stiffer material for the 10:90 PEGDA: HDDA network and the 25:75 PEGDA:HDDA network. The networks show a decrease in mechanical properties (ultimate strength and failure strain) during the degradation at 2 weeks (Figure 8.5B), which is due to fatigue of the materials. Other poly(β-amino ester) networks have shown increased loss of mechanical properties when under cyclical stress in aqueous environments, which lead to abrupt failure [44]. For biodegradable poly(ortho ester)s,
exposure to saline or dry cyclic loads have minimal impact on its mechanical properties; however, the combination of exposing the material to saline and cyclic loading conditions caused a 75% decrease in strength and modulus [153]. Two samples of the 25:75 PEGDA:HDDA networks at 8 weeks failed inside of the host, thus the implantation site is mechanically active and loads inside the host are sufficient for failure. During the degradation and Tg shift, this network reached a state where its mechanical properties were not sufficient to withstand failure. The samples are undergoing multiple loading cycles in the host, which would decrease the mechanical properties of the material. The cyclical loading would decrease the mechanical properties at 2 weeks (lower toughness) before the Tg could further increase. At 8 weeks, the Tg would have increased sufficiently to increase the stiffness and strength (higher toughness). Silicone rubber is considered to be nondegradable, yet it showed decreases in all of its mechanical properties after in vivo degradation. Initially, its toughness was near 4 MJ/m$^3$, but decreased by 25% within the first two months [154]. The in vivo environment is a more mechanically demanding environment; however, these poly(β-amino ester)-co-MMA materials only lost 1-2 MJ/m$^3$. Overall, this range of toughness lies within the range for known biological tissues, and is not a significant decrease in toughness [155].

The behavior of 25:75 PEGDA: HDDA network demonstrates how degradation can result in a network transitioning from a rubbery material to a glassy material. The possibility exists to transition from a glassy material to a rubbery material, where the biodegradable component would need a higher Tg than the nondegradable component. While this method is possible, it may not be practical because decreasing the Tg will decrease the toughness of the material. These shifts in Tg and resulting changes in
mechanical properties could be further controlled by using a linear chain builder that has a Tg lower than Tg of MMA. This may slow the rate of change in mechanical properties, but more linear chain builder would have to be used to increase the Tg to match optimal conditions. Since these networks have 55 wt% MMA, they are not expected to be completely degradable. If the poly(β-amino ester) crosslinker had a higher Tg than the current crosslinkers (−40°C), then less MMA would be needed, which would allow for more degradation. Currently, these poly(β-amino ester) crosslinkers combined with a high Tg linear chain builder allow for temporal tailoring of mechanical properties by shifting the Tg via degradation of the low Tg component.

In order to serve as a multifunctional biomaterial, these networks must have tailorable properties, be able to sustain toughness and degrade, where degradation often serves to release a drug or therapeutic agent. The ability to sustain toughness is pivotal in designing future biomedical devices. The networks designed within this current study are a combination of multiple components with varying Tgs that can undergo shifts in thermal transitions, which allow for temporal control of mechanical properties. These materials are transitioning from rubbery to viscoelastic to glassy at 37°C, but the degradation is in fact decreasing the crosslinking density and increasing the network spacing because the rubbery modulus is proportional to the crosslinking density (Figure 8.7). The degradation and decrease in crosslinking density allows for therapeutic release of the model drug (Figure 8.8). The release rate was varied by changing the degradation rate via PEGDA: HDDA ratio. Similar to the PEGDA:HDDA networks without MMA in Chapter 6, rhodamine B has anomalous transport characteristics in these networks. A decrease in n was seen at higher PEGDA concentrations for networks without MMA in
Chapter 6, but here an increase in $n$ is observed as PEGDA concentration increases at the 25:75 PEGDA:HDDA network. The 25:75 PEGDA:HDDA degrades quickly within a week, thus raising its Tg. This increase in Tg hinders polymer relaxation, thus the transport is controlled more by Case II transport and an increase in $n$. Acrylate based hydrogels have typically shown Fickian release behavior, but changes in crosslinking density changes or hydrophilicity change the value of the diffusional exponent, thus non-Fickian behavior occurs [48, 50]. The diffusional exponent values for these networks are comparable to more highly crosslinked polystyrene, where increasing the crosslinking density increased the diffusional exponent at a set temperature below Tg [49]. Common biodegradable polymers like PLLA and PCL degrade and allow for therapeutic release, but lose their mechanical properties quickly. This temporal control of mechanical properties furthers these poly(β-amino ester)-co-MMA networks as multifunctional materials, where they are able to sustain mechanical properties \textit{in vivo} and elute a model therapeutic agent.

\textbf{8.5 Conclusions}

Semi-degradable networks were synthesized in order to assess their properties under \textit{in vitro} and \textit{in vivo} degradation conditions. The amount of erosion displayed no difference between the two environments, but hydrolysis occurred earlier \textit{in vivo}. The PEGDA concentration controls the amount of degradation, where low Tg components leave the network structure, thus shifting Tg to higher temperatures. These shifts in Tg significantly alter the mechanical properties \textit{in vitro} and \textit{in vivo}. Toughness increased \textit{in vitro} as the material degraded, but decreased \textit{in vivo} due to the mechanically active environment, but values were within range of biological tissues after 8 weeks. The shift
in Tg also affected the release behavior of a model drug, where increased degradation transitioned the drug transport from anomalous to more Case II transport behavior. These networks represent a new class of multifunctional biomaterials that have temporal control of mechanical properties via shifts in Tg and the ability to release therapeutic agents.
CHAPTER 9

CONCLUSIONS

9.1 Introduction

A novel method for treating AAA has been investigated, where a drug-eluting, biodegradable, mechanically-durable photopolymerizable polymer would surround the exterior of the AAA. Not only would it mechanically constrain the AAA from further expansion without detrimental restriction, but it would release a drug to address the biological cause of AAA formation. In order to find a suitable polymer composition for this application, a host of interconnected structure-property relationships had to be established for poly(β-amino ester) networks, an emerging class of biodegradable polymers.

Two design criteria of this proposed treatment put serious restrictions on the type of materials that could be used: (1) mechanical properties and (2) photopolymerization in situ. The modulus of the material must match closely to the modulus of the native tissue, thus a number of current biodegradable polymers like PLLA, PGA, PCL are unacceptable as their semicrystalline structure has a modulus too high for soft tissue like a blood vessel. The material should be more elastomeric, such as a lightly crosslinked polymer network. For instance, crosslinkable polyanhydrides could have been a possible solution, but they have mechanical properties more similar to bone, thus leading to another modulus mismatch.

Photopolymerization in situ works only if the material is a liquid or viscous fluid at operating temperature. Again, this restriction rules out other common biodegradable polymers, such as PLLA and PCL, because they are solid at body temperature, and do not
have reactive groups for polymerization in situ. Because current materials did not meet these requirements, a new material system with tailorable mechanical properties and degradation, while remaining amorphous needed to be examined.

Three research objectives were explored in order to establish the structure-property relationships of poly(β-amino ester) networks. Fundamental relationships between diacrylate structure, macromer molecular weight, diacrylate to amine ratio, polymerization, thermo-mechanical properties, and degradation were established in Chapter 4. This study was pivotal in defining the key relationships between chemical structure and properties. As an extension of Chapter 4, Chapter 5 explored networks composed of two diacrylates to control network properties, which lead to a method to easily tailor degradation and mechanical properties. Chapter 6 explored the effect of doxycycline, the selected drug, on network properties and its release behavior from the networks. While doxycycline inhibits photopolymerization, initiator concentration can be adjusted to allow photopolymerization to fully occur. Also, the release amount and mechanism were tailored by controlling the chemistry of the network.

Due to the poor mechanical properties found in Chapters 4 and 5, the effect of MMA concentration was established in Chapter 7. MMA increased the Tg, which allowed for increased toughness and deformation capacity; however it decreased the degradation rate. Shifts in Tg due to the erosion of low Tg components from the network lead to changes in the mechanical properties. Chapter 8 further examined these poly(β-amino ester)-co-MMA networks’ mechanical properties under degradation conditions in vitro and in vivo. The shifts in Tg were increased by increasing the degradation rate, which allowed networks to undergo vast mechanical changes while degrading. These
semi-degradable networks allow for drug release, and undergo temporal changes in mechanical properties due to changes in network structure.

9.2 Fundamental Advances

The most fundamental structure property-relationships dealt with the starting materials of diacrylates and amine. It was known that increasing the diacrylate to amine ratio decreased the macromer molecular weight, resulting in increased crosslinking density (high modulus, slower degradation) [42]. The effect of diacrylate molecular weight on network properties was unknown, thus a systematic approach was used, where two homologous series of diacrylates were studied. The formation of poly(β-amino ester) networks was found to be highly dependent on both step-growth polymerization and photopolymerization, where the step-growth polymerization was not fully analyzed previously. Low molecular weight diacrylates have an increased tendency to form macromolecular cycles since their reactive groups are closer together [76]. These diacrylates had faster reaction times during step-growth polymerization, which limited their conversion during photopolymerization. Thus, networks were formed that exhibited decreased mechanical properties and higher sol fractions. High molecular weight diacrylates did not convert to a high extent during step-growth polymerization, thus high conversion was reached during photopolymerization. Currently there are several types of network formation via photopolymerization. Traditional network formation uses a mixture of nondegradable acrylates and diacrylates to form a crosslinked network [93]. A typical method to allow biodegradation is to use degradable thermoplastic macromers are endcapped with a (meth)acrylate unit to allow photopolymerization into a network [33, 123]. Alternatively, a mixed chain-growth/step-growth photopolymerization is used for
thiol/acylate system, which allows for enhanced control of structure and degradation profiles [136, 156]. In essence, poly(β-amino ester) networks form their own custom biodegradable crosslinker before photopolymerization with the step-growth polymerization. The research community is no longer limited to commercially available diacrylates for crosslinking now, but can choose from a myriad of different poly(β-amino ester) crosslinkers.

An interesting relationship between diacrylate chemistry and degradation resulted from this systematic study. Diol diacrylates have degradation profiles that are only dependent on their diacrylate molecular weight, such that networks formed from low molecular weight diacrylates degraded faster. Diacrylate to amine ratio controls the macromer molecular weight, crosslinking density, and resulting modulus. This dependence on diacrylate molecular weight, not diacrylate to amine ratio, creates networks that have modulus independent from their degradation rate, which is rare because crosslinking density typically limits water infiltration and slows degradation rate. The PEGDA-based networks follow this trend, where increasing crosslinking density (modulus) reduces degradation. The in vitro cellular response to degradable tissue scaffold materials of varying mechanical properties is often complicated by materials having different degradation profiles. With these networks, a variable could be held constant in those studies to provide further insight on the mechanism directing cellular response.

The degradation profiles were further tailored in Chapter 5, where using a more hydrophilic monomer, PEGDA, increased the water content and allowed for increased degradation. The use of two polymers of varying hydrophilicity to control degradation
rate has been studied in other degradable polymers [11, 23, 121, 157]. While the decrease in mechanical properties was expected for networks containing PEGDA, it was the HDDA behavior that was most interesting. The HDDA network had lower hydrophilicity and water content, combined with a linear degradation profile, and minor change in mechanical properties, which are indicative of surface erosion behavior. This combination of HDDA and PEGDA allowed facile tailoring of degradation rate, which proved key for release studies.

Previously, doxycycline had been encapsulated in PGLA microspheres for treatment of periodontal diseases, but the drug had yet to be used in a photopolymerizable polymer [138]. While the inhibition of the polymerization reaction is detrimental to properties, an increase in photoinitiator concentration can ensure network formation takes place. Due to the increased degradation rate and water content from PEGDA networks, doxycycline release was greater in these networks. The large changes in release mechanism were unexpected, where small amounts of PEGDA (0, 5, 10, 25%) altered the mechanism from Case II, to anomalous transport, to Fickian. Previously, networks composed of 2HEMA and MMA changed from Fickian to anomalous transport (0.5 to 0.6) as 2HEMA concentration was decreased from 100% to 60% network content [48]. The ability to change the release mechanism adds another dimension of tailorability to these networks, where Case II release was deemed more desirable because the elution profile is more predictable.

The most important fundamental advances are found in Chapters 7 and 8, where MMA is shown to have a large impact on mechanical properties. It was known that optimum mechanical properties of amorphous methacrylate network exist when the Tg
matches the operating temperature under hydrated conditions [96]. Also, infiltration of water into the nondegradable networks can shift the relative Tg in a manner to increase or decrease toughness [98]. The poly(β-amino ester) networks needed to increase their Tg by 80°C to reach this goal. Chapter 7 confirmed the basic tailoring of mechanical properties of poly(β-amino ester)-co-MMA networks, where a rule of mixtures applied to increasing the Tg. As the networks were exposed to simulated physiological conditions, they underwent temporal changes in mechanical properties, where some networks saw an increase in toughness. This increase in toughness was linked to an increase in Tg caused by the degradation and erosion of the low Tg network component, the poly(β-amino ester) crosslinker. Current biodegradable materials always lose mechanical properties while they degrade due to erosion and a decrease in molecular weight [16, 18]. Now, mechanical properties could be increased, sustained, or decreased now during degradation. These semi-degradable networks established a template for further materials design by controlling the mechanical properties temporally while degradation occurs.

Chapter 8 furthered this approach of using a semi-degradable network with components of vastly different Tg in vitro and in vivo, where the low Tg component would degrade from the network and increase the Tg. The networks hydrolyzed at earlier time points in vivo, but erosion was nearly the same for both environments, suggesting erosion is the limiting factor. Since networks with PEGDA have higher water contents and mass loss, it was expected to see a large shift in Tg during degradation. These large shifts established relationships between composition, degradation, Tg, and mechanical properties. The temporal mechanical changes were exaggerated in the network with the most PEGDA, which showed rapid transition from rubbery to elastic-plastic to brittle
behavior. Ideal changes in network structure occurred because the \textit{in vitro} samples were not mechanically active during degradation. Unlike the \textit{in vitro} samples, the \textit{in vivo} degraded samples decreased in toughness while implanted, yet still shifted Tg becoming more elastic-plastic and glassy. Biodegradable polymers that undergo mechanical loading show an accelerated decrease in mechanical properties compared to unloaded polymers [18, 44, 153]. These networks do not differ from that trend, yet they have the possibility to sustain toughness if their shift in Tg increases mechanical properties to counteract the loss of mechanical properties due to degradation. This work established a significant fundamental advance by demonstrating a method for temporal control of mechanical properties during degradation of a semi-degradable polymer network.

\textbf{9.3 Practical Applications}

The proposed treatment of AAA with this class of polymers is the main practical application, but several other possibilities exist. The poly(β-amino ester)-co-MMA networks are multifunctional biomaterials, which have several key properties: photopolymerizable, temporal control of mechanical properties, and controlled release capabilities. This combination of attributes could be applied to several biomedical devices. Besides direct application on the aneurysm, this device could be used as a graft interface coating in order to promote healing at the interface site between a Dacron graft and the native tissue during open repair. Current soft tissue fixation devices for orthopedic surgery only address the mechanical demands of the surgery. A semi-degradable polymer system could not only release a drug to promote healing, but also remain mechanically stable for longer time periods. Doxycycline releasing implants would promote more native collagen organization during healing for ligament or tendon
repair since it is a MMP-inhibitor. The networks could be processed into porous tissue scaffolds with porogens, remain mechanically stable during degradation, and release a therapeutic agent, thus promoting tissue infiltration. While not directly addressed in this work, the poly(β-amino ester)-co-MMA networks display shape-memory behavior, which would allow for a further dimension of multifunctional behavior. These materials could elute drugs, change shape, and have temporal control of their mechanical properties while degrading.

Since the networks are both photopolymerizable and drug eluting, they could be used as novel drug delivery devices that could to be polymerized in situ into complex geometries, especially post-operation when enhanced healing is needed at device implantation sites. With some tuning of viscosity, light source intensity, and polymerization time, it could be a rapid-forming, degradable durable skin covering for trauma patients. This could be extended to a liquid bandage product for wound closure during surgeries, which would degrade by itself over time, thus reducing the need for suture removal. Also, an injectable sealant for internal incisions is another possibility.

9.4 Future Directions

Further development of this polymer system for this treatment method should address these key issues: (1) doxycycline interaction with the degradation products, (2) long-term studies in vivo and in vitro, (3) creating a network with limited use of MMA to allow complete degradation, yet have a Tg near operating temperatures.

The interaction of doxycycline with the degradation products should be further studied in order to ascertain which degradation product(s) are changing the structure. Systematic evaluation of the degradation products interaction could occur, where
solutions of doxycycline doped with hexanediol, oligo(ethylene glycol), or poly(acrylic acid) could be analyzed by FTIR. Then acceptable diacrylates that do not have degradation products that interact with doxycycline would be used. While this is an ideal benchtop setup, the experiment could be further adapted with the use of an in situ FTIR, where real time data of any possible interactions between degradation products and doxycycline could be evaluated. Another method would be to encapsulate the doxycycline in microspheres, then further studies on microencapsulation loading efficiency and optimal particle amount in the matrix would be necessary. Also, different MMP inhibitors could be used, such as minocycline or marimastat.

In order to gain more clinical acceptance, long term studies of drug release, degradation, and mechanical behavior are necessary in vivo. The networks are defined as semi-degradable because it is believed that they will not completely degrade. The crosslinkers hold the network together and a decrease in crosslinking density was observed in Chapter 8, which suggests that complete network degradation may be possible, but long term testing will be necessary both in vitro and in vivo. Since the drug release and temporal changes in mechanical behavior from the HDDA-based networks are very slow, the timeframe could be near a year or more. Also, subcutaneous implantation site was used for this study, but the site of implantation should vary depending upon the desired biomedical application. For the AAA treatment, samples should be implanted or polymerized in situ in the peritoneal cavity adjacent to the aorta. For orthopedic applications, the material should be polymerized on the benchtop and then implanted in a load-bearing site.
The use of MMA to increase the Tg addresses the problem of weak mechanical properties; however, it decreases viscosity, increases polymerization time, and decreases degradation. These are all concerns for the clinician that will ultimately use the device. Thus the ideal solution is a monomer solution that has little to no MMA, amorphous liquid at room temperature, viscous, polymerizes quickly, but forms a polymer with a Tg at body temperature with a low crosslinking density. Van Krevelen established a semi-empirical method for predicting the Tg of polymers based upon the segments in the monomer repeating unit through group additive theory [158]. This method allows calculation of the Tg from knowing the structure of the repeating unit and the Tg of each segment in that structure. Using this method, the Tg of the poly(β-amino ester) can be predicted before it is synthesized; however, this does not take into account crosslinking or amine structures. In general, Tg increases when large bulk groups are in the backbone or close to the backbone and decreases when long flexible groups are tethered to the backbone. The diacrylates used in this study did not provide any steric hindrance to increase Tg due to their aliphatic unbranched structure. Using aromatic diacrylates would be a likely way to increase Tg, but small aromatic degradation products would be a cause for concern in vivo. This creates a complicated matter since most commercially available diacrylates have either simple aliphatic structures or aromatic ones. Tert-butyl structures, like tert-butyl acrylate, increase steric hindrance and are non-aromatic. If these structures were incorporated as sidegroups off the diacrylate backbone, then an increase in Tg would be possible without MMA. Also, the use of branched sidegroups from the amine would further increase Tg. This is a possible method to create amorphous, high Tg, degradable, photopolymerizable networks with tailorable mechanical properties.
9.5 Conclusion

This study explored the structure-property relationships of poly(β-amino ester) networks in order to enhance the scientific knowledge of degradable networks, while gaining insight on important design parameters for a novel cardiovascular therapy. The chemical composition, network structure, and environmental effects on a number of network properties were established. Effects of a therapeutic agent on network properties and controlled release behavior were investigated. Finally, networks with temporally-controlled mechanical properties were evaluated in vitro and in vivo. These results further the development and design of poly(β-amino ester) networks as multi-functional biomaterials for advanced biomedical applications.
APPENDIX A

EXEMPLARY RAW DATA

Figure A.1 Raw ATR-FTIR spectra of poly(β-amino ester) during step-growth polymerization.
Figure A.2 Exemplary ATR-FTIR spectra at 812 cm\(^{-1}\) for poly(\(\beta\)-amino ester) during 24 hours of step-growth polymerization.

Figure A.3 Exemplary polymerization profile as measured by ATR-FTIR during UV photopolymerization of poly(\(\beta\)-amino ester) network.
Figure A.4 Exemplary DSC curves of HDDA-co-MMA networks.
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VITA

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David was born in Wilmington, DE and resided in nearby Newark for his childhood. He attended St. Mark’s H.S. and excelled in math and science, where he was voted most studious of his class. He started research early, where he interned at the University of Delaware Department of Physics with Dr. Bonder. The opportunity to do basic research and use a SEM as a high school student was quite appealing. Knowing that science was in his future, he explored the different options for engineering majors. He decided upon Materials Science and Engineering because it was a mixture of engineering disciplines and all things were made of materials and this drew his fascination. Due to the lack of an undergraduate materials science program at University of Delaware, David went to Georgia Tech for his B.S. degree. At Georgia Tech, David spent much of his time exploring Atlanta restaurants, working out, cheering on the football team as a Goldfella, and being a teaching assistant to feed that restaurant habit. During his sophomore year, David was accepted into the joint 5 year B.S./M.S. program, but had not pursued research at Tech. Between his sophomore and junior year, David was awarded the Department of Homeland Security Scholarship, which provided him the opportunity of summer research with Dr. Simmons at Sandia National Lab. During his junior year, David was invited to start research with Ken Gall, a new professor at Tech, which proved to be most fortunate. David quickly learned the field of shape memory polymers, devoted many hours to the lab, and excelled as an undergraduate researcher. He realized that ‘smart’ polymers and biomedical devices were part of his future, Dave decided to continue with Ken for a M.S. on the thermo-mechanical properties of (meth)acrylate shape-memory polymer networks. After completing his B.S. and M.S. degree with thesis, David decided to pursue a Ph.D. with Ken Gall and Bob Taylor on a proposed treatment for abdominal aortic aneurysms. When not working in the lab, David prefers to be having a water ice, drinking wine, exercising, watching college football, or simply eating pulled pork.