

5-2013

# EVALUATING MECHANICAL PERFORMANCE OF HYDROGEL-BASED ADHESIVES FOR SOFT TISSUE APPLICATIONS

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EVALUATING MECHANICAL PERFORMANCE OF HYDROGEL-BASED  
ADHESIVES FOR SOFT TISSUE APPLICATIONS

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A Thesis  
Presented to  
the Graduate School of  
Clemson University

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

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by  
Nitin Balakrishnan  
May 2013

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Accepted by:  
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## ABSTRACT

According to the National Center for Health Statistics, an estimated 22 million women have undergone a hysterectomy procedure in the United States. The most common complication during hysterectomies is accidental laceration of the urinary bladder during the surgery with incidence between 0.2-8.3% with the current gold standard wound repair method being sutures. Yet, sutures come with their own limitations in that they necessitate use of a catheter and collection bag during healing due to preventing proper distention of the bladder tissue at normal pressures. The long-term goal of our study is to eliminate the need for suturing by creating a surgical adhesive that provides a combination of strength, compliance, and biocompatibility for application to the bladder. However, current FDA-approved, commercially available tissue adhesives and sealants each have specific limitations that make them unsuitable for bladder application. While addressing the shortcomings of its predecessors, polyoxamines called Tetronic® (BASF Corporation) have been used as the backbone of a novel tissue adhesive. Previous studies using Tetronic® have exhibited higher strengths with increasing hydrogel content, but need further refinement to meet the mechanical requirements for bladder wall tissue mechanics. Thus, it is hypothesized that incorporation of a low molecular weight polyoximine, Tetronic® 304 (T304, MW: 1650 Da) would allow further increasing of hydrogel content and could yield higher bonding and bulk strength for a tissue adhesive while not compromising gelation time. Briefly, Tetronic® 304, and 1107 (MW: 15030 Da) were reacted with acryloyl chloride to form terminal acrylate groups using previously established methods with the final product characterized through NMR spectroscopy to determine the acrylation

conversion percentage. The Tetronic® acrylate was crosslinked using dithiothreitol (DTT) with the bulk strengths of various blends of the Tetronic®-based adhesive determined via calculating maximum tensions from end-to-end tensile adhesion tests on collagen sheets. In addition, punctured rat bladder specimens sealed with two blends of the hydrogel adhesive were subjected to increasing intravesicular pressure until failure.

The maximum tension achieved from the tensile tests was for the 75/25 at 60wt% T304-acrylate/T1107-acrylate group with a value of  $0.65 \text{ N/cm} \pm 0.06 \text{ N/cm}$  (n=6). In addition, the rat bladder puncture sealed with a 50/50 blend at 50wt % withstood pressure at an average of  $45.45 \pm 3.96 \text{ cm H}_2\text{O}$ . These results have demonstrated that using T304 has allowed for a higher content of hydrogel to be incorporated into the Tetronic®-based adhesive, thus increasing overall bulk strength. To further test the efficacy of this Tetronic®-based adhesive, testing the hydrogel-based adhesive on the bladder both *ex vivo* and *in vivo* using larger animal models will be necessary before moving onto clinical trials

## DEDICATION

This document is dedicated to my parents, Meena and Raju, as well as my sister Nandita, for their continual love, support, and encouragement throughout my academic career.

## ACKNOWLEDGEMENTS

I would like to first and foremost thank the Department of Bioengineering for the opportunity to pursue a graduate degree. Specifically, I have a high level of gratitude for my advisor Dr. Jiro Nagatomi for his assistance, patience, and guidance throughout the research process during both my undergraduate and graduate degrees. This would not have been possible without him. Additionally, I would like to mention Drs. Ken Webb and Jeoung Soo Lee for being kind enough to provide the raw materials, reagents, glassware, and laboratory space for the chemical aspect of my project. Their insight into interpreting results as well as accompanying suggestions was invaluable. Next, I would like to acknowledge Dr. Naren Vyavahare, whom inspired me to pursue a college degree in bioengineering from a young age. I will forever be grateful for his mentorship in this field. Additionally, thanks to Dr. Amod Ogale and Sam Lukubira in the Chemical Engineering department for their assistance during the rheological portion of my project. I would also be remiss to not mention the members of the Cell Mechanics and Mechanobiology lab, especially Lindsey Sanders, Srikanth Sivaraman, Brad Johns and formerly Ben Fleishman for their assistance with my research. A special thanks to Atanu Sen as well.

Acknowledgements to facilities supported by NIH Grants 5P20RR021949-04 and 8P20GM103444-04 for my mechanical testing and ERC Shared Facilities supported by the National Science Foundation under Award Number EEC-9731680 for rheology work.

Lastly, but certainly not least, I would like to thank my friends and family for all their support over the years.

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## CHAPTER ONE

### LITERATURE REVIEW

#### **A. Clinical Motivation**

According to the National Center for Health Statistics, 617,000 hysterectomies were performed in 2004, and an estimated 22 million women have undergone this procedure in the United States.<sup>1</sup> Overall, an average of 622,000 hysterectomies have been performed in the last decade nationally.<sup>2</sup> The most common complication during hysterectomies is accidental laceration of the urinary bladder during the surgery with incidence between 0.2-8.3%.<sup>1</sup> Currently, the gold standard wound repair method for such injuries is suturing. Yet, sutures come with their own limitations in that they necessitate use of a catheter and collection bag during healing because they prevent proper distention of the bladder tissue at normal pressures. Moreover, the uses of catheters can decrease overall patient quality of life. Thus, an alternative approach for effective sealing of wounds is being investigated in the present study.

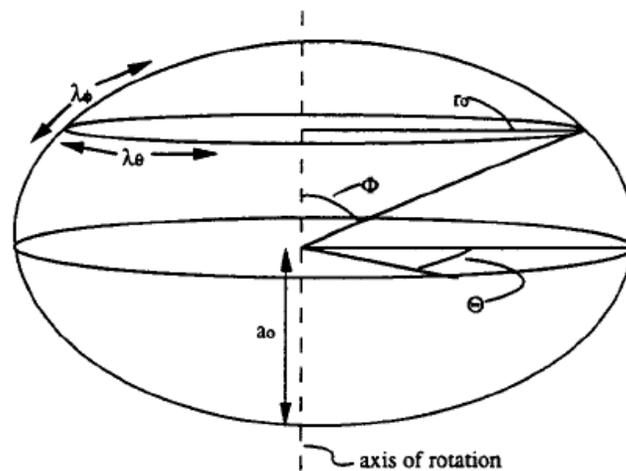
#### **B. Urinary Bladder Mechanics**

When considering the suitability of a tissue adhesive for bladder application, it is essential to understand the mechanical demands of the urinary bladder. Primary functions of the urinary bladder are to contain large volumes of urine at low pressures<sup>3</sup> and to void upon smooth muscle contraction when bladder capacity is reached.<sup>4,5</sup> The mechanical behavior of the bladder wall tissue is reliant on the properties of smooth muscle cells and the

extracellular matrix for their contractile function and tensile properties, respectively.<sup>5,6</sup> Specifically, the microstructure, the interaction of the tissue components, and the resulting overall response of the tissue changes from moment to moment.<sup>7</sup> In one study, Nagatomi et al. found that compared to normal rat bladders, 3-week spinal cord injury (SCI) rat bladders exhibited a decrease in collagen content by 43%, and an increase in elastin content by 260%, which explained the increased compliance of SCI rat bladders.<sup>8,9</sup> Subsequent studies found that spinal cord injury (SCI) in rats prompted smooth muscle hypertrophy ( $p < 0.05$ ) and changes in muscle orientation.<sup>6</sup> While normal rat bladders exhibited smooth muscle direction mostly in the longitudinal direction, 10 days after spinal cord injury smooth muscles oriented in both the longitudinal and circumferential direction.<sup>6</sup> Toosi et al. expanded the scope of the experiment from 10 days to 10 weeks, reporting changes in the biomechanical response of the urinary bladder wall tissue went further than changes in overall bladder compliance. Particularly, changes in material class from anisotropic (in normal rats) to isotropic (in 10-day SCI rats) to anisotropic (in 10-week SCI rats), bladder composition (more collagen present in SCI rats), and architecture (smooth muscle orientation from longitudinal to bimodal) were observed, indicating constant remodeling of bladder wall tissue after the initial injury.<sup>10</sup>

Although mechanical testing of the bladder at the tissue level has provided valuable information about its functional state<sup>8</sup>, the mechanical behavior of the whole bladder is governed not only by the tissue properties, but also by bladder shape and size.<sup>11</sup> Testing of the whole bladder will further elucidate the physiological nature of its smooth muscle and

ECM components under loading *in vivo*.<sup>5</sup> Damaser and Lehman were the first to use mathematical modeling and experimental data from dog bladders *in vivo* to predict the effects of bladder shape on the pressure–volume relationship.<sup>3</sup> They established equilibrium equations for two types of spheroids: prolate and oblate to predict stress, strain, and volume of the bladder tissue at various pressures. The oblate spheroid (**Figure 1.1**) made of urinary bladder material was shown to be more compliant than a prolate spheroid or sphere. Defining bladder capacity as the volume of the bladder at the greatest pressure (22 cm H<sub>2</sub>O in this study; normally between 20-60 cm H<sub>2</sub>O), oblate spheroids had up to 70% more capacity than other models of the bladder.<sup>3</sup> Greater bladder compliance indicates a greater capacity to distend in response to pressure without disruption. Since the use of sutures for wound repair restricts proper distension of the whole bladder at normal physiological pressures, an alternative method of wound repair is necessary.



**Figure 1.1:** Oblate Spheroid Model of Urinary Bladder

### **C. Tissue Adhesives**

Today's most common methods for wound closure are sutures and surgical staples despite a variety of limitations, including risk of infection, the requirement for anesthesia, and difficulty in application and retention in soft tissues. These shortcomings in current methods have led to the development of polymeric tissue adhesives that are capable of being cured *in situ*.<sup>12</sup> Tissue adhesives are defined as any substance that either acts as a tissue connector or as a barrier for leakage.

In the past, tissue adhesives were only used in conjunction with sutures during wound closure procedures.<sup>13</sup> However, in order for surgical adhesives to serve as a viable alternative to sutures and staples, they need to exhibit the ability to rapidly adhere as well as maintain a close proximity to edges of wounds for an appropriate period of time.<sup>14</sup> In addition, an effective tissue adhesive should possess strong binding strength, ease of application, biocompatibility with tissue, minimal reactivity with tissue, and practical cost.<sup>15</sup> Moreover, hemostatic potential, infection control capability, and internal sealant bonding strength will add value to the tissue adhesives.<sup>16</sup> An adhesive can furthermore serve as a temporary scaffold while new tissue remodels the wound over the course of time.<sup>12</sup>

### **D. Current Types of Tissue Adhesives**

Although not specifically approved for urological applications, a variety of FDA-approved adhesives and sealants are currently available on the market for surgical purposes. Major

varieties of these include cyanoacrylates, BioGlue, fibrin glues, polyethylene-glycol (PEG) based hydrogels.<sup>13</sup> Additionally, collagen-based hydrogels are less commonly used, but emerging as a viable option.<sup>13</sup> These types of tissue adhesives have been thoroughly analyzed as hemostatic agents and sealants in animal models as well as in clinical studies of urinary fistula, cystotomy, and partial nephrectomy.<sup>17,18</sup> Although there are benefits to each of these tissue adhesive substances, there are noteworthy drawbacks such as mismatch between the mechanical properties of the adhesives and native tissue, complications related to residual toxicity, and difficulties with crosslinking.

#### **a. Cyanoacrylate**

Cyanoacrylate or “super glue” was first used as a tissue sealant in the 1940s, but its use was limited due to the high levels of inflammation and histotoxicity it caused.<sup>17</sup> The first cyanoacrylate tissue adhesive was used for minor lacerations, but had limited physical properties. Later, with further improvement, cyanoacrylate adhesives were used in treatments for cornea perforation and wound leak keratotherapy<sup>19</sup>, as well as in facial plastic and reconstructive surgery.<sup>15</sup>

In addition to reconstructive surgery, cyanoacrylates are generally used as an alternative to suture closure. Cyanoacrylates are hard and brittle and may have insufficient flexibility for the vibrant nature of *in vivo* conditions. Thus, these polymers are only used in external applications such as skin closure and repair of corneal perforations.<sup>14</sup> However, they do possess numerous favorable characteristics including their ability to rapidly form flexible bonds, act as an occlusive protective dressing, decrease inflammation, and ease of

application. Many studies have noted that cyanoacrylate application only takes between 30-60% of the time as suture repair.<sup>15</sup>

Methyl-2-cyanoacrylate was the first cyanoacrylate compound to be used as a surgical tissue adhesive.<sup>15</sup> Over the years, histotoxicity levels of this adhesive have been shown to be proportional to the length of their monomer side chain.<sup>15</sup> More specifically, the toxic effect of synthetic polymers is linked to types of breakdown products and the release rate.<sup>19</sup> Newer cyanoacrylate-based tissue adhesives, such as butyl-2-cyanoacrylate, have longer side-chain derivatives, and thus, undergo a slower release and more efficient clearing of the toxic byproducts from the local tissue.<sup>15</sup> Moreover, the usage of inhibitors of prostaglandin H synthetase, such as aspirin and indomethacin, has been shown to decrease the cytotoxicity of cyanoacrylates up to eightfold *in vitro*.<sup>20</sup>

Today, the most widely used type of cyanoacrylate is octyl-2-cyanoacrylate, which was approved by the FDA for use in topical wound closure in 1998.<sup>15</sup> There are only two cyanoacrylate brands currently authorized for use in the United States: Dermabond (Ethicon, Inc.), a octyl-2-cyanoacrylate and Trufill n-BCA (Cordis Neurovascular, Inc.), a combination of n-butyl cyanoacrylate and tantalum powder.<sup>13</sup> Studies have shown that tissue injury occurs because of cyanoacrylates' poor compliance which motivated the use of etheric oxygen to allow for the production of flexible monomers, enabled chain rotation, and improved adhesive strength.<sup>14</sup>

## **b. BioGlue**

The next major group of tissue adhesives is based on the combination of purified bovine serum albumin and glutaraldehyde glue, and is known as BioGlue (CryoLife Inc).<sup>13</sup> It is a proprietary FDA-approved (2000) compound generally used as a sealant aid to standard methods of hemostasis in adults undergoing vascular surgery.<sup>17</sup> At this time, BioGlue is the only compound used in the United States for the restricted use of assisting in the repair of aortic dissection by filling in the dissection; thereby closing the open cavity and providing a stronger arterial wall.<sup>13</sup> Strong bonds are created by the glutaraldehyde, which initiates chemical reactions between the aldehyde groups of glutaraldehyde and the amine groups, particularly lysine, of the albumin and tissue surfaces.<sup>21</sup> More specifically, glutaraldehyde links the amine groups from the bovine serum albumin to extracellular matrix proteins of the target tissue, thus creating a covalent bond between the tissue and surgical adhesive.<sup>17</sup>

Although when compared with other similar currently available product, BioGlue has greater bonding and sealing capabilities<sup>22</sup>, it does have limitations as well. For example, researchers always consider possible tissue-related toxicity as a major drawback.<sup>17</sup> In one study, polymerized BioGlue with saline solution contained 100-200 µg/ml of glutaraldehyde and was shown to be cytotoxic to both cell lines used. When BioGlue was applied to a partial lung resection model, minimal edema and necrosis were present after 2 days and severe inflammation was apparent after 7 days.<sup>23</sup> Other safety issues include local tissue necrosis, pseudoaneurysm formation, and risks of eye, nose, throat, and skin

irritation from the presence of glutaraldehyde.<sup>21</sup> Moreover, LeMaire et al. reported that BioGlue reinforcement in pigs impairs vascular growth and causes stricture when applied circumferentially around an aorto-aortic anastomosis.<sup>22</sup> Therefore, it was concluded that since the use of polymerized BioGlue releases high amounts of toxic, residual glutaraldehyde when not used on the aorta, it should not be used in pediatric patients until further studies are conducted.<sup>22,23</sup> It was also noted that even when BioGlue appears to present many positive features as a product, the significant toxic potential of the glue should be evaluated carefully.<sup>23</sup> Finally, the inflammatory effects and long-term reaction to BioGlue need to be better described and tested before the FDA will approve the further expansion of its application.<sup>13</sup>

### **c. Fibrin Glue**

The use of fibrin as an adhesive was first reported in 1940 by Young and Medawar, who applied it as an adjunct to sutures of the peripheral nerves in an animal model during microsurgery, and then subsequently by Seddon and Medawar in humans.<sup>24</sup> Since 1998, fibrin glue products, which are composed of thrombin and fibrinogen, have been approved by the FDA for use in the reinforcement of colonic anastomoses, splenic trauma, and cardiothoracic surgery.<sup>17</sup> Fibrin sealants meet the requirements of an ideal tissue adhesive to the greatest extent because both the adhesive and its resulting degradation products are biocompatible and have properties that offer topical hemostasis, provide tissue approximation, and possess sealant properties.<sup>13,17,25</sup>

Current brands of fibrin glues available in the United States are local blood bank products as well as the commercial products, Hemaseel APR (Haemacure Corp.) and Tisseel VH (Baxter Healthcare Corp.).<sup>13</sup> Both products use human thrombin and aprotinin as an antifibrinolytic agent.<sup>15</sup> Like cyanoacrylates, fibrin glues are used to treat corneal injuries, such as perforations and ulcers.<sup>26</sup> When compared, 79% of eyes had successful healing of corneal perforation treated with fibrin glue and 86% eyes treated with N-butyl-2-cyanoacrylate, indicating that both tissue adhesives are effective in the closure of corneal perforations up to 3 mm in diameter.<sup>26</sup> While fibrin glue displayed faster healing and generated less corneal vascularization than the cyanoacrylate, it required a much longer time for adhesive plug formation.<sup>26</sup>

#### **d. PEG Hydrogels**

The final major category of current commercially available tissue sealants is polyethylene glycol (PEG) hydrogels. Synthetic hydrogels, such as PEG-based polymers, can drastically improve the support of soft tissue organs e.g. the heart or hard tissues e.g. bone.<sup>27</sup> PEG hydrogels as a whole are readily functionalized, nontoxic, non-immunogenic, and blood compatible.<sup>28</sup> Some commercially available PEG-based sealants are made up of PEG diols modified with ester linkages that are degradable by water, and terminal acrylates for photopolymerization.<sup>12</sup> They are used for a vast array of clinical applications such as tissue regeneration, drug delivery, coating, and postoperative adhesions prevention.<sup>28</sup> In addition, PEG hydrogels are used in research applications, such as model scaffolds for cell migration.<sup>29</sup>

CoSeal (Cohesion Technologies, Inc.) and AdvaSeal-S (Genzyme Corp.) are both PEG-based sealants approved by the FDA in 2000 for use as pulmonary sealants and as an additive to blood hemostasis.<sup>17,21</sup> CoSeal has demonstrated the ability to form adhesive bonds with PTFE grafts and biological tissues *in vitro* while simultaneously exhibiting good tissue compatibility and rapid gelation.<sup>30</sup> Its efficacy has been demonstrated in that hemostasis was achieved within 10 minutes in 86% of 148 patients in need of vascular sealing.<sup>21</sup> Another PEG-based sealant, Duraseal, is approved for dural sealing and forms a hydrogel when its PEG ester component combines with trilisine amine with FD&C blue No. 1 dye, thus creating a water tight seal.<sup>21</sup> Duraseal was shown to be 100% successful in preventing cerebrospinal fluid leaks from dural closures.<sup>21</sup> Reported tensile strengths have demonstrated that both CoSeal and Duraseal are suitable as sealants for internal organs, specifically arteries.<sup>30</sup>

Although PEG hydrogels are effective in sealing tissue, there are some associated shortcomings as well. The primary safety concern of PEG-based sealants is swelling. Because PEG hydrogels are water-based, they continue to absorb water and swell *in vivo*, relative to initial volume dispersed.<sup>12,31</sup> CoSeal swells up to 400% and is therefore never used in place of sutures, staples or other mechanical closures. It is also not used in doses of more than 16 milliliters since the safety of larger doses has not yet been verified.<sup>21</sup> Comparatively, Duraseal only swells to 50%, but may be associated with other complications such as inflammatory and allergic reactions if there is a known allergy to its blue dye.<sup>21,32</sup> Another limitation of PEG-based hydrogels lies in that they may contain a

water-soluble agent that requires a light source to polymerize and activate adhesion.<sup>13</sup> This photoactivation, a time-consuming process, makes these hydrogels difficult to work with for surgeons in situations where immediate curing is required (i.e. massive hemorrhaging). However, newer PEG hydrogels, such as CoSeal do not require this activation source.<sup>13</sup>

Recently, swellable PEG amine/dextran aldehyde composite materials have emerged as controlled, biocompatible tissue adhesives.<sup>28</sup> Together, the authors concluded that PEG:dextran hydrogels can effectively adhere to tissue in a tunable manner, while not compromising biocompatibility.<sup>28</sup>

## **E. Use of Tissue Adhesives in Urological Applications**

Tissue adhesives have been tested in urological applications such as cystotomies and partial nephrectomies with varying levels of success in both humans and animals.<sup>17</sup>

### **a. Cyanoacrylate**

Studies related to urologic applications for cyanoacrylate-based adhesives have yielded mixed results. For example, Grummet et al. concluded 2-octyl cyanoacrylate is not fit to be used singularly for vesicourethral anastomoses during radical retropubic prostatectomy in mongrel hounds.<sup>33</sup> In contrast, Seifman and colleagues found that 2-octyl cyanoacrylate was appropriate for closure of a large cystotomy in a rabbit model while demonstrating an inflammatory response similar to traditional suture sealing.<sup>34</sup> Although 2-octyl cyanoacrylate (OCG) has been withheld from areas subject to repetitive movement (such as the bladder), its performance in sealing large bladder wounds on pigs was proven to not be

affected by changing bladder volumes during normal filling.<sup>35</sup> However, a disadvantage of OCG was found in its low rate of biodegradability and potential to cause callus formations.<sup>35</sup>

#### **b. BioGlue**

BioGlue's role in urological applications has not been assessed to date. However, researchers hypothesize that it may prove to be beneficial in controlling bleeding in procedures where chronic inflammation is present as well as in procedures where major blood vessels need to be exposed, such as post-chemotherapy retroperitoneal lymphadenectomies.<sup>17</sup> Yet, there is the worry that application of BioGlue could cause urinary obstruction.<sup>36</sup>

#### **c. Fibrin Glue**

Fibrin glue has been used as a sealant in a variety of urological applications such as sutureless colposuspension in patients with stress incontinence and as an aide to hemostasis in partial nephrectomy.<sup>25</sup> It has also been used to close bladder fistulae and displayed comparable results to microsurgery when treating vasovastotomies.<sup>24</sup> The use of fibrin glue for ureteral surgery has also been assessed in a number of animal models. For example, Barrieras et al. performed a pyeloplasty in a porcine model using a suture-assisted fibrin glue anastomosis. The immediate anastomotic leak point pressure was significantly lower in the fibrin group ( $3.5 \pm 1.5$  mm Hg) versus the control ( $17.3 \pm 5.4$  mm Hg;  $P = 0.038$ ).<sup>17</sup>

Marcovich and associates compared 2-octyl cyanoacrylate (OCG), fibrin glue (FG), and sutures for both open and laparoscopic repair of bladder wounds, using a pig model of cystotomy. Their results showed that at 4 weeks, none of the six bladders of pigs treated with OCG leaked at less than 200 mmHg pressure. In contrast, of the six pigs treated with FG, three died from a huge urine leak, one leaked, and two did not leak at less than 200 mm Hg at 4 weeks. From these findings, it was concluded that OCG is the only viable alternative to sutures for sealing of large bladder wounds and fibrin glues may be effective in bladder injury closure when tissue approximation is small and intraluminal pressures are low.<sup>35</sup> However, it was also noted that reduction in adhesive capacity upon contact with urine due to fibrinolytic activity of urokinase would remain one of the concerns associated with the use of fibrin glue in urological applications.<sup>25</sup>

#### **d. PEG Hydrogels**

PEG hydrogels have been tested in porcine laparoscopic partial nephrectomy (LPN) models and provided hemostasis at physiological pressures up to 100 mmHg.<sup>37</sup> In addition, Park and colleagues found that PEG hydrogels provided hemostasis for LPN in a porcine model and detected no cell-mediated immune responses to the sealant after 2 weeks, with the hydrogel being completely absorbed by the animal within 6 weeks.<sup>38</sup> However, compared to fibrin glue, the PEG hydrogel-based CoSeal did not adhere as well to the nephrectomy bed during LPN, signifying that further refinements need to be made before synthetic hydrogel sealants are suitable for urological applications.<sup>36</sup>

## **F. Use of Tetronic® as a Tissue Adhesive**

In order to address the issues associated with currently available tissue adhesives and sealants, Cho et al. began exploring thermosensitive polymers called poloxamines or Tetronic® (BASF Corp.) as the backbone material of a soft tissue adhesive.<sup>12</sup> Poloxamines are a family of 4-arm polypropylene oxide (PPO) - polyethylene oxide (PEO) block copolymers with a hydrophobic PPO core surrounded by a hydrophilic PEO shell and can be used to prevent swelling of hydrogel-based tissue adhesives.<sup>12,38</sup> Previously, Tetronic® was studied as a network material to encapsulate human foreskin fibroblasts by way of “tandem gelation”, a two-stage process combining reverse, thermally-induced gelation and covalent crosslinking of termini functionalized with reactive groups.<sup>40,41</sup> A study by Cho et al. tested a hypothesis that the combination of noncovalent and covalent gelation of Tetronic® hydrogels would provide an improvement for mechanical properties of tissue sealant/adhesive applications compared to PEG-based hydrogels that were created only by covalent crosslinking.<sup>12</sup> Specifically, Tetronic® 1107 (T1107; MW 15,030 Da) and Tetronic® 904 (T904; MW 6700 Da) were acrylated via dichloromethane and acryloyl chloride and polymerized by Michael-type addition.<sup>12</sup> Both hydrogels, T1107-acrylate and T904-acrylate, were then characterized for mechanical and tissue bonding properties, gelation point, swelling behavior, viscosity, and cytotoxicity.<sup>12</sup> The results of this study provided evidence that all blends (100% T1107-acrylate, 75/25 T1107-acrylate/T904-acrylate and 50/50) containing T1107-acrylate chemically crosslinked below physiological temperatures (37°C), while 100% T904-acrylate and the control 4-arm acrylated PEG did not gel within the temperature range of 4-40°C. Gelation time increased with decreasing

T1107-acrylate content ( $23.5 \pm 2.12$  s for 100% T1107;  $153.33 \pm 5.77$  s for 100% T904). The stiffness and strength of Tetronic® hydrogels were greater than the PEG control and increased with increasing temperature and T904-acrylate content to a maximum value of 400 kPa at 37°C. Tissue bonding strength also increased with increasing T904 content (maximum of 25 kPa), which was comparable to commercial fibrin glue and far exceeded the control PEG hydrogel.<sup>12</sup> Together, it was concluded that all mechanical properties of the hydrogels were temperature dependent and that using tandem gelation contributed positively to material properties.

More recently, Barrett et al. reported that Tetronic® backbones terminated with catechols including glutaric anhydride, pyridine, dopamine and TEA, served to increase both adhesive and cohesive strengths of the hydrogel tissue adhesive.<sup>41</sup> Briefly, the results of lap shear testing revealed that strength values for modified Tetronic® 1107-acrylate gels with varying levels (150-250 ml/mg) of these catechols ranged from 31.9-48.7 kPa, which was marginally stronger than a DOPA-modified PEG ( $30.4 \pm 3.39$  kPa) from a previous study.<sup>42</sup> However, the adhesive strength was much higher than that of the catechol-modified 4-arm PEO control (12.7 kPa).<sup>41</sup> Although the highest lap shear adhesive strength from this study is well above the mean value reported by Cho et al. (25 kPa), when comparing results parameters such as testing methods, crosslinking mechanisms, and equipment must be taken into account.<sup>12,41</sup> For example, Cho et.al and Barrett et al. both used lap shear testing on animal specimens. However, Cho's samples were subject to overnight curing at 37°C in a humidified atmosphere and soaked in PBS before testing.<sup>12</sup> In contrast, Barrett's samples

were initially cured for one hour at 37°C while glued to aluminum fixtures and covered by PBS-soaked gauze.<sup>41</sup> Additionally, a 100 gram weight was placed on the aluminum fixture assembly after which the samples were submerged in PBS for one hour before testing.<sup>41</sup>

## CHAPTER TWO

### PROJECT RATIONALE

The long-term goal of our study is to eliminate the need for suturing by creating a surgical adhesive that provides a combination of strength, compliance, and biocompatibility for application to the bladder. However, current FDA-approved, commercially available tissue adhesives and sealants each have specific limitations that make them unsuitable for bladder application. While addressing the shortcomings of its predecessors, the more recent attempt to use polyoxamines as the backbone of a novel tissue adhesive needs further refinement to meet the mechanical requirements for bladder wall tissue mechanics. Given that the lower molecular weight T904-acrylate exhibited higher strengths with increasing hydrogel content, it is hypothesized incorporation of an even lower molecular weight polyoximine would allow further increasing of hydrogel content and could yield higher bonding and bulk strength for a tissue adhesive. This Master's thesis focused on increasing the overall bulk strength of the Tetronic® adhesive by using a low molecular weight Tetronic® 304 (T304, MW: 1650 Da), while not compromising the strength and gelation time. This study is divided into three project aims:

- 1) To develop a synthesis route for T304-acrylate with high conversion efficiency and product yield.
- 2) To determine blend ratios and weight percentage of T304-acrylate and T1107-acrylate with the highest mechanical strength.
- 3) To demonstrate the efficacy of the Tetronic® tissue adhesive in an *ex vivo* bladder test.

## CHAPTER THREE

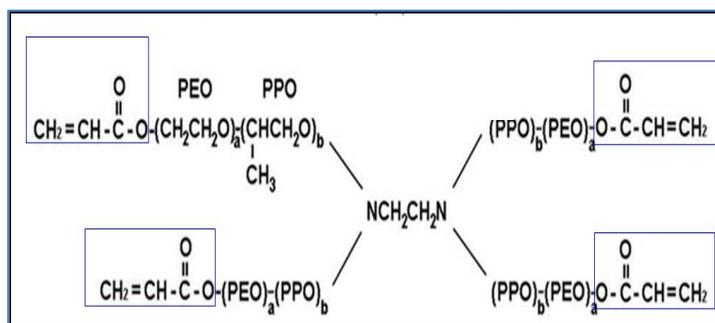
### MATERIALS AND METHODS

#### A. Materials

Tetronic® T1107 (MW 15030 Da) and T304 (MW 1650 Da) were acquired from the BASF corporation (USA) as free samples. Acryloyl chloride, celite fine 500 and 4-methoxyphenol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Toluene (HPLC grade), anhydrous ether (BHT stabilized), hexanes (HPLC grade), and anhydrous sodium sulfate were purchased from Fisher Scientific (NJ, USA). Dichloromethane (HPLC grade), triethylamine (TEA), dithiothreitol (DTT), sodium bicarbonate, calcium hydride and  $\text{CDCl}_3$  were purchased from Acros Organics (NJ, USA), a branch of Fisher. Dichloromethane was dried with calcium hydride and stored over molecular sieves (Grade 514, Type 4A, 8-12 mesh, Acros Organics).

**Table 3.1:** Characteristics of specific Tetronics

<b>Tetronic Type</b>	<b>Molecular Weight (Da)</b>	<b>Mean Number of EO Units per PEO Block</b>	<b>Mean Number of PO Units per PPO Block</b>	<b>Physical State at 25°C (RT)</b>
<b>T304</b>	1650	3.7	4.3	Liquid
<b>T1107</b>	15030	60	20	Powder



**Figure 3.1:** General Structure of Tetronic® Acrylate.

Blue boxes indicate terminal acrylate groups

## B. Acrylation of Tetronic®

### a. Preparation of T1107-acrylate

Acrylated T1107 was prepared by reaction of the raw Tetronic® terminal hydroxyl groups with acryloyl chloride. All glassware was cleaned with sodium hydroxide solution, rinsed with DI water followed by one acetone rinse, and completely dried in a 60°C oven overnight. T1107 (30 g) was dehydrated by azeotropic distillation with toluene over two hours in an oil bath at 130°C, which was then allowed to reflux and then subsequently removed by rotary evaporation (Buchi Rotavapor®, Switzerland) at 90°C. After cooling to room temperature, dehydrated T1107 was dissolved in 120 ml of dry dichloromethane and TEA (4 mmol, 1.115 ml) was added. On an ice bath, acryloyl chloride (8 mmol, 1.30 ml) in 60 ml of dry dichloromethane was then added dropwise over two hours and the reaction was allowed to continue at room temperature for 24 hours. The reactant was filtered through celite fine 500 to remove TEA-HCl salt, and then paper-filtered to remove the celite. The solvent was removed by rotary evaporation at

room temperature. The residue was precipitated in 500ml of cold ethyl ether (-20°C), recovered by filtration, and dried under vacuum for a few hours. The product was redissolved in dry dichloromethane (300ml), washed repeatedly with 30ml of 10% w/v sodium bicarbonate solution until the pH was neutral, and then washed with deionized water (30 ml each) until the pH of the water fraction was neutral. Next, the product was dehydrated with anhydrous sodium sulfate until the solution became clear. After paper filtration, the remaining solution (dichloromethane+ product) was decanted and removed by rotary evaporation at 25°C. The residue was precipitated and washed 3 times with cold ethyl ether (-20°C). The final product was recovered by filtration and dried in a vacuum desiccator overnight. The final structure and acrylation efficiency (%) were determined by <sup>1</sup>H-NMR (Bruker Avance-300MHz) using a CDCl<sub>3</sub> (chloroform) solvent.<sup>43</sup>

#### **b. Preparation of T304-acrylate**

T304-acrylate was synthesized in a similar manner to T1107-acrylate with minor changes to account for reduced thermal stability and different solubility properties. Prior to experimentation, unmodified T304 was dissolved in dichloromethane, subjected to rotary evaporation and precipitated in hexane to test product recoverability. Following the initial reaction setup, the residue was precipitated in a total volume of 500ml of the cold hexane/ethyl ether (50/50 volume ratio), and stored at -20°C to allow the precipitated product to settle overnight. After approximately 24 hours, the hexane/ethyl ether supernatant solution was quickly and gently decanted and the product was redissolved in 300 ml dichloromethane. Subsequently, the solution was washed repeatedly with 30 ml of

10% w/v sodium bicarbonate solution until the pH was neutral. This was followed by deionized water washes with 30 ml each until the pH of the water fraction was neutral, and the remaining product was dried with anhydrous sodium sulfate (approximately 4-6 times or until the solution became clear). Relatively small amounts of sodium sulfate was used in order to preserve as much of the product as possible. The product dissolved in dichloromethane was again subjected to rotary evaporation at 30°C and concentrated in the presence of 2 chips of 4-methoxyphenol. The residue was precipitated in a total volume of 500ml of hexane/ethyl ether and washed 3 more times with cold ethyl ether. The final product was recovered by decanting of the ethyl ether and dried under vacuum within a desiccator at 4°C for 6 to 8 hours. <sup>1</sup>H-NMR was performed to analyze the final structure and acrylation efficiency (%) using 25 mg of product in 1 ml chloroform solvent (Cho and Cribb).

### **C. Mechanical Testing of Tetronic® Adhesive**

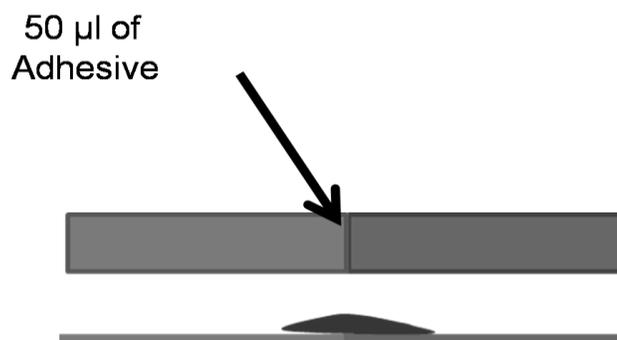
Prior to mechanical testing, the appropriate weight of Tetronic® acrylates (**Table 3.2**) was first mixed with 1ml of PBS (50mM) on a rocker in a cold room, protected from light for 1-2 days.

**Table 3.2:** T304-acrylate/T1107-acrylate blend ratios with corresponding DTT amounts

Wt%	ratio	T1107 (g)	T1107 wt% check	T1107 DTT (mg)	T304 (g)	T304 wt% check	T304 DTT (mg)	Total wt (g)/ 1ml PBS	Total DTT(mg)/ 100ul PBS (T1107/T304)	Total wt% check
30	50/50	0.225	0.15	4.62	0.225	0.15	42.1	0.45	46.7	0.31
40	0/100	0	0	0.00	0.7	0.4	130.9	0.7	130.9	0.41
40	25/75	0.175	0.1	3.59	0.525	0.3	98.2	0.7	101.8	0.41
40	50/50	0.35	0.2	7.18	0.35	0.2	65.4	0.7	72.6	0.41
40	75/25	0.525	0.3	10.78	0.175	0.1	32.7	0.7	43.5	0.41
50	0/100	0	0	0.00	1	0.5	187.0	1	187.0	0.50
50	50/50	0.5	0.25	10.26	0.5	0.25	93.5	1	103.8	0.50

**a. Testing with a Model Tissue**

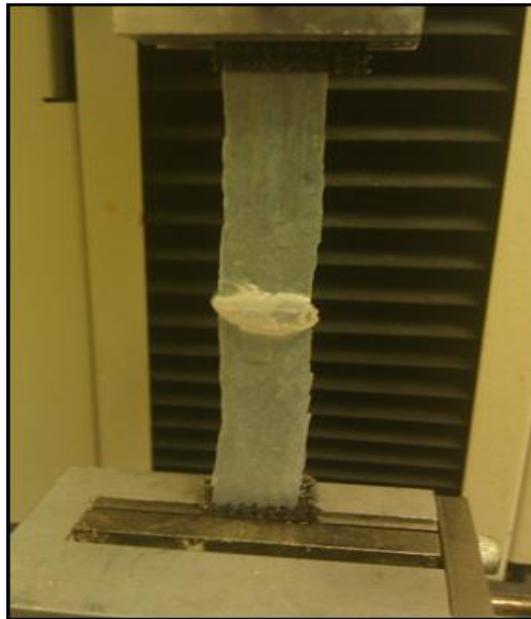
Collagen casing was sectioned into ½ inch-wide strips using a previously created printed template and placed in a fixture that maintained end-to-end contact between two pieces (**Figure 3.2**). The strips were then hydrated in PBS within a 37°C water bath for one hour.



**Figure 3.2** End-to-End Configuration

For testing of each specimen, 50 microliters of T304-acrylate/T1107-acrylate blend was mixed with appropriate volumes of dithiothreitol (DTT) solution (10%) according to **Table 3.2** and vortexed until the solution crosslinked reached a certain viscosity, where it formed a gel-like substance, but was still in a liquid enough state for extraction by a 1 cc syringe.

The solution was judged to be in this “gel-like” state” when it did not flow within the mixing tube when subjected to tube inversion. Reproducibility was created through application of set vortex speeds correlating to gelation times among samples from the same Tetronic® blend. 50  $\mu$ l of the mixture was then applied to the joint between the two pieces of collagen casing and allowed to cure under hydrated conditions in the 37°C water bath for 30-45 min. Immediately before testing, specimen thickness was measured using a digital caliper. Using an MTS Synergie 100 (**Figure 3.3**) with a 10 N load cell, specimens were subjected to uniaxial tensile load at a rate of 5 mm/min until bonded joints broke. To avoid slippage, the ends of the specimen were wrapped with sandpaper (P400 grade) within the grips of the testing system. The data were reported as maximum tension to failure (N/cm).



**Figure 3.3:** Tensile Testing Configuration

## **D. Viscoelastic Characterization of T304 and T1107**

### **a. Gelation Analysis**

To characterize the viscoelastic behavior of the Tetronic® hydrogels, unmodified (reverse thermal gelation) and acrylated (tandem crosslinking via reverse thermal gelation and Michael-type addition)<sup>12</sup>, T304 and T1107 were dissolved in PBS at three different ratios of 100/0, 0/100, and 50/50 (at 30wt % and 50wt % concentration).

#### **1. Reverse Thermal Gelation**

Rheological analysis on unmodified Tetronic® macromers was performed using the AR2000 rheometer (TA Instruments, Newcastle, DE) with a parallel plate configuration (40 mm diameter). 60  $\mu$ L of the test specimens were placed on the bottom plate (pre-cooled to 4°C via ice bath), and then the top plate was lowered to create a 0.15 mm gap between the two plates. A temperature sweep from 4-40°C at 2°C/min was conducted at a constant frequency of 0.1 Hz. The temperature at which the storage ( $G'$ ) and loss ( $G''$ ) moduli met was determined as the gelation point.<sup>12</sup>

#### **2. Tandem Crosslinking**

Parallel plate geometry was used again to analyze the crosslinking characteristics of acrylated Tetronic® macromers. The macromers were dissolved in PBS and then covalently crosslinked with DTT, a donor of the thiol group. The T304-acrylate/T1107-acrylate solutions (60  $\mu$ l of 100/0, 50/50 at 30 and 50wt %, 0/100 only at 30wt %) and DTT (10 $\mu$ l stored at 4°C until testing) were mixed and vortexed at room temperature. The mixture was placed on the bottom plate (pre-warmed to 37°C) of the AR2000 rheometer,

and the top plate was lowered to create a 0.15 mm gap between the two plates.

Oscillatory shear strain of 1% was applied immediately at a constant frequency of 0.1 Hz.

Storage and loss moduli were recorded as a function of time and gelation time was determined as the instance at which the lines for  $G'$  and  $G''$  crossed over.<sup>12</sup>

### **E. *Ex Vivo* Adhesive Testing on Rat Bladder**

Rat bladders were obtained from Pel-Freez Biologicals Inc. (Rogers, AR) and stored in 30% sucrose solution at 4°C until testing. Prior to testing, a small, approximately 2 mm puncture was made on the dome of the bladder using an 18 gage needle. Using a 1 cc syringe, the Tetronic®/DTT mix (500 µl to 50 µl ratio) was applied to the hole and allowed to cure for 1 hour within a saline solution-containing petri dish in the 37°C water bath. After curing, the rat bladder was mounted via sutures and superglue on a shaved 18 gage needle within a plastic container which was filled with saline solution until the bladder was submersed. The specimen was subjected to increasing intravesicular pressure using a Harvard Apparatus 11 Plus Pump (Harvard Apparatus; Holliston, MA) and a 60 ml syringe at a flow rate of 0.1 ml/min until failure, which was evidenced by saline solution colored with calcein blue dye, indicating leakage.

### **F. Statistical Analysis**

Quantitative data for maximum tension and maximum intravesicular pressure withstood by the Tetronic®-based adhesive were statistically analyzed using the commercial statistics software, SigmaStat 3.5. Following a one-way analysis of variance (ANOVA)

test, when statistical significance was observed, post-hoc comparisons were made using the Tukey method. Data sets with  $p$  values of less than 0.05 were considered to be statistically significant.

## CHAPTER FOUR

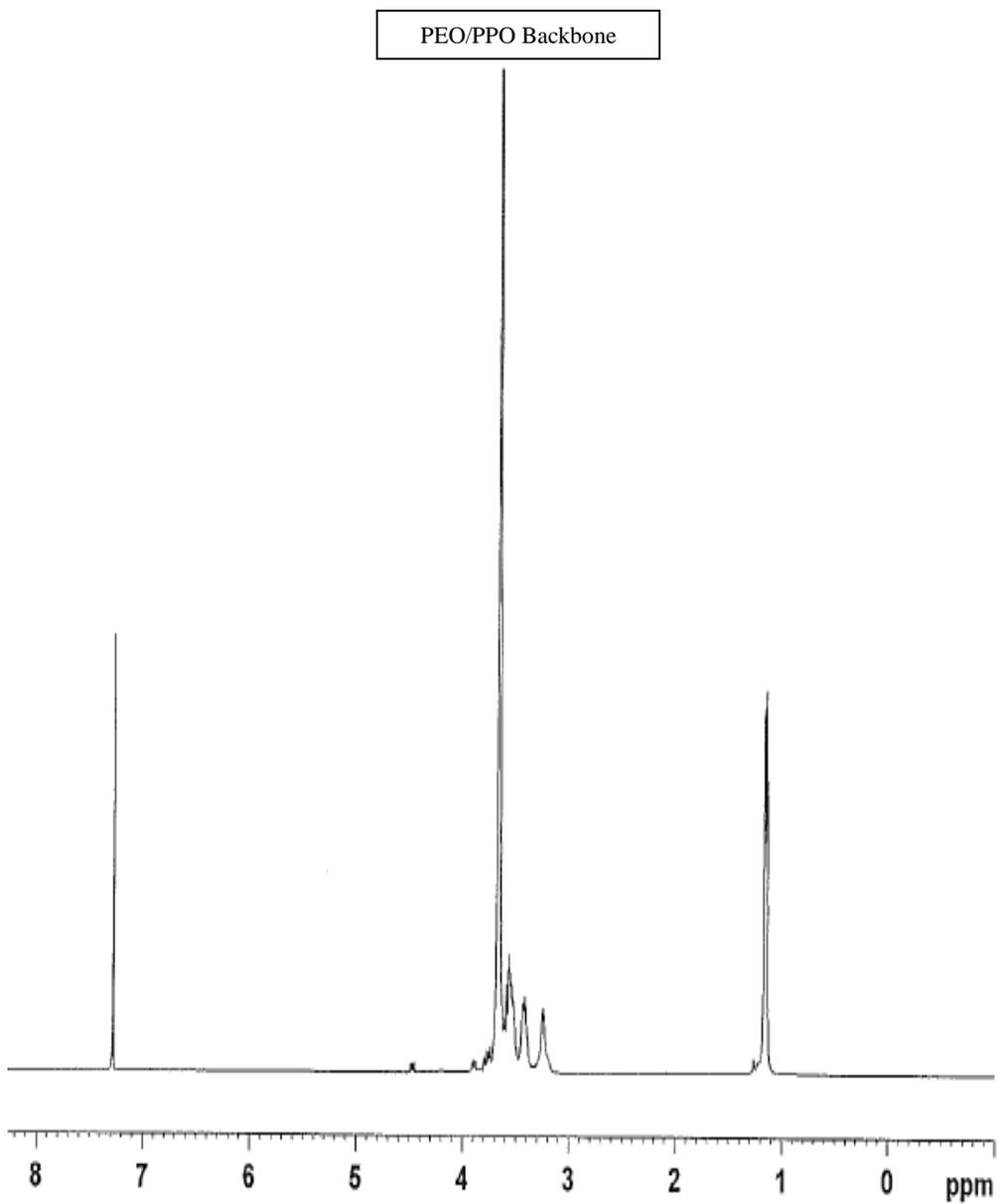
### RESULTS

#### A. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) Characterization of Tetronic® Acrylate

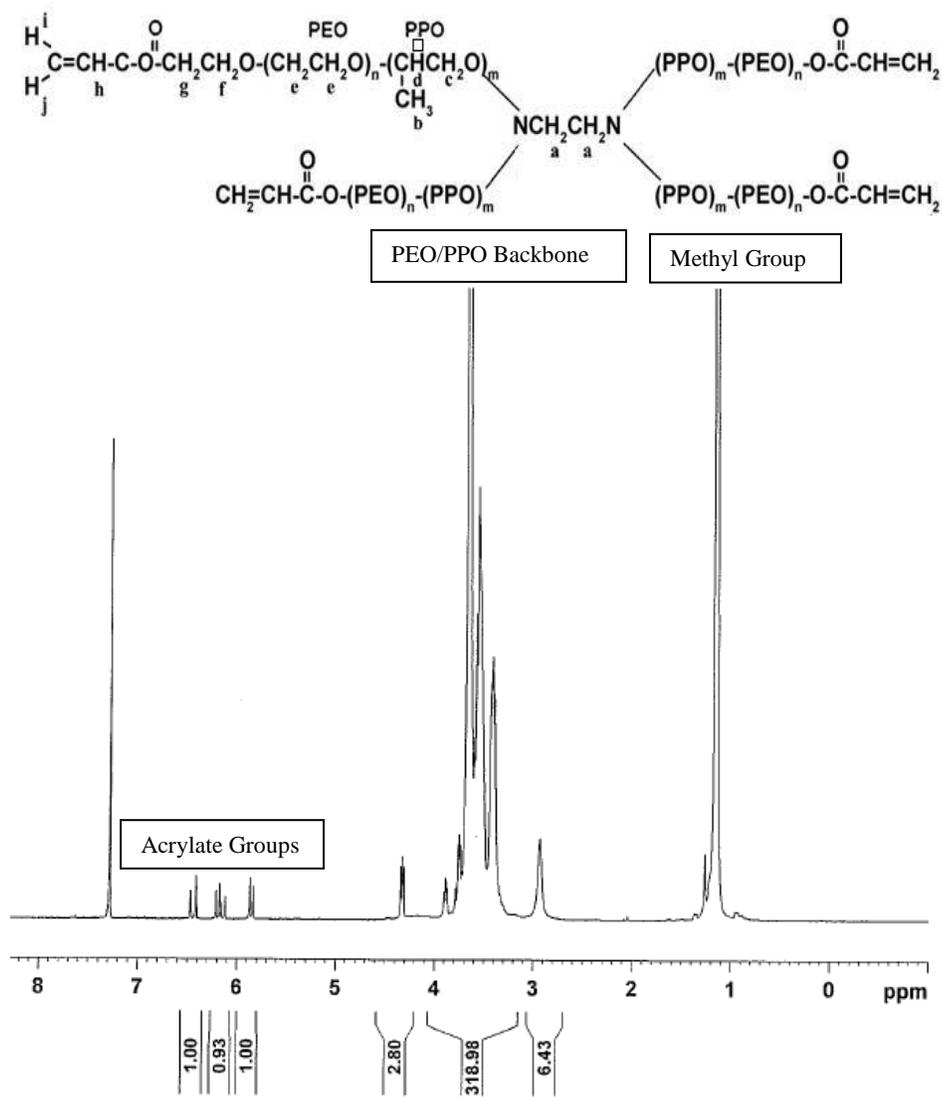
T1107-acrylate (unmodified: **Figure 4.1**, acrylated: **Figure 4.2**) and T304-acrylate (unmodified: **Figure 4.3**, acrylated: **Figure 4.4**) were characterized via NMR spectroscopy, with peaks present at locations including  $\delta = 1.2$  (PPO CH<sub>3</sub>), 3.65 (PEO CH<sub>2</sub>), 4.32 (-CCH<sub>2</sub>OC (=O)), and 5.8-6.4 (2H, acrylic -CH<sub>2</sub>) ppm. The acrylation efficiency conversion percentage, calculated based on the ratio of the integrals of the PEG backbone ( $\delta = 3.4\sim 3.7$ ) and the acrylate peaks, ( $\delta = 5.8\sim 6.4$ )<sup>12</sup> were 93-95% for T1107-acrylate and 62-74% for T304-acrylate over two synthesis reactions for each Tetronic® type (**Table 4.1**).

**Table 4.1:** Result summary of acrylation for Tetronics (mean $\pm$  SD; n=2 reactions)

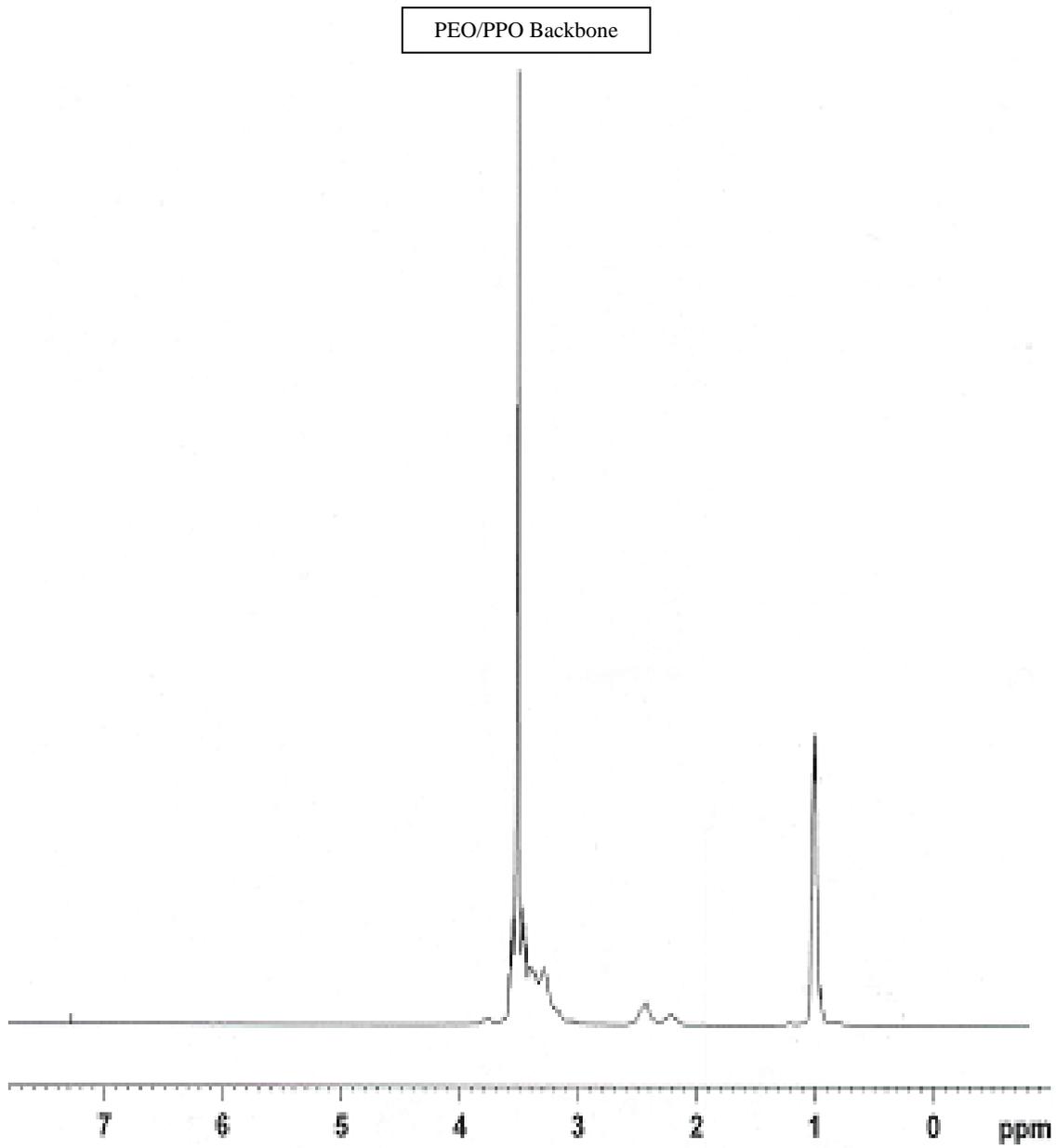
<b>Tetronic Type</b>	<b>Average Acrylation Conversion Acquired via NMR Spectra</b>	<b>Average Overall Product Yield (%)</b>
<b>T304</b>	68 $\pm$ 8.5%	53 $\pm$ 7.1%
<b>T1107</b>	94 $\pm$ 1.4%	73 $\pm$ 8.9%



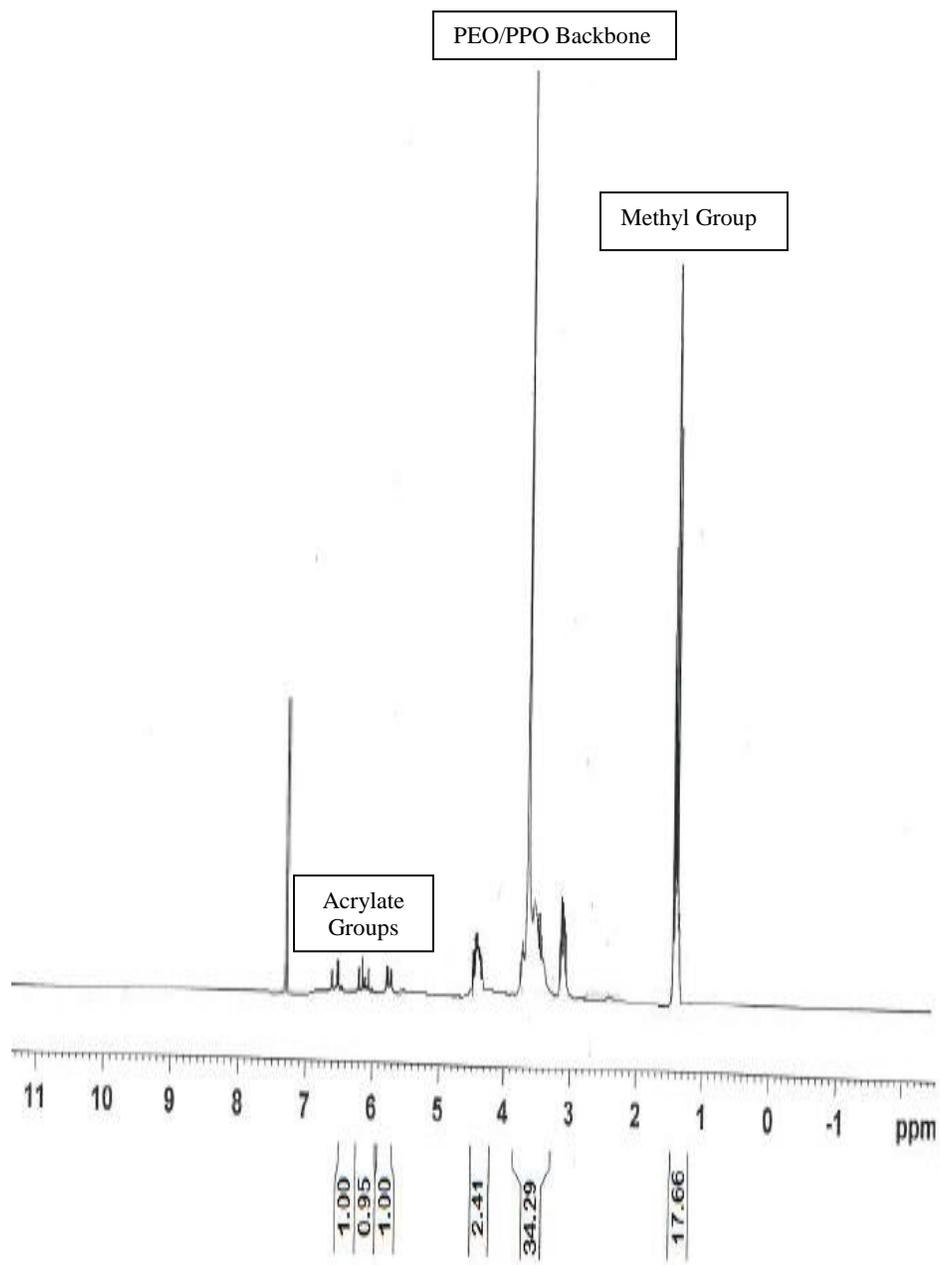
**Figure 4.1:**  $^1\text{H-NMR}$  spectrum for unmodified T1107 (T1107:  $n = 60$ ,  $m = 19$ )



**Figure 4.2:** Tetronic® structure (T1107:  $n = 60$ ,  $m = 19$ ) and  $^1\text{H}$  NMR spectrum of T1107-acrylate



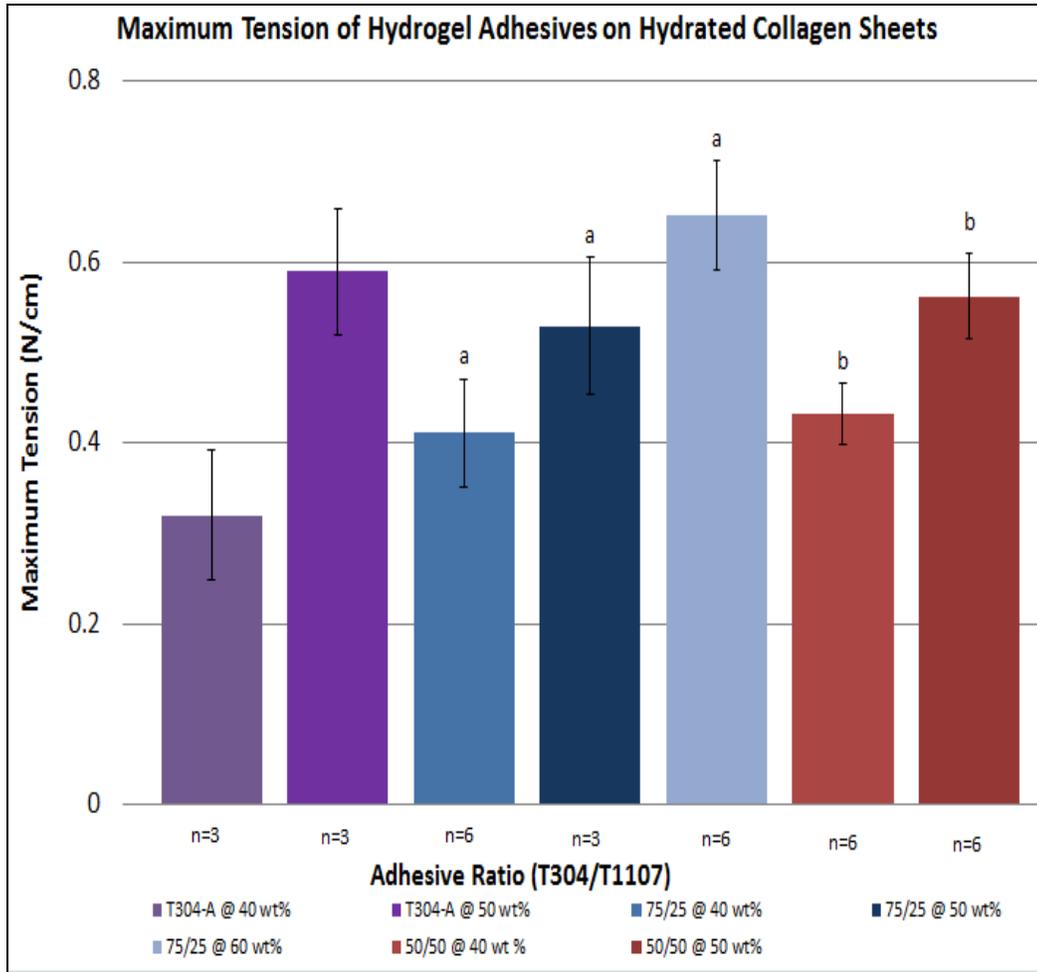
**Figure 4.3:** <sup>1</sup>H-NMR spectrum for unmodified T304 (T304: n =3.7, m =4.3)



**Figure 4.4:**  $^1\text{H}$  NMR spectrum of T304-acrylate (T304:  $n = 3.7$ ,  $m = 4.3$ )

## **B. Mechanical Testing of Tetronic® Adhesive**

The end-to-end tensile testing of collagen sheets bonded with Tetronic® adhesives revealed that at 40wt % (w/v), T304-acrylate/T1107-acrylate at 75/25 and 50/50 ratio blends exhibited a similar adhesion strength to T304-acrylate alone ( $p > 0.05$ ) with an average maximum tension of  $0.41 \pm 0.06$  (mean  $\pm$  SEM,  $n=6$ ) and  $0.43 \pm 0.03$  N/cm ( $n=6$ ), respectively, compared to  $0.32 \pm 0.07$  N/cm ( $n=3$ ) (**Figure 4.5**). In comparison, at 50wt %, the adhesive strengths were also similar ( $p > 0.05$ ) for all three blends tested (T304-acrylate alone:  $0.59 \pm 0.07$  N/cm for  $n=3$ , 50/50:  $0.56 \pm 0.05$  N/cm for  $n=6$ , and 75/25:  $0.52 \pm 0.08$  N/cm for  $n=3$ ), which were greater (but not significantly) than that of the 40wt % formulations. The highest strength value achieved was for the 75/25 T304-acrylate/T1107-acrylate group at 60wt % with a value of  $0.65$  N/cm  $\pm 0.06$  N/cm ( $n=6$ ), which was similar to the 50wt % blend groups, but exhibited significantly stronger ( $p < 0.05$ ) adhesive strength than the other 50/50 blend ratio groups at both 40 and 50wt %. There was also significant statistical difference among the 75/25 blend ratios for 40, 50, and 60wt %.



**Figure 4.5:** End-to-End Testing Mechanism for T304-acrylate/T1107-acrylate ratios at 40wt %, 50wt %, and 60wt% (ratio/wt%), (n=6 for 75/25/40 50/50/40, 50/50/50 and 75/25/60; n=3 for all other groups). Letters indicate significant difference between groups.

## C. Viscoelastic Characterization of Tetronic®

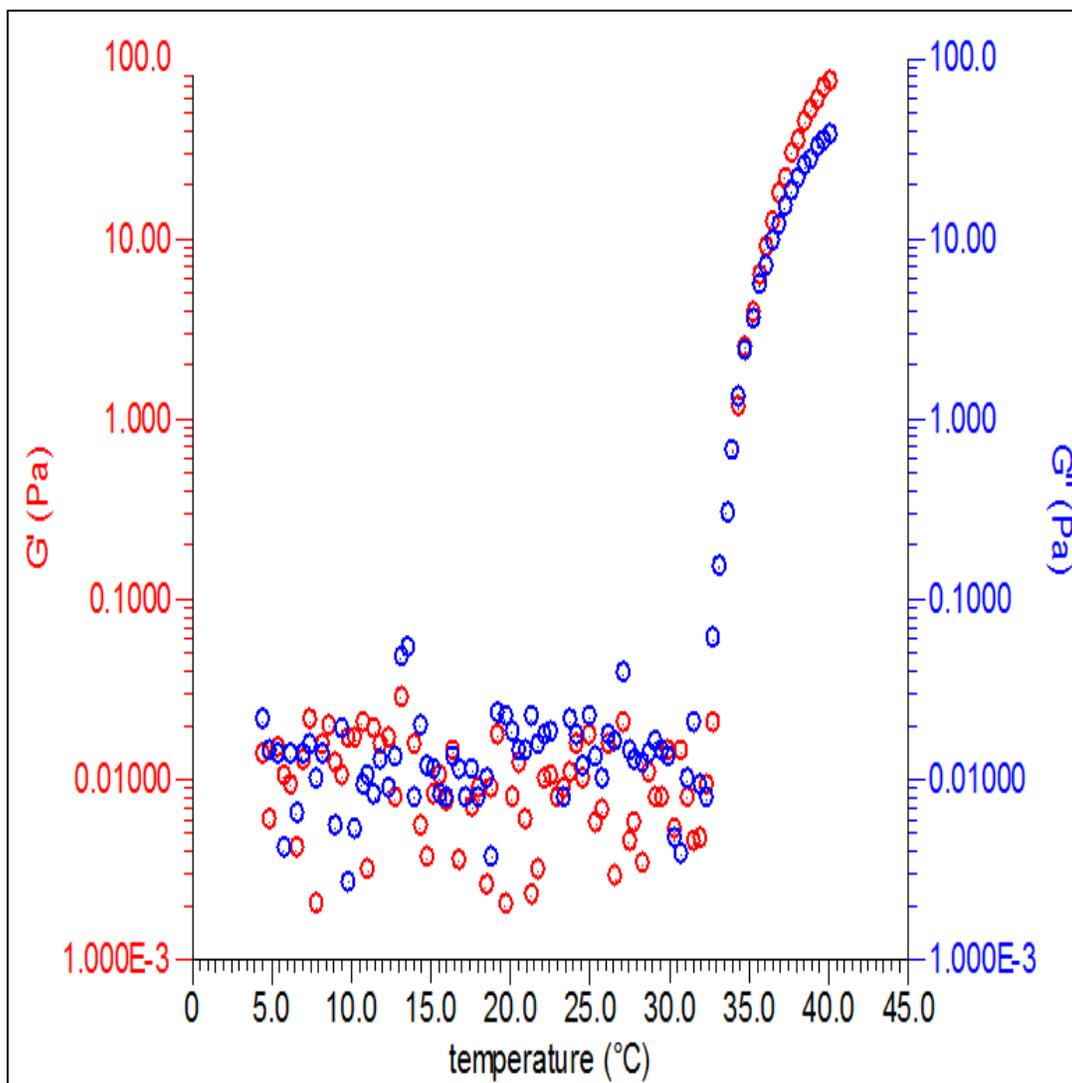
### a. Gelation Analysis

#### 1. Reverse Thermal Gelation

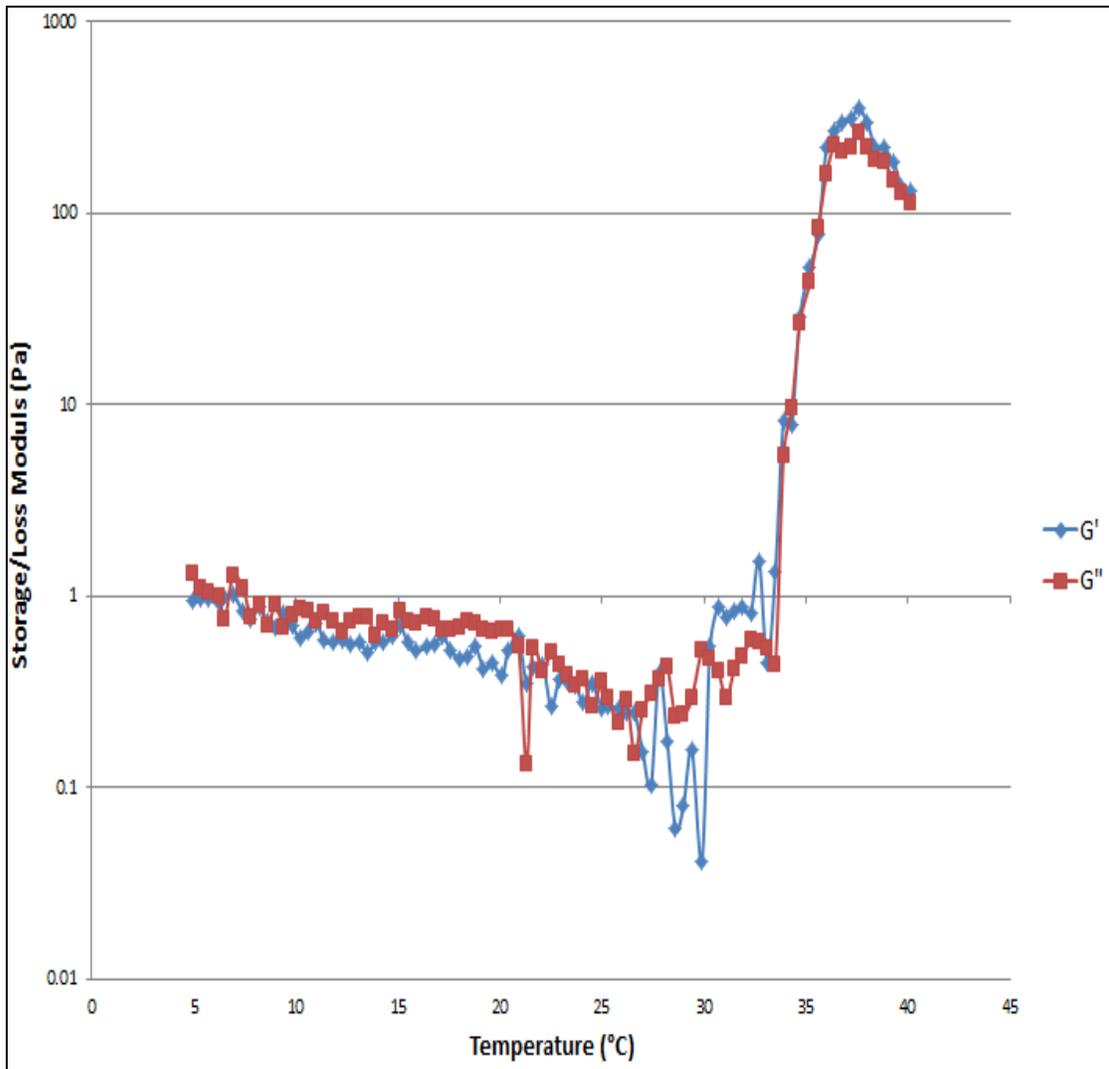
A temperature sweep from 4-40°C at 2°C/min was performed for the 100% T304 (at 30 and 50wt %), 100% T1107 (at 30wt %) and a 50/50 blend (at 30 and 50wt %). Sol-gel transition temperatures (at which the storage ( $G'$ ) and loss ( $G''$ ) moduli met) were  $20.05 \pm 0.28^\circ\text{C}$  for 100% T1107 and  $36.2 \pm 1.64^\circ\text{C}$  for the 50/50 blend at 30wt %. In contrast, gelation temperatures at 50wt % for the 50/50 blend were  $36 \pm 1.03^\circ\text{C}$  (**Figure 4.6**) and  $34.7 \pm 0.45^\circ\text{C}$  (**Figure 4.7**) for the 100% T304 (**Table 4.2**). Data were not acquired for 100% T1107 at 50wt % and 100% T304 at 30wt %, as it either gelled too fast to test or did not gel within the temperature range tested.

**Table 4.2:** Sol-gelation temperature of three Tetronic® blends % for a temperature sweep of 4-40°C (n=2)

<b>T304/T1107 composition (total 100%)</b>	<b>Reverse Thermal Gelation: Gelation Point (<math>^\circ\text{C}</math>) @ 30wt%</b>	<b>Reverse Thermal Gelation: Gelation Point (<math>^\circ\text{C}</math>) @ 50wt%</b>
<b>100/0</b>	N/A	$36.0 \pm 1.03$
<b>50/50</b>	$36.2 \pm 1.64$	$34.7 \pm 0.45$
<b>0/100</b>	$20.1 \pm 0.28$	N/A



**Figure 4.6:** Temperature sweep of 50/50 blend of T304-acrylate/T1107-acrylate at 50wt %



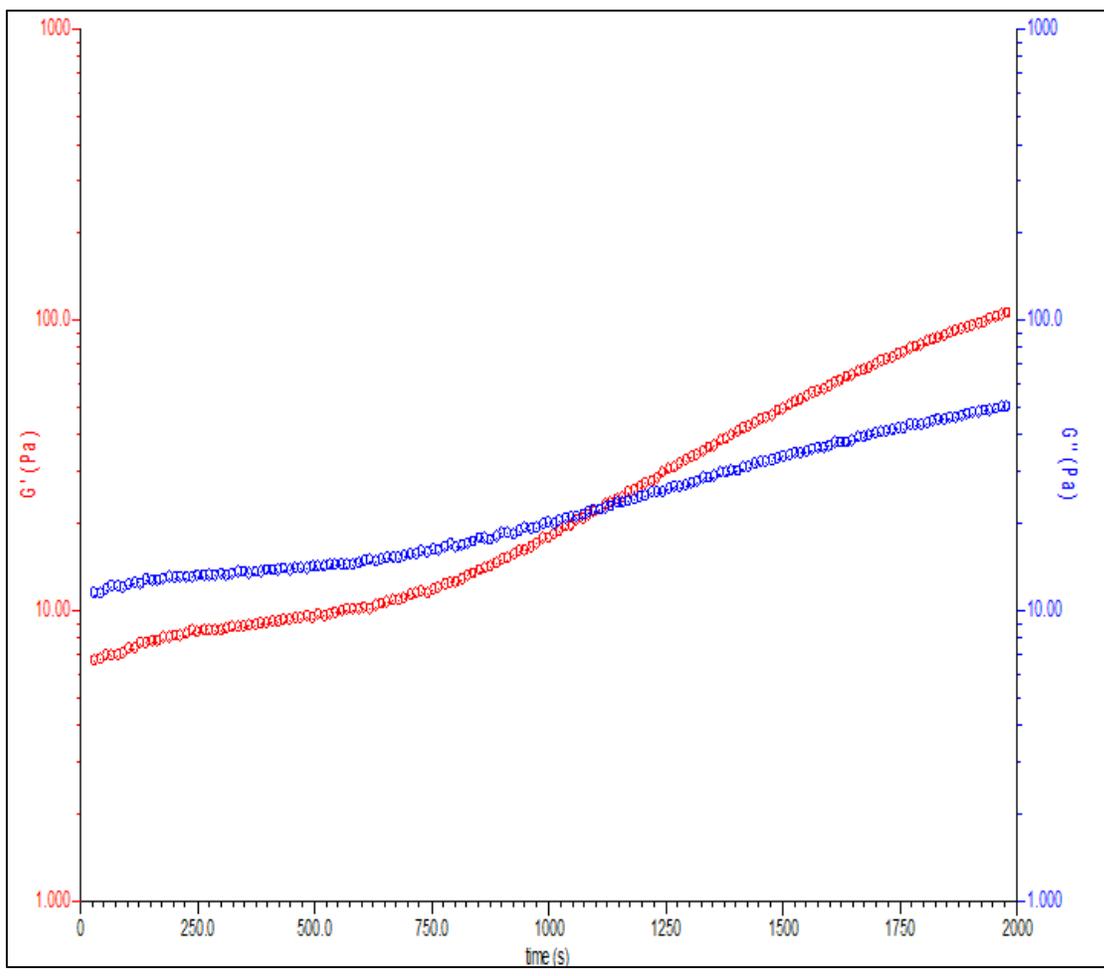
**Figure 4.7:** Temperature sweep of 100% T304-acrylate at 50wt %.

## 2. Tandem Crosslinking

The gelation times were measured for three blends of T304-acrylate/T1107-acrylate at 50wt % when covalently crosslinked with DTT at 30 and 50wt%. The 100% T1107-acrylate's gelation time was  $26.6 \pm 2.28$  seconds for 30wt %, but did not yield viable results at 50wt % due to rapid gelation. Gelation times 7 minutes and 19 minutes on average (**Table 4.3**) for the 50/50 blend and 100% T304-acrylate at 30wt % (n=2), (**Figure 4.8**) respectively. In comparison, the average was 5 minutes and 11 minutes for the same blend ratios at 50wt %.

**Table 4.3:** Gelation times of three Tetronic® blends when crosslinked with DTT (n=2)

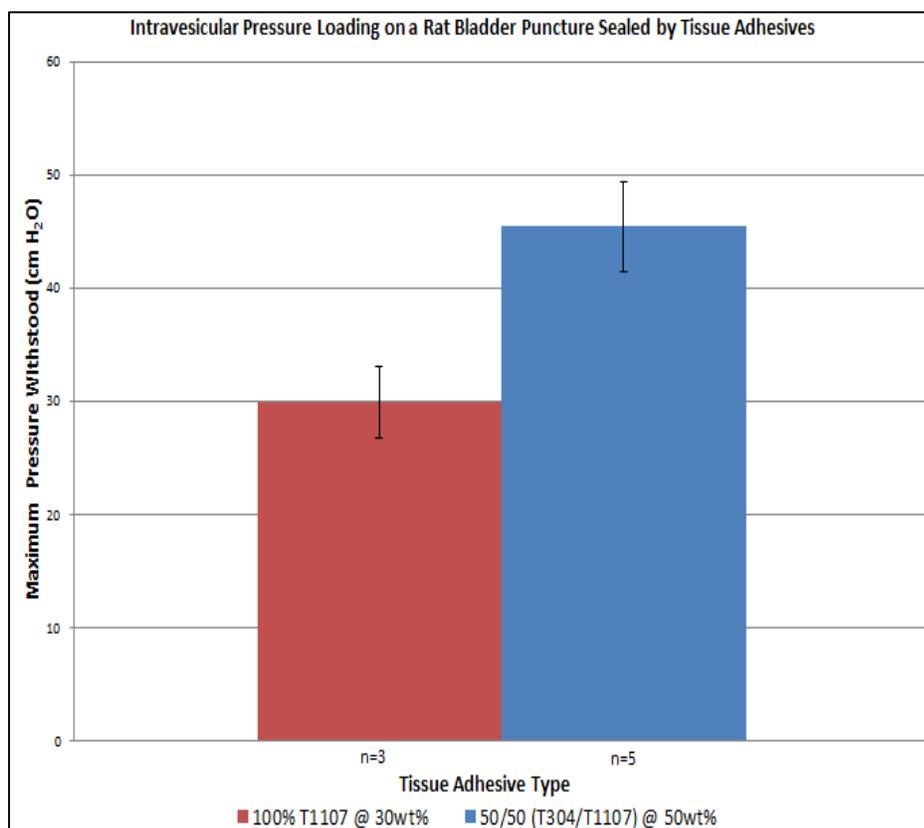
<b>T304-acrylate/T1107 - acrylate composition (total 100%)</b>	<b>Tandem Crosslinking: Gelation Time (s) @ 30wt%</b>	<b>Tandem Crosslinking: Gelation Time (s) @ 50wt%</b>
<b>100/0</b>	$1143.8 \pm 12.14$	$629.3 \pm 6.02$
<b>50/50</b>	$474.1 \pm 17.31$	$304.5 \pm 8.49$
<b>0/100</b>	$26.6 \pm 2.28$	N/A



**Figure 4.8:** Chart of 100% T304-acrylate subjected to oscillatory shear until gelation at 30wt %

#### **D. *Ex Vivo* Adhesive Testing on Rat Bladder**

Rat bladders with approximately 2 mm punctures sealed with 100% T1107-acrylate at 30wt % withstood an average of  $29.91 \pm 3.21$  cm H<sub>2</sub>O before failure (n=3). In contrast, T304-acrylate/T1107-acrylate (50/50) at 50wt % withstood significantly ( $p < 0.05$ ) higher pressure at an average of  $45.45 \pm 3.96$  cm H<sub>2</sub>O (mean  $\pm$  SEM, n=5) (**Figure 4.9**)



**Figure 4.9:** Effect of intravesicular pressure-loading rat bladder punctures sealed with two different Tetric® adhesives (n=3 or 5)

## CHAPTER FIVE

### DISCUSSION

#### A. Characterization of Tetronic® Acrylate

The goal of the present study was to investigate the effect of blending a low MW T304 on overall bulk strength of a Tetronic® 1107-based tissue adhesive, which was previously investigated<sup>12,39,40,41</sup> For T1107, the  $94 \pm 1.41$  % average percentage conversion of the terminal hydroxyls to acrylates obtained in the present study was on par with that reported previously by Cho et (97% average)<sup>12</sup>, indicating that a similar level of chemical crosslinking and bulk strength of this component could be expected. In contrast, average acrylate conversion percentage for T304 was  $68\% \pm 7.49$  with a relatively low product yield compared to T1107-acrylate (53% vs. 73%), which may be attributed to the differences in physical state (solid for T1107 and liquid for T304 at RT). We speculated in early stages of the study that large amounts of T304 were lost during the sodium sulfate drying and ethyl ether/hexane wash steps of the acrylation process based on differences in the amount of product observed before and after these specific steps. Therefore to improve both acrylation efficiency and product yield for T304, we modified the synthesis protocol to include longer reflux durations for the distilled product, efficient rotary evaporation by allowing more time to elapse in order to fully evaporate either toluene or DCM, and more sodium sulfate drying steps at smaller volumes. Using this modified protocol, T304-acrylate product yield almost tripled from 17% to an average of 53%. Additionally, acrylation percentage increased from 39% to an average of 68%. Further modification of

the synthesis process such as even slower addition of acryloyl chloride to the product may lead to an even higher acrylation percentage for the T304-acrylate.

## **B. Mechanical Testing of Tetronic® Adhesive**

The results of the present study demonstrate that T304-/T1107-acrylate blends (**Figure 4.5**) were mechanically stronger compared to the T904-/T1107-acrylate blends previously tested (**unpublished data, Appendix C, Figure C.1**). More specifically, the average maximum adhesive strengths at a 75/25 and 50/50 ratio of T304-/T1107-acrylate values,  $0.41 \pm 0.06$  N/cm and  $0.43 \pm 0.03$  N/cm (**Figure 4.5**), were much greater than the same ratios for T904-/T1107-acrylate (40wt %),  $0.15 \pm 0.02$  N/cm (n=9) and  $0.07 \pm 0.01$  N/cm (n=9) (**Figure C.1**), respectively. A similar trend was also observed at 50wt %.

Differences in strength between the T304 and T904 are attributed to the much lower MW of the T304 (1650 Da vs 6700 Da), which enables more Tetronic® content to be incorporated into the hydrogel adhesive. Among the blends tested to date, the 75/25 ratio of T304-/T1107-acrylate at 60wt % exhibited the highest bulk strength, corroborating with our original hypothesis that the higher content of hydrogel by incorporation of the low MW T304-acrylate would increase the strength of the tissue adhesive. Statistical analysis verified this by showing significant differences ( $p < 0.05$ ) among various weight percentages of the same blend ratio (i.e. 75/25 at 40, 50, and 60wt % and 50/50 at 40 and 50wt %), but not among specific blend ratios within one weight percentage category. However, it must be noted that T1107-acrylate did contribute to the bulk strength of the adhesive as evidenced by increases from the 75/25 to 50/50 ratios by manipulating the

amount of T1107-acrylate content for both 40wt % ( $0.41 \pm 0.06$  N/cm to  $0.45 \pm 0.05$  N/cm) and 50wt % ( $0.52 \pm 0.08$  N/cm to  $0.56 \pm 0.05$  N/cm). Yet, a “ceiling” has been established for how much T1107 content can be included in the surgical adhesive (30wt %) before rapid thermal gelation occurs. Catalyzed by the large molecular weight of 15,030 Da and PEO/PPO ratio of the gel, T1107 has a high capacity for reverse thermal gelation in both its unmodified and modified forms.<sup>12</sup> This theory is also illustrated in the fact that mechanical testing results were unattainable for high weight concentrations of the T1107 (i.e. 25/75 T304-/T1107-acrylate at 40wt % and up) due to gelling of the Tetronic® hydrogel before it could be applied to the collagen casing surface.

To estimate a target strength for the Tetronic®-based adhesive, the Law of LaPlace was applied at physiological conditions of 40 cm H<sub>2</sub>O voiding pressure, 600 ml capacity, and 12 cm distended bladder diameter. Calculated as a function of bladder thickness, diameter and intravesicular pressure, it was determined that the surgical tissue adhesive must withstand tensions approximately between 3.2-4.8 N/cm. While these values were not reached in the present study, failure during mechanical testing was observed as “cohesive failure” as the material broke at the interface between the two collagen strips, but was still present on the surfaces of the debonded pieces. This type of failure indicates that the adhesive strength of the Tetronic® hydrogel is stronger than cohesive strength. It is predicted based on results generated by mechanical testing of the adhesive that higher weight percentages of T304-acrylate (in the range of 70-80wt %) in the hydrogel-based tissue adhesive will aid in yielding even higher bulk strengths. Additionally, it is projected

that bond strength can also be improved through copolymerization of the Tetronic® hydrogels with PEG/dextran hydrogels or acrylate/aldehyde difunctional polyoxamers, in addition to functionalizing the Tetronic® hydrogel with catechols.<sup>12,27,28,41,45,46</sup>

### C. Viscoelastic Characterization of Tetronic®

Concentrated aqueous solutions of Tetronic® have been shown to undergo physical gelation at temperatures in the 20-35°C range due to the hydrophobic transition associated with their PPO segments.<sup>41</sup> At 30wt %, the 100% T1107 gelled at 20.05±0.28°C (**Table 4.2**). In contrast, the 100% T304 at 30wt % did not gel under the temperature range tested in (4-40 C) as Cho et al. observed for the 100% T904 in a previous study, suggesting that both T304 and T904 lack reverse thermal gelation at relatively low concentrated solutions.<sup>12</sup> Both the 100% T304 and 50/50 blend at 50wt %, however, transitioned from a liquid to solid state at physiological temperatures of 36±1.03°C and 34.7±0.45°C (**Table 4.2**), respectively.<sup>12</sup> Moreover, T304/T1107 at 30wt % and a 50/50 blend ratio gelled at a higher temperature, 36.2±1.64°C, than the same blend ratio for T904/T1107 which gelled at 23.67±1.22°C. This demonstrates that adhesives containing T304 can gel upon contact with tissue at physiological temperatures (37°C).<sup>12</sup> Yet, it is projected that since the 100% T304 at 30wt % failed to gel at physiological temperatures, either a higher molecular weight Tetronic should be used in conjunction with the T304 or a higher weight percentage of T304 alone should be used to create the Tetronic®-based tissue adhesive.

The increase in gelation time with increasing T304-acrylate content observed in the present study (**Table 4.3**) is associated with the diminishing contribution of the T1107-acrylate component to reverse thermal gelation.<sup>12</sup> This is evidenced in the drastic difference in gelation times between the 50/50 blend ( $304.5 \pm 8.49$  sec) and the 100% T304-acrylate ( $629.3 \pm 6.02$  sec), demonstrating the impact T1107 has on gelation time. When comparing the gelation times for 100% T304-acrylate and the 100% T304-acrylate in Cho et al.'s study<sup>12</sup>, the 7.5-fold difference  $153.33 \pm 5.77$  sec versus  $1144 \pm 12.1$  sec, was thought to be attributed to the difference in acrylation efficiency (87% vs. 68%), and in molecular weights. This was because neither product contained T1107, and the gelation is due mainly to the covalent crosslinking, mediated by the presence of DTT. Moreover, the present study demonstrated that the gelation time of the 50/50 blend at 50wt % was lower than that the same blend at 30wt %. These results illustrate the impact of hydrogel content and an increased number of crosslinking sites on the ability to adjust gelation time. Finally, since the Michael-type addition is a pH-dependent reaction, the gelation times and reaction rates for covalent crosslinking can be further tuned for desired applications by adjusting the pH of the solution reactants.<sup>12</sup>

#### **D. *Ex Vivo* Adhesive Testing on Rat Bladder**

Because the adhesive developed in the present study was originally meant for repair of bladder injuries, *ex vivo* testing was conducted using a custom device<sup>44</sup> on rat bladders submersed in saline solution with the purpose of approaching more physiological conditions than tensile testing with tissue segments. The 50/50 blend of T304-

acrylate/T1107-acrylate at 50wt % withstood pressures at an average of  $45.45 \pm 3.96$  cm H<sub>2</sub>O, higher pressures than the control group, the 100% T1107-acrylate at 30wt % ( $29.91 \pm 3.21$  cm H<sub>2</sub>O). These results showed that this blend of Tetronic® adhesive was effective at sealing a rat bladder puncture at physiological pressures. However, further tests need to be conducted following repeated cycling of the bladder in order to measure long-term durability of the adhesive. It also must be noted that in the present study, only two blend ratios of T304-acrylate/T1107-acrylate were tested in these conditions. Therefore, it is predicted that blends with higher levels of Tetronic® contents (75/25 at 60wt %), will withstand higher levels of intravesicular pressure.

## CHAPTER SIX

### CONCLUSION

The results from the present study demonstrated the impact of a low molecular weight Tetronic®, T304, on the bulk strength of a hydrogel-based bladder adhesive. Specifically, using T304 instead of T904 has allowed for a higher content of hydrogel to be incorporated into the Tetronic®-based adhesive, thus increasing the weight percentage to increase overall bulk strength. Additionally, the contribution of T1107 to the bulk strength and gelation time of the Tetronic®-based adhesive has been demonstrated. Therefore, further fine tuning of T304-acrylate/T1107-acrylate blends ratios as well as improving the acrylation efficiency of the T304 should allow for achieving the balance between high overall bulk strength and clinically relevant gelation times.

## CHAPTER SEVEN

### LIMITATIONS AND FUTURE WORK

#### A. Limitations and Potential Solutions

##### a. Characterization of Tetronic® Acrylate

- Acrylation efficiency of the T304 (68%) was lower in comparison to T1107 (94%) possibly due to rapid addition of acryloyl chloride. This conversion percentage may be improved by adding the acryloyl chloride much more slowly which will provide more time for the reaction and more efficient filtration of the TEA-HCl salt to remove excess chlorides.
- Product yield of T304 was also low in comparison to T1107 mainly due to the product loss during the sodium sulfate drying steps. This could be improved by more sodium sulfate drying steps at smaller volumes.
- In the present study, PBS was used to dissolve Tetronic® without further adjustment of pH. Since crosslinking rate of Tetronic®-acrylate is highly sensitive to pH variability, in future studies, pH levels of the T304 should be monitored (and adjusted accordingly) at regular intervals and maintained in the desired range (ideally 7.2-7.4) before it is used as a tissue adhesive.

##### b. Mechanical Testing of Tetronic® Adhesive

- Since the main goal of the present study was to improve the bulk strength of the Tetronic®-based adhesive, mechanical testing was performed in the end-to-end configuration. By completing shear adherence tests of T304-acrylate/T1107-acrylate

with the same blend ratios as T904-acrylate/T1107-acrylate previously tested, a direct comparison could be made as to how incorporation of T304 in the hydrogel-based adhesive improves the bond strength.

- Tetronic® adhesives on collagen casings were cured under a hydrated condition for one hour prior to mechanical testing for all blend ratios and weight percentages to maintain consistency between the previous and present studies. Since the viscoelastic characterization demonstrated that gelation of T304/T1107 (50/50) occurs in 5 minutes, future mechanical testing may be performed with shorter curing times in order to be more clinically relevant.

#### **c. Viscoelastic Characterization of Tetronic®**

- Temperature sweep tests were only performed on unmodified Tetronic® solutions to characterize the reverse thermal gelation behavior of the polymers. Conducting these tests on modified Tetronic® macromer solutions would provide further insight into the effect of terminal acrylate groups on gelation temperatures.

#### **d. *Ex Vivo* Adhesive Testing on Rat Bladder**

- Pressure testing of rat bladders was limited to a single loading cycle until failure. Subjecting the rat bladder to repeated cycles of pressure loading will allow for assessing long-term strength and durability of the adhesive.

## **B. Future Work**

- **Evaluating the swelling behavior of T304-acrylate**
  - Rationale: In order to ensure the adhesive does not lose its mechanical strength or cause compression in surrounding tissues
- **Testing the hydrogel-based adhesive on the bladder both *ex vivo* and *in vivo* using larger animal models**
  - Rationale: Tests on larger animal models are a necessary step after small animal models (rats) before ultimately moving onto clinical trials.
- **Incorporation of a controlled biodegradable feature to the formulation**
  - Rationale: Tetronic® polymers go through hydrolytic degradation, so a feature that enables controlled biodegradation will extend the amount of time for which the adhesive will be effective.
- **Development of a user-friendly application tool for surgical procedures**
  - Rationale: Ease of application is one of the characteristics of an ideal surgical adhesive. The application mechanism must take into account the two separate parts of the hydrogel-based adhesive: the Tetronic® component and the thiol-containing crosslinker component.

## APPENDICES

## **Appendix A: Additional Literature Review on Tissue Adhesives**

### **a. Cyanoacrylate**

#### **1. Leggat et al. (2007)**

The application of adhesive cyanoacrylate film strengthens by rapid polymerization, usually within 5–60 seconds, catalyzed by hydroxyl groups on the surface being glued. Earlier research work proposed that cyanoacrylate adhesives may generate lipid hydroperoxides, which in turn activate prostaglandin and thromboxane biosynthesis. These adhesives have also demonstrated the capacity to oxidize and lyse cell membranes, somewhat explaining the reason behind the presence of particular thrombotic events associated with necrosis when cyanoacrylates are used *in vivo*. In general, cyanoacrylate has been shown to cause inflammation and tissue necrosis *in vivo* when used as a tissue adhesive.<sup>20</sup>

#### **2. Mizrahi et al. (2011)**

Methodology in one study involved synthesizing the cyanoacrylates by condensation and Knoevenagel reaction followed by characterization of the product by <sup>1</sup>H NMR spectroscopy. Mechanical testing was initially performed using an Instron universal testing machine on aluminum specimens, with 5 microliters of the monomer applied to each of the two specimens. The peak detachment force (N) and modulus were determined after the probe was withdrawn at a rate of 0.1 mm/min. Similar experiments were performed using strips of fresh skin harvested from 10 rats. 25 microliters of each glue type was applied to cross-sectional cuts of 2 x 6 cm strips of skin with specimens

stretched at a rate of 10 mm/min. Furthermore, the cytotoxicity of the different cyanoacrylates was evaluated by exposing cells to the polymerized glues either by direct contact (“direct”) or indirectly by exposing cells to the medium (DMEM) in contact with the polymerized glues (“indirect”). And finally to test biocompatibility, Skin incisions of 1.5 cm were made in anesthetized rats with 20 microliters of cyanoacrylate applied to the resulting pouch. After 12 days, the rats were killed via carbon dioxide, and the skin surrounding the glues was analyzed using histology.<sup>14</sup>

Results showed that yields of the synthesized monomers reduced with increasing side-chain length, possibly due to higher boiling points and larger degrees of side-chain entanglement with increasing length. For mechanical testing, load values at rupture were between 20 and 30 Newtons, consistent with previous studies for other cyanoacrylate glues used *ex vivo*. Also, increasing side chain length was proven to increase the elasticity of the polymer as well as decrease adhesive strength (although all strengths were still classified as “useful”. With regard to cytotoxicity, monomers with longer side chains had less toxicity both *in vivo* and *in vitro*. Biocompatibility findings demonstrated that animals injected with cyanoacrylates comprised of longer side-chains had inflammation without necrosis while those injected with monomers comprised of shorter side-chains displayed serious tissue responses with prominent inflammation and necrosis.

### **3. Mobley et al. (2002)**

The binding strength of cyanoacrylates improved when the octyl-2 isomer was introduced due to its approximately 4-fold three dimensional breaking strength increase in comparison with its predecessor. Another advantage of this newest cyanoacrylate type is that it possesses an antimicrobial activity that have been shown to have positive effects on posttraumatic laceration.<sup>15</sup>

### **4. Reese et al. (2001)**

Additionally, data acquired from numerous clinical trials have shown that rates of wound dehiscence, hematoma formation and infection in cyanoacrylate-based adhesives are nearly identical to those found in sutures repairs.<sup>13</sup>

## **b. BioGlue**

### **1. Furst et al. (2005)**

One study sought to evaluate the amount of glutaraldehyde released from BioGlue, the effect of cytotoxicity on cultured cells (both *in vivo* and *in vitro*), and the local reaction of rabbit lung, liver and aortic tissues to BioGlue. In the rabbit lung and liver models, a fibrin sealant was used as the control group. With respect to experimental procedure, BioGlue was prepared according to manufacturer instructions, allowed to polymerize for 2 minutes, and then overlaid with 5 ml of saline solution. The resulting supernatants were then harvested and analyzed to determine content of glutaraldehyde. The cytotoxic effect of BioGlue was evaluated by adding the supernatants to either human embryo fibroblasts

(MRC5) or mouse myoblasts (C2C12). *In vivo* toxicity was measured on three different tissues by applying BioGlue onto a rabbit partial lung resection, a liver abrasion, or an intact abdominal aorta. Transverse tissue samples were stained with hematoxylin and eosin and histologically assessed 2 and 7 days after application.<sup>23</sup>

## **2. LeMaire et al. (2002)**

To test their hypothesis, LeMaire et al. first subjected ten 4-week-old domestic piglets ( $8.0 \pm 1.4$  kg) to general anesthesia. Next, under sterile conditions, the infrarenal abdominal aorta was exposed through a midline laparotomy after which the aorta was transected at the midpoint between the renal arteries and the bifurcation. A 2 mm ring of aorta was removed from the proximal stump and cut longitudinally to allow for accurate measurement of the aorta's circumference. Finally, an end-to-end aorto-aortic anastomosis was performed with interrupted 6-0 polypropylene sutures. Following verification of patency using an aortogram, five piglets were assigned to either the BioGlue group or control group. For the experimental group, a layer of BioGlue was applied around the entire anastomosis as per manufacturer instructions. After a 7-week growth period, the aortas were excised for morphometric analysis and histopathology. Histologic sections of 5 microns were prepared using hematoxylin and eosin as well as Geske' stains, while for the morphometric analysis, aortic growth was evaluated by calculating the changes in outer circumference and aortic area over the 7-week growth period.

Initial results from this study showed that weight gains were similar in both the experimental and control groups. Yet, in pigs treated with BioGlue, aortic circumference increased less,  $1.5 \pm 0.8$  mm, versus  $2.7 \pm 0.8$  mm for the control group ( $p=0.054$ ). Moreover, BioGlue animals developed a 33.9% stenosis of the aortic lumen area against 3.7% in the controls ( $p = 0.038$ ). Histological results demonstrated normal postoperative changes in the anastomotic sections of the control animals, including suture granulomas and a mild increase in adventitial connective tissue. In comparisons, similar sections in the BioGlue animals showed macrophage infiltration, dystrophic calcification, and presence of foreign body giant cells. The group also found that the piglets treated with BioGlue showed evidence of major fibrosis in the area surrounding the aorta.<sup>22</sup>

### **c. Fibrin Glue**

#### **1. Radosevich et al. (1997)**

Fibrin glues are biological, biodegradable products that do not cause serious negative reactions of tissue or induce necrosis/inflammation, which is in contrast to synthetic sealants. Fibrin binds to biological tissues either by covalent, hydrogen or other electrostatic bonds. Additionally, fibrin binds covalently to fibronectin and collagen by way of factor XIIIa.<sup>24</sup>

## **2. Cho et al. (2011)**

There are certain drawbacks with naturally derived products such as fibrin glue and BioGlue. These risks involve the possibility of viral transmission, hypersensitive reactions to bovine proteins, and relatively low mechanical properties.<sup>12</sup>

## **3. Traver et al. (2006)**

The composition of the commercial types of fibrin glue varies, but there are distinct similarities such as incorporation of a 2-vial system containing fibrinogen, thrombin, factor XIII, and calcium. The mechanism by which fibrin glue operates is actually a mimic of the final steps of the natural coagulation cascade experienced by the human body: thrombin activates factor XIII, which stabilizes the blood clot, by stimulating crosslinking of the fibrin chains to long fibrin strands. Preparation takes 15 minutes and application is performed using a double-barrel syringe system.<sup>17</sup>

## **4. Mobley et al. (2002)**

This type of tissue adhesive has the longest history, and therefore, the broadest range of applications. Recently, enhanced tensile strength by using pooled plasma has been shown to increase fibrinogen production leading to more versatility for fibrin products in facial reconstruction. Also, higher concentrations of thrombin in fibrin lead to quicker clot formation and are ideal for hemostasis. Finally, an advantage of the double-barrel syringe method of mixing is uniform mixing of the components, but disadvantages lie in complexity and needle clogging. As a result, a more sophisticated system of application,

the Hemamyst aerosol spray device (Haemacure) is used. This is a gas pressurized system using pressures of 5 to 10 liters/ min. The components are mixed in a pressurized gas stream, allowing for more thorough mixing of the components compared to the older syringe systems.<sup>15</sup>

#### **5. Reese et al. (2001)**

Fibrin glues have displayed the ability to be effective in cardiovascular surgery for hemostasis, sealing air leaks in lung procedures, control burn bleeding, and in treating lymphatic leaks in the neck. Additionally, they are the only type of tissue adhesives that promote healing.<sup>13</sup>

#### **6. Artzi et al. (2009)**

Fibrin glue contains an antiproteinase that in turn hinders the growth of vascular granulation tissue and healing of surgical anastomoses.<sup>28</sup> It is also characterized as having relatively non-specific and minimally adhesive tissue interaction while demonstrating a mild tissue response.<sup>45</sup>

#### **7. Shazly et al. (2008)**

Finally, fibrin glues are generally more biocompatible than cyanoacrylates, but introduce a moderate risk of infectious transmission due to their biological origins.<sup>46</sup>

#### **d. Tetronic®**

##### **1. Cho et al. (2011)**

Acrylated macromers were covalently crosslinked using dithiothreitol (DTT) as a donor of the thiol group. Solutions of T1107/T904 acrylate ratios and DTT at 4°C were mixed at a 1:1 thiol:acrylate molar ratio (30% weight/volume final Tetronic® concentration). Also, viscosity of the polyoxamine solutions were characterized at three different temperatures using a rheometer (Physical MCR 300). The macromers were also subjected to thermally-induced noncovalent gelation assessed by a tube inversion method. Tissue bond strength was examined using rat skin; various hydrogel solutions mixed with DTT were applied to a 1 cm x 1 cm area of one skin strip and another skin strip was placed over it. Bonding strength was calculated as the maximum load divided by the bonded area. Tensile testing on all samples was performed after overnight equilibration at 4 and 37°C where samples were cut into dog-bone shaped and subjected to uniaxial testing on an MTS Synergie 100 machine at 20% strain and relaxation. Finally, cytotoxicity tests were conducted using normal human dermal fibroblasts.<sup>12</sup>

In general, Tetronic®-based hydrogels are expected to bond to native tissue by way of mechanical interactions with the tissue microarchitecture. Additionally, they can bond through covalent crosslinking of free thiols present in ECM proteins. The amount of free thiols present may vary based on exposure time to DTT during gelation (larger time periods increase thiol number).<sup>12</sup>

## Appendix B: Complete Tetronic®/DTT Mixing Table

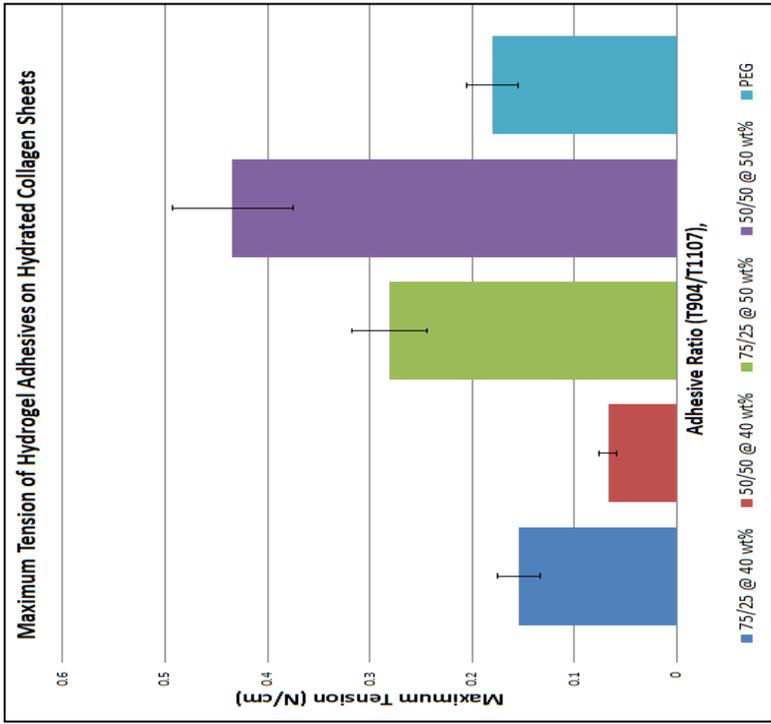
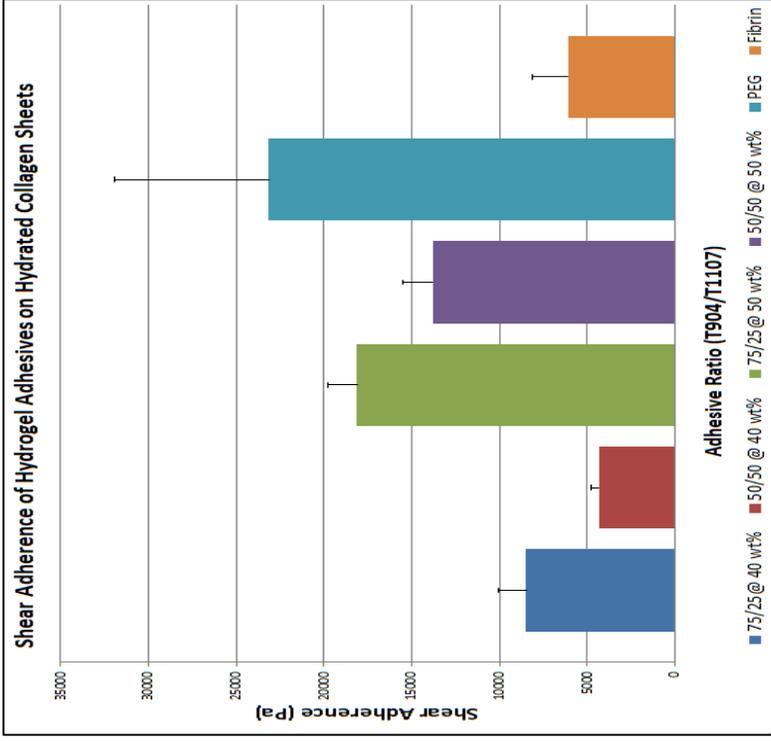
**Table B.1:** Full Tetronic®/DTT mixing table (**bolded** formulations used in mechanical testing)

Wt%	ratio	T1107 (g)	T1107 wt% check	T1107 DTT (mg)	T304 (g)	T304 wt% check	T304 DTT (mg)	Total wt (g)/ 1ml PBS	Total DTT(mg)/ 100ul PBS (T1107/T 304)	Total wt% check
30	50/50	0.225	0.15	4.618563	0.225	0.15	42.1	0.45	46.7	0.3
<b>40</b>	<b>0/100</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0.7</b>	<b>0.4</b>	<b>130.9</b>	<b>0.7</b>	<b>130.9</b>	<b>0.4</b>
<b>40</b>	<b>25/75</b>	<b>0.175</b>	<b>0.1</b>	<b>3.592216</b>	<b>0.525</b>	<b>0.3</b>	<b>98.2</b>	<b>0.7</b>	<b>101.8</b>	<b>0.4</b>
<b>40</b>	<b>50/50</b>	<b>0.35</b>	<b>0.2</b>	<b>7.184431</b>	<b>0.35</b>	<b>0.2</b>	<b>65.4</b>	<b>0.7</b>	<b>72.6</b>	<b>0.4</b>
40	75/25	0.525	0.3	10.77665	0.175	0.1	32.7	0.7	43.5	0.4
50	0/100	0	0	0	1	0.5	187.0	1	187.0	0.5
50	50/50	0.5	0.25	10.26347	0.5	0.25	93.5	1	103.8	0.5
50	25/75	0.25	0.125	5.131737	0.75	0.375	140.2	1	145.4	0.5
50	30/70	0.3	0.2	6.158084	0.7	0.35	130.9	1	137.0	0.5
50	40/60	0.4	0.2	8.210778	0.6	0.3	112.2	1	120.4	0.5
<b>60</b>	<b>25/75</b>	<b>0.375</b>	<b>0.2</b>	<b>7.697605</b>	<b>1.125</b>	<b>0.45</b>	<b>210.4</b>	<b>1.5</b>	<b>218.1</b>	<b>0.6</b>
60	50/50	0.75	0.3	15.39521	0.75	0.3	140.2	1.5	155.6	0.6
70	25/75	0.583	0.2	11.96721	1.75	0.525	327.2	2.333	339.2	0.7
80	25/75	1	0.2	20.52695	3	0.6	560.9	4	581.5	0.8
90	0/100	0	0.2	0	9	0.81	1682.8	9	1682.8	0.9

### **Appendix C: Previous Mechanical Tests Using Tetronic®**

Unpublished results from previous experiments conducted in our lab are presented below (**Figure C.1**). The graphs display the maximum tension (N/cm) for n=9 tests per ratio and shear adherence (Pa) for n=6 tests per ratio achieved for various blend ratios of T904-acrylate/T1107-acrylate. Maximum tension results were obtained via end-to-end tests while shear adherence testing followed a different protocol whereby a 4.9 mm-diameter circular defect was created in a wide, ¾ inch-wide collagen sheet and placed over a narrow, ½ inch-wide sheet with a stainless steel washer used to limit excessive spreading. Next, 75 µl of the Tetronic®/DTT mix was applied to the center of the defect to form a hydrogel rivet that maintained contact between the two sheets. Finally, a similar curing time and mechanical testing procedure as the end-to-end test was observed.

Largest average maximum tension values achieved for end-to-end tests of T904-acrylate/T1107-acrylate ratios were  $0.43 \pm 0.06$  N/cm (mean  $\pm$  SEM) at a ratio of 50/50 and  $0.28 \pm 0.04$  N/cm for a ratio of 75/25, respectively at 50wt %. In comparison, the highest average maximum shear adherence values were  $18,123 \pm 1650$  Pa for the 75/25 ratio and  $13,801 \pm 1733$  Pa at a ratio of 50/50, also both at 50wt %. However, the PEG control was found to have the largest value for these tests with  $23,152 \pm 8782$  Pa.



**Figure C.1:** End-to-End (left) and Shear Adherence (right) configuration results for T904-acrylate/T1107-acrylate ratios at 40wt %, 50wt %, and PEG control

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