ABSTRACT
THE DEVELOPMENT OF 3D PRINTABLE MATERIALS

by Katherine Jean Bootsma

An interpenetrating polymer network (IPN) is a material that possesses unique physical and mechanical properties. These unique properties give rise to the use of IPNs in a variety of applications including drug delivery, tissue engineering scaffolds, and as actuators in soft robotics. This thesis reports two separate applications of IPNs where 3D printing could prove useful. The first study focuses on the use of an alginate-polyacrylamide IPN hydrogel as a potential material for use in a medical simulator. The chemistry of a previously reported biomimetic IPN was altered so that 3D printing could be achieved. The elastic and viscoelastic behaviors of the 3D printed IPNs were quantified and found to be on the range of various biological tissues. The second study focuses on the development of a PVDF-poly(glycidyl methacrylate-co-methyl methacrylate) IPN as a separator membrane in a flexible lithium ion battery. A second polymer network was added to a preexisting separator formulation to create a semi-IPN. This separator membrane was characterized with dissolution tests, tensile mechanical tests, and rheology.
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CHAPTER I - Introduction*

Katherine Bootsma

*References formatted according to Springer guidelines for science reviews.
I.1. General Introduction

The material of interest in this thesis is an interpenetrating polymer network (IPN). An IPN is a material composed of two polymer networks where at least one of the networks has been synthesized or crosslinked in the presence of the other [1]. Ideally, the networks do not interact with one another aside from physical entanglement [2]. IPNs possess robust mechanical properties which can be attributed to this unique composition. Typical IPNs reported in literature [3–7] combine a covalently crosslinkable network with an ionically crosslinkable network. Under an applied force, the crosslinks break. Ionic crosslinks can break more easily than covalent crosslinks, so damage accumulates here and energy is dissipated before covalent crosslinks are broken [8]. When the force is removed, however, the ionically crosslinked network rezips and the crosslinks are reformed [9], though they may not be in the original locations. Unbroken covalent crosslinks maintain the IPN’s elasticity [5], but when broken, rupture occurs. Owing to these unique properties, IPNs find a wide range of applications including, but not limited to, drug delivery [10], tissue engineering scaffolds [11], and actuators in soft robotics [12]. Additionally, medical simulation and separators in flexible lithium ion batteries are fields where IPNs could be useful. This thesis work focuses on medical simulation and flexible lithium ion battery separators due to existing gaps in knowledge in these fields.

I.2. Application to Medical Simulation

Medical simulation is a technique used in medical education to teach, practice, and validate medical procedures. It reduces medical errors [13] and the associated costs [14] and it increases patient safety [13]. A decrease in resident work hours [13] and the limited availability of cadaveric tissues [14] have driven the expansion of medical simulation. As the need for medical simulation grows, so does the need for biomechanically realistic tissue analogue materials for use in medical simulators. IPNs are a good candidate material as they have been shown to have properties tunable to those of biological tissues [9], and other studies have demonstrated their 3D printability [15, 16]. This makes them attractive as a 3D printable tissue simulant material that could be implemented in a medical simulation device. An extensive review on materials used in medical simulation, mechanical characterization methods, and current commercially available medical simulators is given as CHAPTER II of this thesis. This review titled “Materials Used as Tissue Phantom in Medical Simulation” was submitted to be published by Springer as a chapter.
in a textbook titled *Body and Tissue Phantoms in Biomedical Science and Industry*. Dr. Jessica Sparks, Dr. Jason Berberich, Elizabeth Dimbath, and I collaborated in the writing of this review chapter. My contributions included writing the sections on mechanical characterization of materials, the structure and mechanical properties of materials used in medical simulators, and rewriting the published hard and soft tissue simulator models section and its corresponding table. Dr. Sparks wrote the introduction, the conclusion, and the section on the structure and mechanical properties of biological tissues. Elizabeth Dimbath did extensive literature searches, contributed the table listing the mechanical properties of selected human tissues and the table listing selected commercially available simulators with biomechanical relevance, and wrote the preliminary draft for the published hard and soft tissue simulator models section. Both Dr. Sparks and Dr. Berberich provided editing help throughout the writing process. Finally, John Kromer provided assistance in conducting initial literature searches.

The first study done in this work, *3D Printing of an Interpenetrating Network Hydrogel Material with Tunable Viscoelastic Properties*, was designed and carried out in an effort to contribute to the medical simulation and 3D printing knowledge base. The resultant paper from this work is given as CHAPTER III of this thesis.

### 1.3. Application to Flexible Lithium Ion Battery Separators

Presently, wearable electronics and other flexible devices are becoming increasingly popular. This rise in popularity necessitates flexible energy storage in the form of lithium ion batteries, but typical materials used in lithium ion batteries are rigid and inflexible. Previous studies have shown that a composite anode layer and a composite cathode layer can be made flexible and are able to maintain electrochemical performance under mechanical abuse [17]. The 3D printing of flexible lithium ion batteries is desired to be able to achieve unique product geometries, but because the battery is composed of three distinct components, it is currently difficult to print an entire battery cell sequentially. Two problems encountered when printing sequentially are the seepage of the inks into the previously printed component(s) and the dissolution of previously printed components in the common printing solvent. Using an IPN as the separator layer could prove to be a solution to both of these issues due to increased crosslinking and a tighter entanglement of polymer chains. The work done to address these issues is presented as CHAPTER IV of this thesis.
I.4. Overview of Study 1 (CHAPTER III)

CHAPTER III presents a peer-reviewed paper published in the *Journal of the Mechanical Behavior of Biomedical Materials*. It focuses on the creation of biomimetic IPNs in various compositions and the subsequent 3D printing of the IPN formulation exhibiting the most stress-relaxation. The objectives were: to assess the effects of different functional groups on the mechanical properties of the IPNs in a cast-molded state; to develop an IPN 3D printing method that allows for near-simultaneous crosslinking of the two networks; and to determine which network components can tune the elastic modulus and stress-relaxation properties of the 3D printed IPN.

This work was started in late 2014 by Martha Fitzgerald. She and Brandon Free did the printer hardware modifications and determined the software workflow necessary for a viable printing process. Further, Martha optimized the IPN chemical formulation in order to obtain prints with good shape fidelity. Martha performed the ImageJ analyses to quantify the shape fidelity of the prints. Elizabeth Dimbath assisted Martha with 3D printing, and she ran prints and mechanical tests that led to the creation of Figure 3B. Liz assisted Joe Conjerti and me in making, swelling, and mechanically testing many IPN formulations with different functional groups and compositions. Dr. Konkolewicz provided chemical expertise with regards to the various functional groups. I performed the gel time experiments. Martha, Dr. Sparks, Dr. Berberich, and I collaborated to write and edit this paper.

I.5. Overview of Study 2 (CHAPTER IV)

CHAPTER IV presents a summary of the work that was carried out with aims of achieving sequential 3D printing of a flexible lithium ion battery. The study focuses on the modification of an existing separator layer formulation that was provided to us by researchers at Wright-Patterson Air Force Research Lab (Soft Materials Branch, Materials and Manufacturing Directorate). The setbacks preventing sequential 3D printing were that separator ink seeps into the printed electrode layer when the separator is printed on top of the electrode and electrode ink seeps through the printed separator when an electrode is printed on top of the separator. Additionally, all three layers of the battery are printed using the same solvent so experiments were carried out to ensure that sequential printing did not result in dissolution of the previously
printed layer. We hypothesized that by adding a second crosslinkable polymer network to the existing formulation to yield a semi-IPN, that these issues could be fixed. We worked to prove our hypothesis through dissolution experiments, mechanical testing, and rheological characterization.

Aaron Blake, Ryan Kohlmeyer, James Hardin, and Dan Berrigan at the Air Force Research Lab initially presented us with and explained their problems in sequential 3D printing of full cell lithium ion batteries. Dr. Berberich and Dr. Konkolewicz provided expertise as to what the composition of the second polymer network should be and Progya Chakma performed initial polymerizations of this second network. I experimented with the polymerization conditions. Dr. Konkolewicz assisted me in performing GPC on the polymers. Additionally, I fabricated the separator membranes in many different compositions, performed the dissolution tests, and with Dr. Sparks’ help, I carried out mechanical testing and rheological characterization. We sent our results to our collaborators at AFRL and they helped to make Figure 1 and Figure 3 in this chapter. Dr. Sparks helped me to edit this chapter.

I.6. References


CHAPTER II - Materials Used as Tissue Phantoms in Medical Simulation*†

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†References are formatted according to Springer guidelines for science reviews.
II.1. Introduction

In the United States healthcare system, as many as 98,000 people die in hospitals each year due to preventable medical errors [1, 2]. This number exceeds the number of U.S. deaths per year due to diabetes, Alzheimer’s disease, or influenza [3]. Costs associated with medical errors have been estimated at $17 billion per year [1, 4]. Medical simulation is an important strategy for reducing these adverse events [5]. Medical simulation offers a safe environment in which a physician can practice a procedure repeatedly without placing patients at risk [5].

Simulation has been an integrated part of world culture for many centuries and has been incorporated into many different fields of study including military, aviation and space programs, as well as medicine [6–8]. Medical simulation has existed for as long as people have worked to treat human ailments, but it was only recently that the industry for medical simulation devices began experiencing rapid growth [5, 6, 8]. The first medical simulators were static models and have since advanced to include realistic mannequins and virtual reality simulators [5, 9]. The increase in medical simulation relevance is due in part to the decrease of resident work hours to 80 hours per week, which limits time to finish required training. Reduction in resident work hours has put pressure on simulator development with the expectation of being able to teach and refine skills to make up for lost time in clinical settings [5]. The Joint Council for Thoracic Surgery Education has made recommendations, including the use of simulation, for ways to overcome these regulations [10]. This will likely be a trend in medical regulatory bodies as regulations continue to change. As a result of the move towards simulators as teaching devices, there is anticipation for the learning curve on complex procedures to be shortened and for better-equipped physicians to be produced [11–13].

A second driving force for simulator development is the limited availability of cadaveric tissues. Human and animal cadaveric tissues have been the primary materials used in teaching and practicing medical procedures and skills for many years. Now, as resident work hours are decreasing and the expectation for competence is higher, there is a need for other types of simulation that offer valid learning tools that are easily accessible and provide minimum risk to patients [5, 7, 8, 14]. Cadavers are expensive to use and can be hard to acquire [7]. Teaching and training schedules in medical programs are often dependent on limited availability of cadavers,
which decreases students’ access to practice and instruction [8]. Cadaveric tissues also no longer have the same properties as living human tissue, so students do not get a realistic feel for the way tissues will react in a live procedure. Animal tissues have many of the same limitations as human tissues with the added high cost of housing the animals. Although tissues can be tested quickly after death, for animals and human cadavers, the results do not always extrapolate to live human tissue [15]. Cadaveric tissues can often only be used once, which further increases the cost of investment [7].

There is a great need for the development of low-cost, high fidelity, biomechanically realistic tissue analogue materials for use in medical simulators [16]. Training medical professionals on simulators with realistic mechanical and geometrical properties has been shown to increase the safety of the patient, and to maintain and develop confidence and good practice for trainees [5, 6, 9, 10, 14, 16]. The result is lower cost training with less associated risk for all parties [5, 8]. The goal of this chapter is to provide an overview of materials used as tissue phantoms for medical simulation. The emphasis will be on medical simulators used for training on manual tasks or other procedures for which tissue mechanical behavior is an important consideration for the simulator design. The chapter will discuss: (1) basic concepts in mechanical characterization of materials; (2) structure and mechanical properties of biological tissues; (3) structure and mechanical properties of medical simulator materials; (4) simulator designs currently available or reported in the literature; (5) recent and future developments in this field.
II.2. Mechanical Characterization of Materials

II.2.1. Stress and Strain

The mechanical properties of materials are determined by subjecting material samples to mechanical tests, such as tension or compression experiments. The two primary variables in mechanical testing are force (load) and displacement. Mechanical tests may involve applying a controlled displacement and measuring the resulting force, or applying controlled force and measuring the resulting displacement. The choice depends on the desired information and the capabilities of the mechanical testing equipment. Force and displacement data collected during an experiment are converted to stress and strain, where stress is defined as the intensity of the internal force on a particular plane (area) passing through a point, and strain is defined as the amount of deformation per unit length [17]. Nominal stress, $\sigma$, and nominal strain, $\varepsilon$, can be calculated using Eqs. 1 and 2:

$$\sigma = \frac{F}{A_0}$$

$$\varepsilon = \frac{\delta}{L_0}$$

where $F$ is the measured force, $A_0$ is original cross sectional area of the specimen, $\delta$ is change in length, and $L_0$ is original length. Several important material properties can be derived from the resulting stress-strain curve (Fig. 1). These are defined briefly below.
II.2.2. Elastic and Plastic Deformation

The elastic modulus, $E$, also known as Young’s modulus, is defined as the slope of the initial linear portion of the stress strain curve (Fig. 1 A). It is a measure of stiffness or a material’s resistance to deformation. During the initial linear regime the material is said to exhibit elastic behavior and to obey Hooke’s law (Eq. 3):

$$\sigma = E\varepsilon$$  \hspace{1cm} (3)

Elastic behavior of a material assumes that the material will spring back to its original shape if the load or stress is removed (the deformation is reversible). At higher strain levels, the material may begin to elongate in a fashion that is no longer reversible and exhibit plastic deformation. The yield strength or elastic limit describes the point where the linear elasticity no longer applies and any additional load will lead to some fraction of deformation that will be permanent and irreversible (Fig. 1 B). Knowledge of the yield strength is important since it often represent the upper limit of a load that the material can withstand during operation. Once a material begins to undergo plastic deformation during tensile testing, it may begin to exhibit what is referred to as strain hardening where the material becomes stronger due to the rearrangements at the molecular level in the material. At a point referred to as the ultimate strength of the material, the nominal stress will no longer increase and will begin to decrease due to necking (Fig. 1 C). Necking occurs when there is a decrease in the cross-sectional area of the sample and the strain begins to increase rapidly. Many thermoplastics and ductile metals exhibit plastic deformation.

II.2.3. Failure

Ultimately, if a large enough load is applied to a material it will undergo failure or fracture due to a break in the material. This may happen in the elastic region for more brittle materials such as ceramics and some metals or it may occur in the plastic deformation region for materials such as bone, ductile metals, or polymers. Fracture stress (Fig. 1 D) is simply the stress at which the material fails or fractures.
II.2.4. Work of Extension

Another important material property that can be determined from the stress-strain curve is the work of extension or strain energy storage (Fig. 1 E). This is a measure of the amount of work per unit volume that is absorbed by the material as it extends. Brittle materials such as glass and ceramics have low work of extension due to their inability to deform without failure. Elastomers and some biological tissues can have large work of extension since they can withstand significant deformation.

II.2.5. Mathematical Models of Mechanical Behavior

Biological tissues exhibit highly complex mechanical behaviors such as nonlinear stress-strain relations, dependence on time or rate of loading, and anisotropic effects. In addition, experimental factors such as age, hydration, specimen harvest/storage/preservation procedures, perfusion, length scale, temperature, and other test conditions can all affect mechanical test results. For maximum realism, tissue simulant materials may need to mimic one or more of the complex mechanical features of biological tissue. Therefore, understanding how mechanical behaviors of materials are quantified is an important step toward developing more advanced tissue simulants, so that simulant materials can be compared objectively with native tissues. The following section provides an overview of the mathematical models, known as constitutive models, commonly used to characterize mechanical behavior.

II.2.5.1. Elasticity

An elastic material is a material for which the current state of stress is only a function of its current configuration [18]. When a load is applied to the material, the deformation is instantaneous, and when the load is removed, the original configuration is instantaneously resumed. This indicates that the behavior of elastic materials is independent of time and that no energy was lost in the loading and unloading process [19]. Through the application of Hooke’s law (Eq. 3), the stress in an elastic material is linearly proportional to strain by the elastic modulus. Many soft biological tissues exhibit markedly nonlinear stress-strain relations and thus do not obey Hooke’s law. However, Hooke’s law is often used to describe the mechanical behavior of bone or hard tissue simulant materials [20].
II.2.5.2. Hyperelasticity

Hyperelastic constitutive models are used to describe nonlinear materials that recover their original shape after large deformations. A hyperelastic material stores the energy that was used to deform it as strain energy, and upon removal of the deforming load the stored energy is used to recover the original shape [18]. Many strain energy functions have been developed to describe an array of hyperelastic material behaviors. Among the most common strain energy functions used for biological materials are the Ogden, Mooney-Rivlin, and Neo-Hookean models, which are reviewed in [21]. The Ogden model (Eq. 4) is given here as an example:

\[ W = \sum_{n=1}^{N} \frac{\mu n}{\alpha_n} \left( \lambda_1^{\alpha_n} + \lambda_2^{\alpha_n} + \lambda_3^{\alpha_n} - 3 \right) \]  

where \( W \) is the strain energy, \( \lambda \) is the principle stretch, \( \mu \) is the shear modulus, \( \alpha \) is the strain hardening exponent, and there can be \( N \) terms in the model. To write stress in terms of strain, the strain energy function is differentiated with respect to the applicable deformation measure. The details are beyond the scope of this chapter and are available in [22]. Hyperelastic constitutive laws have been used to describe the nonlinear stress-deformation behavior of many biological tissues, such as muscle [23], tendon [24], ligament [25], skin [26], and brain [27].

II.2.5.3. Viscoelasticity

Viscoelastic materials exhibit behavior of both elastic solids and viscous fluids. Just as elastic solids can be modeled by a spring with completely recoverable deformation, viscous Newtonian fluids are analogous to a dashpot with permanent deformation. In a dashpot model, the stress is linearly related to the strain rate by the fluid viscosity, \( \eta \) [19, 28]. The time dependence of the mechanical properties of viscoelastic solids arises from the viscous fluid contribution and its dependence on strain rate. Because the stress in a deformed material is dependent on the rate at which it was strained [18], viscoelastic materials are said to
have memory, and they will have non-unique stress-strain curves (Fig. 2). Upon the removal of a
deforming load, the original configuration is eventually (as opposed to instantaneously) recovered.
Under continuous loading and unloading cycles, a viscoelastic material dissipates energy.

Linear viscoelastic behavior is modeled by combining springs and dashpots in different
configurations. The Kelvin-Voigt model combines a spring and dashpot in parallel and the
Maxwell model combines the two in series. These models, however, are quite simplified and have
limited ability to describe both the creep and stress relaxation responses typical of biological
materials. The standard solid model (or three parameter solid) combines the Kelvin-Voigt model
in series with a spring, and this model is capable of capturing the behavior of many biological
tissues including cartilage and white blood cell membrane [19]. The stress-strain relation for the
three parameter viscoelastic solid model is given in Equation 5:

\[(E_1 + E_2)\sigma + \eta \frac{d\sigma}{dt} = E_1 E_2 \varepsilon + E_1 \eta \frac{d\varepsilon}{dt}\]  \hspace{1cm} (5)

where \(\sigma\) is stress, \(\varepsilon\) is strain, \(\eta\) is viscosity of the dashpot, and \(E_1\) and \(E_2\) are the elastic moduli of
the series spring and Kevlin-Voigt spring, respectively. An excellent description of viscoelasticity
is available in [29].

II.2.5.4. Poroelasticity
Poroelastic, or biphasic, materials also have time dependent mechanical properties. As its name
implies, the time dependence arises from the flow of fluid through the pores of an elastic or
viscoelastic solid [30]. Equations modeling this behavior are beyond the scope of this chapter
and are discussed in [31, 32]. Poroelastic or biphasic constitutive models have been used to
describe not only tissue mechanics but also tissue perfusion, for numerous biological materials
including cartilage [31], brain [33], and liver [34–37].

II.2.5.5. Tissue Anisotropy
Biological tissues are both heterogeneous and anisotropic. When selecting materials for medical
simulation, the engineer must keep in mind that the compressive or tensile properties of tissue can
be different depending on orientation. Skin [38], skeletal muscle [39], bone [40], and blood vessels
[41] are all heterogeneous with structural features and mechanical properties that are direction dependent. For example, under the same loading conditions, the elasticity of the human brachialis muscle is higher when the probe was oriented parallel to the muscle fibers as opposed to perpendicular to the fibers [39]. Man-made materials are often isotropic unless one is working with composite materials. Most materials used for medical simulation are isotropic; however, with techniques such as 3D printing and the use of composite materials, the creation of anisotropic simulation materials should be possible. Constitutive models to describe anisotropic material behavior are reviewed in [42].

II.2.6. Mechanical Testing Methods
The following section will describe common laboratory testing methods used to evaluate the mechanical response of a material to an applied deformation.

II.2.6.1. Tension
A tensile mechanical test deforms a material by stretching it uniaxially, and it is commonly used because stress and strain levels are uniform throughout the cross section of the material. A specimen of the material of interest is gripped between two clamps on the test frame. Tensile tests require the test specimen to be of a specific geometry, and the most common shape is called a dog bone. The ends of a dog bone specimen have larger cross sectional areas than the middle, or gauge, section. The use of a dog bone shaped specimen allows for better gripping of slippery materials by the clamps [28], however, rectangular shaped specimens can be used for materials that won’t slip out of the clamps on the test frame. The rate of deformation can be specified by the investigator in the test file associated with the test frame. The test frame acquires load and displacement data, which can be converted into stress and strain with geometric relationships in order to obtain the desired mechanical properties. The elastic modulus and the failure strength of the material are typical mechanical properties obtained from a tensile test [30]. In addition to uniaxial tension, biaxial tensile tests can also be performed to simultaneously stretch a material in two directions. This method can be used to test anisotropic materials [28].
II.2.6.2. Compression

A compressive mechanical test deforms a material by decreasing its length uniaxially. Compressive testing is typically used for materials that are porous, brittle, or difficult to grip in tensile clamps [28]. Two modes of compression exist: unconfined and confined. In unconfined compression testing the specimen is deformed between two non-porous platens attached to a load frame, while in confined compression testing the specimen is placed in a container and a single porous platen deforms it. The porous platen allows for fluid to move out of the compressed material, and is typically only used for poroelastic materials [30]. Cylindrical specimens are typically used for both confined and unconfined compression. Occasionally in unconfined compression, friction between the specimen and the loading platens can cause barreling, increasing the cross sectional area of the sample. Barreling can be avoided by altering the cylindrical geometry so that there is a gauge segment with a smaller cross sectional area-similar to a dog bone shape, or the compression platens can be lubricated to reduce friction [28]. Similar to tensile testing, the investigator can specify the rate at which the specimen is compressed. Load and displacement data are acquired by the test frame and stress and strain can be calculated in order to obtain the elastic modulus, failure stress, and other material properties.

II.2.6.3. Creep and Stress Relaxation

Creep and stress relaxation are mechanical tests that are used to measure the time-dependent strain and stress responses of viscoelastic materials. In a creep test, a constant load or stress is applied and maintained while the strain in the material is measured over time [18, 30]. In a stress relaxation test, a constant strain is applied and maintained while the stress is measured over time. Relaxation implies that the stress in the material decays with time. Creep (Equation 6) and stress relaxation (Equation 7) behaviors can be modeled by the three parameter solid model for viscoelastic materials, and are given as:

\[
\varepsilon(t) = \frac{\sigma_0}{q_0} \left[ 1 - \left( 1 - \frac{p_1 q_0}{q_1} \right) e^{-\frac{q_0 t}{q_1}} \right] 
\]

\[
\sigma(t) = q_0 \varepsilon_1 \left( 1 - e^{-\frac{t}{p_1}} \right) + \sigma_0 e^{-\frac{t}{p_0}} 
\]

(6)  

(7)
where $\sigma_0$ is instantaneously applied stress, and $p_1$, $q_0$, and $q_1$ are defined in terms of viscosity $\eta$ and elastic moduli $E_1$ and $E_2$ as:

$$ p_1 = \frac{\eta}{E_1 + E_2} \quad (8) $$

$$ q_0 = \frac{E_1 E_2}{E_1 + E_2} \quad (9) $$

$$ q_1 = \frac{E_1 \eta}{E_1 + E_2} \quad (10) $$

**II.2.6.4. Indentation**

Indentation mechanical testing is localized compression testing [30]. A probe with known geometry is used to compress a small portion of a material’s surface and then is removed. The testing equipment acquires load, depth, and time data which can be used to calculate the reduced modulus. Indentation testing does not require a specific specimen geometry because only a small, flat portion of the sample is necessary for testing. Because only a localized portion of a material is tested, indentation is used to test functionally graded materials or materials whose compositions are inhomogeneous [30].

**II.2.6.5. Frequency Based Testing**

Frequency based testing includes dynamic mechanical analysis (DMA) and rheometry and is performed to determine the viscoelastic properties of materials. Frequency based testing measures a material’s stiffness (modulus) and damping in response to a cyclic application of force or displacement. The in-phase component is the storage modulus, termed $G'$, and is a measure of the material’s elastic behavior. The out-of-phase component, $G''$, is the loss modulus and is a measure of the material’s viscous behavior. Tan delta is the ratio of $G''/G'$. A review of rheological testing methods and applications is available in [43].
II.3. Structure and Mechanical Properties of Biological Tissues

II.3.1 Histological Structure of Tissues

Brief descriptions of the structural organization of human skin, skeletal muscle, bone, and blood vessels will be provided, as these are among the most frequently encountered tissues in current simulator models. More detailed descriptions of anatomical and histological structure are available in other references, such as [44, 45].

II.3.1.1. Skin

Skin is composed of two layers: the epidermis and the dermis. In addition, a distinction is made between thick skin, such as that found on the palms and soles of the feet (Fig. 3), and thin skin, found elsewhere on the body. The epidermis is made up of epithelial tissue of ectodermal origin. The primary cells of the epidermis are keratinocytes (keratin-producing cells) arranged in five layers: the stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum (Fig. 3). Cells of the stratum basale undergo rapid mitosis and drive the constant renewal of the epidermis, while cells of the stratum corneum are non-nucleated cells that are shed continuously at the skin surface [44]. The dermis is a connective tissue layer beneath the epidermis. It contains projections, termed dermal papillae, that interdigitate with projections of the epidermis, known as epidermal pegs or ridges. These interdigitations mechanically reinforce the dermal-epidermal junction [44]. The dermis contains a papillary layer, made up of loose connective tissue, fibroblasts, and immune or inflammatory cells such as mast cells, lymphocytes, and macrophages, and a thicker reticular layer composed primarily of dense irregular type I collagen [44]. Within the dermis are found nerve fibers and a rich network of blood and lymphatic vessels.
II.3.1.2. Skeletal Muscle

Skeletal muscle is primarily composed of long multinucleated cells termed muscle fibers. Muscle fibers contain numerous myofibrils, which consist of contractile proteins arranged in highly organized repeating units known as sarcomeres. Sarcomeres are responsible for the characteristic striations of skeletal muscle visible upon light microscopy (Fig. 4). Muscle contraction results from an increase in overlap between the thick and thin filaments, composed of actin and myosin, within a sarcomere. Connective tissue coverings play an important role in force transmission during muscle contraction [44]. A thin connective tissue layer termed the endomysium encloses each individual muscle fiber. Groups of muscle fibers are clustered into bundles enclosed by a lining termed the perimysium. Finally, a dense connective tissue layer called the epimysium surrounds the entire muscle. Blood and lymphatic vessels and nerves travel within the connective tissue coverings to supply the tissue.

II.3.1.3. Bone

Bone is a connective tissue specialized for mechanical support, protection, and locomotion. It also harbors the bone marrow, where blood cells are formed, and serves as a reservoir of calcium, phosphate, and other ions [44]. Bone may be classified as
dense bone (Fig. 5), such as that found in the diaphysis of long bones, and spongy bone, such as that found in the core region of the epiphyses or bulbous ends of long bones. Bone is composed of three cell types (osteocytes, osteoblasts, and osteoclasts) as well as calcified bone matrix material. Osteocytes are involved in the maintenance of the bone matrix and are found in lacunae, stellate-shaped cavities within the bone matrix (Fig. 5) [44]. Osteoblasts synthesize the organic components of bone matrix, which includes type I collagen, proteoglycans, and glycoproteins [44]. Osteoclasts are responsible for bone resorption and remodeling. In dense bone, the osteocytes in their lacunae are arranged in layers, or lamellae, as shown in Fig. 5. A set of concentric lamellae forms a unit termed an osteon. Blood vessels and nerves are found in the central canal of the osteon, known as the Haversian canal. The vessels and nerves originate in the marrow cavity and can travel between osteons via oblique channels called Volkmann’s canals.

II.3.1.4. Blood Vessels

This summary of blood vessel structure will focus on medium to large sized vessels, since these are most often represented in simulator design. The general organization of a medium sized artery is shown in Fig. 6 (bottom). It consists of three layers: the tunica intima, tunica media, and tunica adventitia. The tunica intima is the innermost layer, closest to the lumen of the vessel, and consists of a single layer of endothelial cells (e) plus the internal elastic lamina. The internal elastic lamina is composed of elastin, a protein which provides elastic recoil ability to the structure. The tunica media (m) is composed primarily of layers of smooth muscle cells. In large elastic arteries, such as the aorta, the smooth muscle cell layers of the tunica media are interspersed with numerous elastic lamina layers (not shown). The tunica adventitia (a), the outermost layer, consists mainly of type I collagen and some elastic fibers [44]. If the vessel is large enough to require its own blood supply, known as vasa vasorum, these are found within the tunica adventitia. Medium sized veins...
follow a similar organizational pattern as medium sized arteries (Fig. 6). Some notable differences are that the internal elastic lamina is absent in veins, and the tunica media is less well developed.

**II.3.2 Mechanical Properties of Human Tissues**

Mechanical properties of tissues are determined by their structure and composition. For example, high water content contributes viscous effects and can impart viscoelastic properties to tissues, and highly aligned collagen fibers can impart strong anisotropic behavior. An understanding of tissue mechanical behavior is important when selecting materials for medical simulation. Skin is known to exhibit features of nonlinearity, anisotropy, and viscoelasticity in its mechanical response [46]. Similarly, the mechanical behavior of passive skeletal muscle has been described as nonlinear, anisotropic, and viscoelastic, with evidence of tension/compression asymmetric behavior and significant fluid contributions at high rates of loading [47]. Many models of bone biomechanics use relatively simple constitutive relations such as linear elasticity with isotropy or transverse isotropy; however, later investigators have attempted to incorporate additional complex features such as rate-dependence, hierarchical structure, interstitial flow and post-yield behaviors [20, 48].

Blood vessels have been modeled as pseudoelastic, randomly elastic, poroelastic, and viscoelastic [49]. A given material property, such as the elastic modulus, may vary over several orders of magnitude when comparing materials such as bone and arteries, or even when comparing the same material tested under different conditions. Thus, when comparing the mechanical behavior of a potential simulant material to that of a target biological tissue, simply matching the elastic modulus may be inadequate. A more advisable approach would be to compare the stress-strain response of the materials when tested under the same or similar conditions. Ideally, the test conditions would be selected to mimic as closely as possible the deformation modes and loading rates that are anticipated in the desired application. Table 1 highlights some important mechanical properties and values for a variety of tissue types. All values in Table 1 are from studies on human tissue.
## Table 1: Mechanical Properties of Selected Human Tissues

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Mechanical Property</th>
<th>Value</th>
<th>Test Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Elastic Modulus</td>
<td>Young: $4.2 \times 10^4$ Nm$^{-2}$</td>
<td>Torque-motor and rotational detector used to apply force of $28.6 \times 10^3$ Nm to forearm.</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old: $8.5 \times 10^2$ Nm$^{-2}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ultimate Tensile Stress</td>
<td>15.6 ± 5.2 MPa</td>
<td>Cadaveric tissue stored in 4 ºC saline, tensile tests performed using Instron with a preload of 1-2N and 10 cycles of preload, Langer lines were perpendicular to direction of stretch and three strain rates tested and averaged (0.06 $s^{-1}$, 57 $s^{-1}$, 167 $s^{-1}$).</td>
<td>[51]</td>
</tr>
<tr>
<td>Skeletal Muscle:</td>
<td>Maximum Tensile Stress</td>
<td>25.6 ± 2.6 N cm$^{-2}$</td>
<td>Eleven angles (16º-116º) of knee extension measured on LIDO dyanometer in sitting position with test duration of 5 s and rest of 10 s.</td>
<td>[52]</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>Shear Modulus</td>
<td>Vastus lateralis: 3.74 ± 0.23 kPa</td>
<td>Right knee held at 30º flexion with MRI used to image acoustic waves propagated through leg using air pressure.</td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rectus femoris: 3.91 ± 0.16 kPa</td>
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<tr>
<td></td>
<td></td>
<td>Vastus Intermedius: 4.23 ± 0.25 kPa</td>
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<tr>
<td></td>
<td></td>
<td>Vastus Medialis: 3.95 ± 0.32 kPa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compact Bone</td>
<td>Elastic Modulus</td>
<td>20.5 ± 0.5 GPa</td>
<td>Agilent Nanoindenter G200 used with strain rate of 0.05 $s^{-1}$ to a depth of 2000 nm, samples surrounded by physiological saline solution.</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td>Hardness</td>
<td>0.68 ± 0.02 GPa</td>
<td>Agilent Nanoindenter G200 used with strain rate of 0.05 $s^{-1}$ to a depth of 2000 nm, samples surrounded by physiological saline solution.</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td>Mineral Density</td>
<td>1.032 ± 0.015 gHA cm$^{-3}$</td>
<td>Raman spectroscopy using a LabRAM HR 800 in wet conditions with a 785 nm laser.</td>
<td>[54]</td>
</tr>
<tr>
<td>Blood Vessels:</td>
<td>Aorta Elastic Modulus</td>
<td>0.24 MPa</td>
<td>Pre-stretched 3 times to 2 N then stretched until failure at rate of 5mm min$^{-1}$.</td>
<td>[55]</td>
</tr>
<tr>
<td></td>
<td>Maximum Tensile Stress</td>
<td>1.5 MPa</td>
<td>Pre-stretched 3 times to 2 N then stretched until failure at rate of 5mm min$^{-1}$.</td>
<td>[55]</td>
</tr>
<tr>
<td>Coronary Artery</td>
<td>Elastic Modulus</td>
<td>1.48 ± 0.24 MPa</td>
<td>Soaked in 0.9% w/v NaCl prior to test, 37ºC and 1mm min$^{-1}$ strain rate using Digital push/pull force gauge ISF-DF50 with 0.05 N pre-load, arterial wall considered incompressible.</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td>Maximum Tensile Stress</td>
<td>1.44 ± 0.87 MPa</td>
<td>Soaked in 0.9% w/v NaCl prior to test, 37ºC and 1mm min$^{-1}$ strain rate using Digital push/pull force gauge ISF-DF50 with 0.05 N pre-load, arterial wall considered incompressible.</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td>Maximum Strain</td>
<td>Maximum Tensile Stress</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.54 ± 0.25</td>
<td>5.38 MPa</td>
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<tr>
<td></td>
<td>Soaked in 0.9% w/v NaCl prior to test, 37°C and 1mm min⁻¹ strain rate using Digital push/pull force gauge ISF-DF50 with 0.05 N pre-load, arterial wall considered incompressible.</td>
<td>Stored in patients’ heparinized blood using Universal Testing Machine samples were stretched to a stretch ratio of 1.1 for 10 cycles with 1% s⁻¹ rate for 200 seconds then repeated until failure.</td>
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<tr>
<td>Saphenous Vein</td>
<td></td>
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<tr>
<td>Umbilical Vein</td>
<td>1.65 MPa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stored in PBS using Universal Testing Machine samples were stretched to a stretch ratio of 1.1 for 10 cycles with 1% s⁻¹ rate for 200 seconds then repeated until failure.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abdominal Organs:**

<table>
<thead>
<tr>
<th></th>
<th>Maximum Tensile Stress</th>
<th>Maximum Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach Transverse:</td>
<td>0.5 ± 0.16 MPa</td>
<td>194.55 ± 27.29%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>178.93 ± 34.39%</td>
</tr>
<tr>
<td></td>
<td>Stored in physiological saline at 4°C until testing. Instron used to stretch sample at loading velocity of 50 mm min⁻¹ with preconditioning of 10 cycles at 30% strain.</td>
<td>Stored in physiological saline at 4°C until testing. Instron used to stretch sample at loading velocity of 50 mm min⁻¹ with preconditioning of 10 cycles at 30% strain.</td>
</tr>
<tr>
<td>Longitudinal:</td>
<td>0.67 ± 0.19 MPa</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Elastic Modulus:</td>
<td>Long term modulus: 20 kPa</td>
<td>Instantaneous modulus: 60 kPa</td>
</tr>
<tr>
<td></td>
<td>Aspiration experiments done on in vivo liver (whole organ with capsule) during surgery.</td>
<td></td>
</tr>
<tr>
<td>Large Bowel Transverse:</td>
<td>0.645 ± 0.165 MPa</td>
<td>176.66 ± 39.48%</td>
</tr>
<tr>
<td></td>
<td>Stored in physiological saline at 4°C until testing. Instron used to stretch sample at loading velocity of 50 mm min⁻¹ with preconditioning of 10 cycles at 30% strain.</td>
<td>Stored in physiological saline at 4°C until testing. Instron used to stretch sample at loading velocity of 50 mm min⁻¹ with preconditioning of 10 cycles at 30% strain.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>205.49 ± 50.4%</td>
</tr>
<tr>
<td>Longitudinal:</td>
<td>1.188 ± 0.302 MPa</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[56] [57] [58] [59]
II.4. Structure and Mechanical Properties of Materials Used in Medical Simulators

As noted above, the mechanical properties of materials depend on their chemical composition. Selected natural and synthetic materials common in medical simulation are discussed in this section with regards to how the structure affects their mechanical performance.

II.4.1. Alginate

Alginate is a naturally occurring linear copolymer [60, 61] of two sugar subunits. The sugars β-D-mannuronate (M) and α-L-guluronate (G) are bound by (1-4) glycosidic linkages and occur in different proportions and sequences depending on the type, age, and section of brown algae from which it is isolated [62]. Sequences that have greater than 20 M sugars in a row are referred to as M blocks and sequences that have greater than 20 G sugars in a row are referred to as G blocks. Sequences with alternating G and M sugars are called GM blocks [60]. Alginates can form gels when placed in solution with divalent [61] or trivalent [63] cations, which form ionic bridges between G blocks [60] of polymer chains and effectively crosslink the alginate polymer into a hydrogel [64]. This is illustrated in Fig. 7. These ionic crosslinks are physical [65] and reversible [66]. Maximum strength of the alginate polymer is achieved when all of the sugar subunits are crosslinked with a cation [61]. A common method for forming alginate hydrogels is the use of a glucono-δ-lactone (GDL) and calcium carbonate (CaCO₃) system [60, 61, 66–68]. This system allows for the in-situ release of calcium ions [60]. Sparingly soluble CaCO₃ is dispersed evenly throughout the alginate. When the GDL is added to the solution, it hydrolyzes, releasing a proton, thus causing the pH of the solution to drop. The CaCO₃ becomes soluble at a lower pH and calcium ions are liberated [60, 61, 66–68]. The amount of GDL in the system controls the gelation time [61]. Other methods of gelling alginates

![Fig. 7 Two alginate chains with G subunits participating in ionic crosslinking by divalent cations (Ca²⁺).](image)
are simpler and use a soluble form of calcium such as a calcium sulfate slurry [69] or calcium chloride [63].

The mechanical properties of alginate gels depend on their molecular composition [62]. Hydrogels made from an alginate with a high G content tend to be strong and brittle, whereas hydrogels made from an alginate with a high M content will be softer and more elastic [60]. In the work done by Mancini, Moresi, and Rancini, it was found that both compressive engineering stress and deformation work increased with an increase in the percentage of G subunits. The ranges for engineering stress and deformation work in their study were 42-506 kPa and 5-72 kJ/m$^3$ respectively. Additionally, they found that the rigidity constant, increased with an increase in the percentage of G subunits [70]. Similarly, the work by Drury, Dennis, and Mooney demonstrated that alginate hydrogels with high G contents had a greater tensile modulus, greater ultimate tensile stress, and greater extensibility than high M content hydrogels [62]. They also reported the compressive modulus for alginate gels can range from 1-1000 kPa and the shear modulus can range from 0.02-40 kPa [62].

**II.4.2. Agarose**

Agarose is a chemical derivative of agar [71] which is found in red algae [68], however red algae is a limited and expensive source [68]. The sugars 3,6-anhydro-α-L-galactose and β-D-galactose are bound by a (1-4) glycosidic linkage to form its disaccharide subunit. These disaccharide units repeat to form a linear polysaccharide [72]. The structure is shown in Fig. 8. Agaroses are classified by their molecular weight and the degree of crosslinking [71]. Agarose can form thermally reversible [73], physical hydrogels when heated up and then cooled to about 35°C [30]. The elastic modulus of agarose hydrogels increases with increasing polymer concentration [74]. For any given polymer concentration, Oyen notes that the elastic modulus can vary up to 3 orders of magnitude. This variation can be attributed to multiple research groups not accounting for the agarose molecular weight. Oyen also notes that agarose is
a poroviscoelastic material and that the time dependence of the material may not have been accounted for when the elastic moduli were determined [30]. The elastic moduli from the groups she reviewed ranged from 1 kPa to 3000 kPa [30]. Normand et al showed that tensile moduli are generally larger than compressive moduli, but agarose hydrogels are able to withstand more strain in compression than in tension [74]. Another factor that is affected by the polymer concentration is the permeability due to pore size. Increasing the polymer concentration decreases the pore size [73], thus decreasing the permeability of the hydrogel [30]. Additional reported mechanical properties of agarose hydrogels are the 0.02 MPa fractures stress and a 52% fracture strain [75].

II.4.3. Gelatin

Gelatin is a water soluble [76] protein polymer [77] obtained from the hydrolysis of collagen [78]. It differs from collagen in that it has low immunogenicity, is more soluble in aqueous media, and contains less tertiary protein structure [78], but it still contains some of the same signaling sequences [77]. Typical animal sources for collagen are porcine, bovine, and fish. The source animal can have an effect on the temperature at which gelatin forms a gel because the difference in the number of certain amino acids [78].

Gelatin can form thermally reversible hydrogels [79] with a gelation temperature around 30°C [78]. The hydrogels can be chemically or physically crosslinked. Chemically, it can be crosslinked by aldehydes and isocyanates which are toxic [77]. Genipin and transglutaminase are slightly less toxic chemical crosslinkers [77]. Amino acid residues with amine or carboxylic acid groups can be covalently crosslinked [78]. Toxic chemical crosslinking can be avoided by physical crosslinking with ultraviolet radiation [78]. Though gelatins are biocompatible, biodegradable, commercially available, and inexpensive [78], they tend to exhibit poor mechanical strength [77].
II.4.4. Chitosan

Chitin is a hydrophobic polysaccharide of the sugar N-acetyl D-glucosamine [80] that occurs naturally in arthropod exoskeletons [81]. Chitosan is derived through the process of chitin deacetylation, which removes the acetyl group from the sugar, resulting in the sugar subunit D-glucosamine [80]. N-acetyl D-glucosamine and D-glucosamine are bound by β (1-4) glycosidic linkages [81] and occur in different proportions depending on the degree of N-deacetylation of the N-acetyl D-glucosamine subunits [80]. In chitin there only exists about 10% deacetylation [80]. Typical chitosan samples are sourced from shrimp and crab [82] and have a percent deacetylation between 50-90% [81]. The structure for chitosan is illustrated in Fig. 9. The degree or percent of deacetylation affects the solubility [80] and the crystallinity [81] of the chitosan polymer. Deacetylation results in a free amino group which increases the solubility and makes chitosan more hydrophilic than chitin [81]. Chitosan is soluble in dilute solutions of acetic or formic acids [80, 82], whereas chitin is insoluble in water and most organic solvents [80]. The resultant amino groups are reactive and also make chitosan a pH responsive polymer [25]. The crystallinity of the chitosan polymer is greatest at 100% and 0% deacetylation [81], meaning that the polymer is either a homopolymer of D-glucosamine or N-acetyl D-glucosamine, respectively.

Chitosans can form hydrogels when chemically crosslinked with glutaraldehyde [73]. Though the chitosan itself is nontoxic and biocompatible [73, 83], residual amounts of the crosslinking agent are undesirable. Other methods of gelling chitosans include increasing the pH and extrusion into a non-solvent [81]. Freeze dried chitosan scaffolds have been shown to exhibit a modulus of about 500 kPa [81].
**II.4.5. Polyacrylamide**

Polyacrylamide is a hydrophilic, linear, bioinert synthetic polymer [84]. Its monomer constituent, acrylamide, is shown in Fig. 10.a. Acrylamide monomers can form polyacrylamide hydrogels by radical polymerization in aqueous solution. Typically, this polymerization is initiated by ammonium persulfate (APS) with tetramethylethlenediamine (TEMED) as an accelerator [66, 85]. Polyacrylamide chains can be crosslinked by N,N’-methlenebisacrylamide (MBAA, structure shown in Fig. 10.b) [30, 66]. Unpolymerized acrylamide monomer is toxic [84].

Polyacrylamide has manifold applications including gel electrophoresis [30], in which the most important mechanical property is pore size, cell culture experimentation [86], as well as food science applications [85]. The mechanical properties of polyacrylamide have been studied by numerous groups, and general trends have emerged. By increasing the amount of crosslinker and/or increasing the total polymer concentration, the pore size can be decreased and the elastic modulus can be increased [30]. Acrylamide hydrogels do not exhibit hysteresis [69] and can be categorized as elastic because creep-recovery tests have demonstrated that the gels return to zero strain upon removal of the stress [85]. Additionally, the hydrogels have shear moduli that depend on temperature, which in conjunction with the creep-recovery follows the theory of rubber elasticity [85]. However, polyacrylamide hydrogels with certain polymer concentrations and a certain monomer to crosslinker ratio can behave viscoelastically [85].

Polyacrylamide hydrogels can have a fracture energy up to 1 J/m² [87], a stretch ratio at rupture of ~7 [69], and a modulus up to 1000 kPa [30], but overall, their properties are considered...
limited [86]. For this reason, they are often incorporated into interpenetrating polymer networks to increase their properties [88].

**II.4.6. Poly(vinyl alcohol)**

Poly(vinyl alcohol) (PVA) is a synthetic hydrophilic polymer [71]. It is non-toxic [71] and biodegradable [89]. It can form hydrogels by crosslinking with glutaraldehyde [73], epichlorohydrin [73], acetaldehyde [64], formaldehyde [64], glyoxal [71], or borate [71]. This method usually leaves residual amounts of the crosslinking agent used. Because the residual crosslinking agents are highly toxic and the extraction of them is time consuming and expensive, this method of crosslinking is forgone in favor of freeze-thaw crosslinking [64]. The freeze-thaw method is a procedure by which the polymer solution is repeatedly frozen and thawed. This introduces crystalline domain formation to chemically crosslink the polymer [71, 90]. The number of freeze-thaw cycles impacts the mechanical properties, where more cycles increases the compressive modulus [91]. The repeated unit in the PVA chain is shown in Fig. 10. c.

The resultant hydrogels demonstrate nonlinear viscoelastic behavior [90], and the stress strain relation exhibited is similar to that of biological soft tissues [91]. The hydrogels also have remarkable properties including high elasticity [73], high tensile strength, and a high tensile modulus [89]. PVA hydrogels have been shown to achieve a tensile strength of 70-100 MPa and a tensile modulus of 2700-3700 MPa [89]. In compression, PVA hydrogels have achieved a modulus of up to 18.4 MPa, and a strain at failure of up to 62% [90]. These properties are dependent on the water content of the PVA hydrogel, and because PVA is a viscoelastic material, it is also dependent on the strain rate at which the material properties are measured [90].

**II.4.7. Poly(ethylene glycol)**

Poly(ethylene glycol) (PEG) is a synthetic, hydrophilic polymer that can either be linear or branched in structure [92]. The basic PEG structure with hydroxyl end groups is shown in Fig. 10. d. The hydroxyl groups can be converted to other functional groups including carboxyl, amines, and acrylates [92]. When the end functional groups are the same, the PEG chain is symmetric and when they are different, the PEG chain is asymmetric. PEG is an attractive
polymer because it is bioinert [92], nontoxic, and non-immunogenic, thus the FDA has approved its use in some clinical applications [64].

PEGs can be made into hydrogels by crosslinking. The typical methods to crosslink PEG are radiation or photopolymerization and free radical polymerization [92]. Where radiation crosslinking does not require a crosslinking agent [71], the free radical polymerization method to crosslink PEG can only occur if the PEG has acrylate functional groups [92]. These acrylate groups form covalent crosslinks [64] and are commonly diacrylates or dimethacrylates [92]. The mechanical properties of PEG hydrogels are dependent on the crosslinking method and/or crosslinking agent used. When PEG is crosslinked with proteolytically degradable groups, the elastic modulus ranges from 0.5-5 kPa, but when PEG is covalently crosslinked by acrylates, the elastic modulus can range from 20-500 kPa [86].

II.4.8. Silicones

Silicones are a group of synthetic polymers containing a backbone of alternating silicon to oxygen bonds, with the repeating unit known as siloxane [71]. The silicon atoms in the siloxane unit bind functional groups that can be methyl [71], vinyl, or phenyl groups [93]. Silicones can have a structure that is either linear or cyclic, and are attractive for their thermal stability, chemical inertness [93], and nontoxicity [71]. The main factor affecting the stability is the high bond energy of the Si-O ionic bond [71]. The linear structure of silicone is shown below in Fig. 11.a.
Silicones can be made into three-dimensional networks by crosslinking between the polymer chains. One method is condensation crosslinking, where the contact with moisture induces crosslinking. A more common method for crosslinking is free radical crosslinking, but the functional groups attached to the silicone must be vinyl groups [71]. The best mechanical properties of silicones arise with the proper selection of crosslinkers and fillers [93]. Some notable mechanical properties of silicones are its low surface tension [71], its ability to elongate to 1250% its original length [93], its tensile modulus up to 1.05 MPa [94], its tear strength of 9.8 kN m$^{-1}$, and its shear modulus ranging from 100 kPa to 3 MPa [95].

**II.4.9. Polyurethanes**

Polyurethanes are a wide range of polymers including thermoplastic elastomers and rigid and flexible foams. The polymer chains possess a high degree of flexibility, and good mechanical properties are obtained at low temperatures. Polyurethanes also exhibit resistance to solvents, oils, and abrasion. Its ultimate tensile strength can range from 28.9-34.4 MPa, it has a flexural modulus of 0.689 kPa, and a tear strength ranging from 47-87 kN/m [93]. The generic chemical structure of polyurethane is shown in Fig. 11.b.

**II.4.10. Interpenetrating Polymer Networks (IPNs)**

The mechanical properties of biological tissues tend to be greater than the mechanical properties that a single polymer or material can achieve by itself, thus creating a need for materials with better properties. In recent years, efforts have been made to increase mechanical properties by forming interpenetrating network (IPN) hydrogels, which show better properties than single component hydrogels [87]. An IPN hydrogel is a hydrogel formed by the entanglement of two polymers that have been synthesized in the presence of one another [88]. Each polymer of the IPN can contain crosslinks, or molecules that bind one polymer chain to another chain of the same kind to form a network. A semi-IPN is an IPN in which only one polymer is crosslinked.
into a network [83] and a full IPN is an IPN in which both polymers have been crosslinked into networks [83]. Ideally there is no interaction between the two polymers and/or polymer networks, but realistically there is some interaction [83]. IPNs can be categorized by the way they are synthesized. A sequential IPN is an IPN where the polymerization of one polymer from its monomers occurred in the presence of an already synthesized polymer [88]. A simultaneous IPN is an IPN where two polymerizations occur in the presence of one another and one polymerization does not interfere with the other [88].

A typical combination for IPN hydrogels is one covalently crosslinked network and one ionically crosslinked network [84]. Under a load, the covalently crosslinked network maintains the hydrogel’s elasticity [66, 69] and the crosslinks in the ionically crosslinked network break and form damage zones that allow for damage accumulation before crack propagation can occur [96]. The breaking of these ionic bonds dissipates energy. This dissipation of energy by the breaking of sacrificial ionic bonds [97] is called hysteresis and this characterizes the toughness of the IPN [63, 69]. Upon removal of the load, the ionic crosslinks reform, or self-heal [66]. Gong, et al have further specified what constituent polymers make for IPNs with the best mechanical properties. They claim that a soft, ductile, loosely crosslinked neutral polymer with 20-50 times the molar concentration of a rigid, brittle, highly crosslinked polyelectrolyte makes for an IPN with the best properties [75]. Other groups have verified the strength of this composition [96, 98]. The loosely crosslinked network can support a high strain while the highly crosslinked network can dissipate energy at these high strains [84, 99]. Additionally, the crosslinking of both networks provides for more network to bear loads and thus increases the elastic modulus [99].

The ratios of the two networks need to be optimized in order to achieve the maximum mechanical properties [69]. Upon optimization of the IPN, mechanical property synergism is obtained, where the properties of the IPN are better than the sum of the properties of its constituent polymers [88]. General tactics to increase the properties of IPNs include increasing the mole or weight percent of the polymers in the IPN (thus reducing the amount of water) and/or increasing the mole or weight percent of one or both of the crosslinkers.

A prominent example in the literature of an IPN consistent with the criteria for increasing mechanical properties [75] is an alginate-polyacrylamide hydrogel [63, 66, 69, 99]. In such IPNs
the ionic network is alginate and the covalent network is polyacrylamide. They are typically crosslinked by divalent calcium cations and MBAA, respectively. While typical hydrogels are brittle and have fracture energies on the order of 10 J/m$^2$, alginate-polyacrylamide IPNs have been shown to have a fracture energy as high as 9000 J/m$^2$ [69]. These IPNs have also been stretched 20 times their initial length before failing [69]. Yang et al [63] further increased the properties of these gels by incorporating different ionic crosslinkers. The hydrogels were initially synthesized as semi-IPNs, where the alginate was uncrosslinked. The gels were then soaked in aqueous solutions of different metal cations including strontium, barium, aluminum, and iron. This allowed for the alginate to crosslink. They found that the trivalent cations aluminum and iron most greatly increased the elastic modulus of the IPN, with iron obtaining the highest elastic modulus at 252.2 ± 34 kPa. This demonstrates a great increase from the group that was soaked in an aqueous sodium ion solution (3.8 ± 0.1 kPa). Additionally, they found that stress at failure and energy dissipation under an applied load was greatest for trivalent cations as crosslinkers. They attribute the better mechanical properties obtained from trivalently crosslinked hydrogels to the fact that a trivalent cation is able to crosslink three carboxylic groups potentially from three different alginate chains, yielding a denser three dimensional network structure. It is known that when hydrogels are immersed in an aqueous solution that they absorb water [99] and their mechanical properties are diminished [66], but these gels were able to increase their properties due to ionic crosslinking occurring simultaneously with swelling. It should be noted, however, that this method of increasing the elastic modulus, energy dissipation, and stress at failure came at a cost of reducing the stretchability of the gels. They were still able to stretch 10 times the initial length, which the authors claim is sufficient for many relevant applications [63].

Another IPN that has shown promising mechanical properties is one composed of poly(2-acrylamide-2-methylpropanesulfonic acid) (PAMPS) and polyacrylamide. This gel was found to exhibit 43 times the fracture stress of the corresponding single network gels and it resisted slicing [75]. This gel also obtained a compressive stress of 17 MPa [75]. Another group working with the same IPN characterized some trends for the IPN as follows: an increase in the mole percent of covalent crosslinker causes an increase in the elastic modulus, but causes a decrease in tensile strength and elongation; and an increase in mole percent ionic crosslinker decreases elongation [99]. An IPN with no ionic crosslinker was found to stretch to 1109% its original
length, but did not have sufficient tensile strength or elastic modulus [99]. This decrease in elongation is similar to the trend observed by Yang, et al [63] in an alginate-polyacrylamide IPN.

II. 4.11. Summary of Mechanical Properties of Tissue Simulant Materials

It is important to quantify the mechanical properties of materials in order to select a proper material for a medical simulator. The properties of selected materials are given in Table 2 with descriptions of the testing conditions used to obtain the properties. Material properties stated in the preceding material sections that do not appear in Table 2 did not have specified testing conditions.

Table 2: Mechanical Properties of Selected Tissue Simulant Materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Mechanical Property</th>
<th>Value</th>
<th>Test Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td>Elastic Modulus</td>
<td>Compression: 1\textsuperscript{a}-120\textsuperscript{b} kPa</td>
<td>(a) Force and deformation measured by Stable Micro Systems TA-XT2 Texture analyser at 20°C. (b) Uniaxial compression using an Instron 4502 mechanical testing machine with crosshead speed of 4.8 mm min\textsuperscript{-1}. Specimens were coated in silicone oil to reduce friction.</td>
<td>(a) [100] (b) [67]</td>
</tr>
<tr>
<td>Shear Modulus</td>
<td>2-9 kPa</td>
<td>An ARES rheometer with parallel plate set up was used to compress the cylindrical specimens to 16% strain. Once equilibrium was reached, shear stress relaxation tests were performed. A shear strain of 0.001 was applied and the specimen was allowed to relax for 20 min. A step change in shear strain to 0.005 occurred over 60 ms, and the specimen was allowed to relax. This was repeated two more times for shear strains of 0.01 and 0.05.</td>
<td>[101]</td>
<td></td>
</tr>
<tr>
<td>Deformation Work</td>
<td>5-72 kJ m\textsuperscript{3}</td>
<td>Uniaxial compression until rupture with an Instron 4301 mechanical testing machine with a 100 N load cell.</td>
<td>[70]</td>
<td></td>
</tr>
<tr>
<td>Tensile Stress at Rupture</td>
<td>3.7 kPa</td>
<td>Gel specimens were glued to polystyrene clamps. A tensile machine with a 500 N load cell applied a constant stretch rate of 2 min\textsuperscript{-1}. Tests were performed in air at room temperature.</td>
<td>[69]</td>
<td></td>
</tr>
<tr>
<td>Stretch at Rupture</td>
<td>1.2</td>
<td>Gel specimens were glued to polystyrene clamps. A tensile machine with a 500 N load cell applied a constant stretch rate of</td>
<td>[69]</td>
<td></td>
</tr>
</tbody>
</table>
Fracture Energy | 4400 J m\(^{-2}\) | Gel specimens were glued to polystyrene clamps. A tensile machine with a 500 N load cell applied a constant stretch rate of 2 min\(^{-1}\). Tests were performed in air at room temperature. An unnotched sample was tested to obtain a force-length curve. A notched sample was tested to determine the length at which the notch started to propagate. [69]

| Agarose | Elastic Modulus | Tension: 83.7-3690 kPa | Dog bone samples of 60 mm gauge length and 6 mm width were tested on an Instron 4501 mechanical testing machine with an environmental chamber maintained at 10°C. The testing rate was 100 mm min\(^{-1}\). [74]

| | Compression: 1-2580 kPa | Cylindrical specimens of 13 mm diameter and 13 mm height were tested on an Instron 4501 mechanical testing machine with an environmental chamber maintained at 10°C. The compression platens were lubricated with dodecane. The testing rate was 50 mm min\(^{-1}\). [74]

| Failure | Tension: 0.11-0.21 | Dog bone samples of 60 mm gauge length and 6 mm width were tested on an Instron 4501 mechanical testing machine with an environmental chamber maintained at 10°C. The testing rate was 100 mm min\(^{-1}\). [74]

| | Compression: 0.33-0.44 | Cylindrical specimens of 13 mm diameter and 13 mm height were tested on an Instron 4501 mechanical testing machine with an environmental chamber maintained at 10°C. The compression platens were lubricated with dodecane. The testing rate was 50 mm min\(^{-1}\). [74]

| Chitosan | Tensile Stress at Failure | 5-145 kPa | Rectangular specimens of dimensions 1 cm x 5 cm were strained at a rate of 0.4 min\(^{-1}\) on an MTS Bionix 100 materials testing system until failure. [102]

| | Tensile Strength | 2.9\(a\)-6.3\(b\) MPa | Tensile test using Instron Model 4201 at an extension rate of 1 mm min\(^{-1}\) on dry (a) and wet (b) samples. [103]

| | Elongation at Break | 10.1\(a\)-78\(b\) % | Tensile test using Instron Model 4201 at an extension rate of 1 mm/min on dry (a) and wet (b) samples. [103]

| Polyacrylamide | Elastic Modulus | Compression: 0.2-1.5 MPa | Unconfined compression tests using a Bose Electroforce 3200 mechanical testing system with a 10 N load cell at room temperature. The gel specimens were immersed in 0.01 M Hepes buffer at pH 8.5. A preload of 0.1-0.2 N was applied to ensure contact between the [104]
Specimens were subjected to ramp loading at 0.02 mm s\(^{-1}\) to a strain of 8%. This strain was held for 360 s to allow for stress relaxation.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile Stress at Rupture</td>
<td>11 kPa</td>
<td>Rectangular gel specimens of dimensions 77 mm x 5 mm x 3 mm were glued to polystyrene clamps and an Instron 3342 mechanical testing machine. The 500 N load cell applied a constant stretch rate of 2 min(^{-1}). Tests were performed in air at room temperature.</td>
<td>[69]</td>
</tr>
<tr>
<td>Stretch at Rupture</td>
<td>6.6</td>
<td>Rectangular gel specimens of dimensions 77 mm x 5 mm x 3 mm were glued to polystyrene clamps and an Instron 3342 mechanical testing machine. The 500 N load cell applied a constant stretch rate of 2 min(^{-1}). Tests were performed in air at room temperature.</td>
<td>[69]</td>
</tr>
<tr>
<td>Fracture Energy</td>
<td>1800 J m(^{-2})</td>
<td>Rectangular gel specimens of dimensions 77 mm x 5 mm x 3 mm were glued to polystyrene clamps and an Instron 3342 mechanical testing machine. The 500 N load cell applied a constant stretch rate of 2 min(^{-1}). Tests were performed in air at room temperature. An unnotched sample was tested to obtain a force-length curve. A notched sample was tested to determine the length at which the notch started to propagate.</td>
<td>[69]</td>
</tr>
<tr>
<td>Compressive Fracture Stress</td>
<td>0.7 MPa</td>
<td>Compressive testing using Tensilon RTC-1310A mechanical testing machine on water swollen gels with 9 mm diameter and 4 mm height at a strain rate of 0.1% min(^{-1}).</td>
<td>[75]</td>
</tr>
<tr>
<td>Poly(vinyl alcohol) Tensile Strength</td>
<td>70-100 MPa</td>
<td>Measurements taken on an AGS-500C automatic tensile test machine at a rate of 10 mm min(^{-1}).</td>
<td>[89]</td>
</tr>
<tr>
<td>Elastic Modulus Tension:</td>
<td>2700-3700 MPa</td>
<td>Measurements taken on an AGS-500C automatic tensile test machine at a rate of 10 mm min(^{-1}).</td>
<td>[89]</td>
</tr>
<tr>
<td></td>
<td>0.7-18.4 MPa</td>
<td>Unconfined compression testing to 65% strain at a rate of 100% min(^{-1}) on an electomechanical material testing machine (DDL, Eden Prairie, MN). Specimens were preloaded and preconditioned for 10 cycles from 1-10 N before testing.</td>
<td>[90]</td>
</tr>
<tr>
<td>Failure Strain Compressive:</td>
<td>45-62%</td>
<td>Unconfined compression testing to 65% strain at a rate of 100% min(^{-1}) on an electomechanical material testing machine (DDL, Eden Prairie, MN). Specimens were preloaded and</td>
<td>[90]</td>
</tr>
<tr>
<td>Material</td>
<td>Property</td>
<td>Value/Description</td>
<td>Source</td>
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<tr>
<td></td>
<td></td>
<td>Preconditioned for 10 cycles from 1-10 N before testing.</td>
<td></td>
</tr>
<tr>
<td>Failure Stress</td>
<td></td>
<td>1.4-2.1 MPa</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Unconfined compression testing to 65% strain at a rate of 100% min$^{-1}$ on an electomechanical material testing machine (DDL, Eden Prairie, MN). Specimens were preloaded and preconditioned for 10 cycles from 1-10 N before testing.</td>
<td>[90]</td>
</tr>
<tr>
<td>Shear Modulus</td>
<td></td>
<td>0.10-0.43 MPa</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cylindrical specimens were fixed with adhesive between plexiglass slides. Plexiglass slides were attached to custom aluminum shear platens. The upper platen was attached to a 5000 N load cell and the lower platen was attached to a water bath in which the specimens were tested. The specimens were preconditioned and subjected to 65% shear strain at 75% min$^{-1}$.</td>
<td>[90]</td>
</tr>
<tr>
<td>Poly(ethylene glycol)</td>
<td>Elastic Modulus</td>
<td>0.5$^{a}$-500$^{b}$ kPa</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(a) Five rounds of unconfined compression from 0-20% strain at a rate of 2 mm min$^{-1}$. Specimens were cylindrical with diameter 10 mm and height 5 mm. Data was taken at 15 Hz. Testing was done with an MTS Synergie 100 machine with a 10 N load cell. (b) Cylindrical gels were allowed to soak in PBS for a week prior to testing. Unconfined compression at a rate of 1 mm min$^{-1}$ on a MTS Synergie 100 machine with a 10 N load cell.</td>
<td>(a) [105] (b) [106]</td>
</tr>
<tr>
<td>Silicones</td>
<td>Shear Modulus</td>
<td>210-300 kPa</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A parallel plate Bohlin rheometer system applied frequencies ranging from 0.005 to 30 Hz and temperatures from 0°C to 70°C on cylindrical specimens of height 1.2 mm and diameter 16 mm.</td>
<td>[95]</td>
</tr>
<tr>
<td>Tensile Modulus</td>
<td></td>
<td>1.05 MPa</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tensile testing using an Instron 4502 mechanical testing machine on specimens of gauge length 10 mm at a rate of 100 mm min$^{-1}$ to failure. Tensile modulus taken at 80% tensile strain.</td>
<td>[94]</td>
</tr>
<tr>
<td>Strain at Failure</td>
<td></td>
<td>325%</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Tensile testing using an Instron 4502 mechanical testing machine on specimens of gauge length 10 mm at a rate of 100 mm min$^{-1}$ to failure.</td>
<td>[94]</td>
</tr>
<tr>
<td>Polyurethanes</td>
<td>Ultimate Tensile Strength</td>
<td>28.9-34.4 MPa</td>
<td>ASTM test D-412.</td>
</tr>
<tr>
<td></td>
<td>Tear Strength</td>
<td>47-87 kN/m</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ASTM test D-624.</td>
<td>[93]</td>
</tr>
<tr>
<td>Material</td>
<td>Property</td>
<td>Value</td>
<td>Notes</td>
</tr>
<tr>
<td>----------</td>
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</tr>
<tr>
<td>Alginate and Polyacrylamide IPN</td>
<td>Flexural Modulus</td>
<td>0.689 kPa</td>
<td>ASTM test D-790. [93]</td>
</tr>
<tr>
<td></td>
<td>Elastic Modulus</td>
<td>3.8-252.2 kPa</td>
<td>Tensile tests of dumbbell shaped samples with 35 mm length, 2 mm width, 12 mm gauge length with a 500 N load cell on a CMT6503 tensile machine at room temperature. The ends of the sample were fixed to the clamps of the machine. The lower clamp was stationary and the upper clamp moved upward at a rate of 100 mm min(^{-1}). Modulus determined from the slope of the stress-strain curve from 0-10% strain. [63]</td>
</tr>
<tr>
<td></td>
<td>Fracture Energy</td>
<td>Up to 8700 J m(^{-2})</td>
<td>Rectangular gel specimens of dimensions 77 mm x 5 mm x 3 mm were glued to polystyrene clamps and an Instron 3342 mechanical testing machine. The 500 N load cell applied a constant stretch rate of 2 min(^{-1}). Tests were performed in air at room temperature. An unnotched sample was tested to obtain a force-length curve. A notched sample was tested to determine the length at which the notch started to propagate. [69]</td>
</tr>
<tr>
<td></td>
<td>Elongation at Break</td>
<td>20</td>
<td>Rectangular gel specimens of dimensions 77 mm x 5 mm x 3 mm were glued to polystyrene clamps and an Instron 3342 mechanical testing machine. The 500 N load cell applied a constant stretch rate of 2 min(^{-1}). Tests were performed in air at room temperature. [69]</td>
</tr>
<tr>
<td></td>
<td>Tensile Stress at Rupture</td>
<td>156 kPa</td>
<td>Rectangular gel specimens of dimensions 77 mm x 5 mm x 3 mm were glued to polystyrene clamps and an Instron 3342 mechanical testing machine. The 500 N load cell applied a constant stretch rate of 2 min(^{-1}). Tests were performed in air at room temperature. [69]</td>
</tr>
<tr>
<td></td>
<td>Stretch at Rupture</td>
<td>23</td>
<td>Rectangular gel specimens of dimensions 77 mm x 5 mm x 3 mm were glued to polystyrene clamps and an Instron 3342 mechanical testing machine. The 500 N load cell applied a constant stretch rate of 2 min(^{-1}). Tests were performed in air at room temperature. [69]</td>
</tr>
<tr>
<td>Poly(2-acrylamido-2-methylpropanesulfonic acid)</td>
<td>Compressive Stress</td>
<td>17.2 MPa</td>
<td>Compressive testing using Tensilon RTC-1310A mechanical testing machine on water swollen gels with 9 mm diameter and 4 mm height at a strain rate of 0.1% min(^{-1}). [75]</td>
</tr>
</tbody>
</table>
and Polyacrylamide IPN & Compressive Fracture Strain & 92% & Compressive testing using Tensilon RTC-1310A mechanical testing machine on water swollen gels with 9 mm diameter and 4 mm height at a strain rate of 0.1% min\(^{-1}\). & [75]

II.5. Medical Simulators with Biomechanical Relevance

II.5.1. Commercially Available Simulators

Numerous companies already manufacture medical simulators that have biomechanical relevance. Medical simulators can be physical, virtual, or a hybrid of the two. Table 3 lists selected simulators intended for various clinical tasks including line placement, wound care, medical imaging, interventional procedures, and full patient simulation.

Table 3: Selected Commercially Available Simulators with Biomechanical Relevance

<table>
<thead>
<tr>
<th>Clinical Task</th>
<th>Company</th>
<th>Representative simulators</th>
<th>Type of Simulator</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Line/Line Placement</td>
<td>VATA Inc.</td>
<td>Chester Chest(\text{TM}), Peter PICC Line(\text{TM}), NITA Newborn(\text{TM}), Advanced Venipuncture Training Aid(\text{TM}), Port &quot;Body in a Box&quot;(\text{TM}), “Bonnie” Bone Marrow Biopsy(\text{TM})</td>
<td>Physical</td>
<td><a href="http://www.vatainc.com/">http://www.vatainc.com/</a></td>
</tr>
<tr>
<td>Wound Care</td>
<td>VATA Inc.</td>
<td>Seymour II(\text{TM}) Wound Care Model, &quot;Wilma&quot; Wound Foot(\text{TM}), &quot;Pat&quot; Pressure Ulcer Staging Model(\text{TM}), &quot;Stan&quot; Stage IV Pressure Ulcer Model(\text{TM}), “Vinnie” Venous Insufficiency Leg(\text{TM}), “Annie” Arterial Insufficiency Leg(\text{TM})</td>
<td>Physical</td>
<td><a href="http://www.vatainc.com/">http://www.vatainc.com/</a></td>
</tr>
<tr>
<td>Medical Imaging</td>
<td>CAE Healthcare</td>
<td>Vimedix, Blue Phantoms</td>
<td>Physical</td>
<td><a href="https://www.bluephantom.com/">https://www.bluephantom.com/</a></td>
</tr>
<tr>
<td></td>
<td>CIRS Inc.</td>
<td>Many models for Diagnostic CT, elastography, fluoroscopy/x-ray, mammography, multi-</td>
<td>Physical</td>
<td><a href="http://www.cirsinc.com/">http://www.cirsinc.com/</a></td>
</tr>
</tbody>
</table>
II.5.2. Published Hard and Soft Tissue Simulator Models

Many research groups have developed and tested hard and soft tissue simulator models intended to approximate various clinical techniques. Several of these are listed in Table 4 along with the constituent materials and the mechanical properties considered in making the simulator. The numeric values of the mechanical properties are given in Table 4 if stated in the reference text. Finally, Table 4 notes if the simulant materials were directly compared to tissue mechanical property values. The design and development of these models are described in more detail in the following sections.

Table 4: Materials Used in Hard and Soft Tissue Simulator Models

<table>
<thead>
<tr>
<th>Technique</th>
<th>Simulator</th>
<th>Material</th>
<th>Mechanical Properties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endoscopy &amp; Laparoscopy</td>
<td>Endoscopy part task trainer</td>
<td>Plastic, polystyrene, cardboard</td>
<td>None stated</td>
<td>[107]</td>
</tr>
<tr>
<td></td>
<td>Laparoscopic simulator</td>
<td>Silicons, latexes</td>
<td>Elastic modulus, density, hardness</td>
<td>[108]</td>
</tr>
<tr>
<td>Pyeloplasty simulator</td>
<td>Organosilicate material</td>
<td>None stated. (Material formulations developed from)</td>
<td>--</td>
<td>[16]</td>
</tr>
<tr>
<td>Category</td>
<td>Model Type</td>
<td>Material</td>
<td>Mechanical Properties</td>
<td>Source</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------------------------</td>
<td>-------------------------------------------</td>
<td>------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Neurosurgery</td>
<td>Pediatric lumbar spine model</td>
<td>Elastomeric powder</td>
<td>No properties stated.</td>
<td>[109]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Used water to represent CSF rheological properties.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Haptic feedback considered when selecting materials</td>
<td></td>
</tr>
<tr>
<td>Brain aneurysm models</td>
<td>1. Photosensitive polymeric liquid-plastic epoxy</td>
<td>a. Tensile strength b. Elongation at break c. Hardness (Shore A)</td>
<td>1. a. 50 MPa, b. 15-20% 2. a. 50 MPa, b. 20% 3. b. 200%, c. 27</td>
<td>[14]</td>
</tr>
<tr>
<td>Orthopaedic Surgery</td>
<td>Temporal bone model</td>
<td>Polyamide nylon with glass beads</td>
<td>Hardness</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Facial bone biomodel</td>
<td>Unspecified plastic</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Endovascular Intervention</td>
<td>Perfused human cadaver</td>
<td>Human cadaver, water, pigment</td>
<td>Vascular pressure, feel</td>
<td>No</td>
</tr>
<tr>
<td>Pulsatile blood vessel</td>
<td>1. Silicone</td>
<td>a. Complex modulus</td>
<td>1. b. ~0.4-1.5 2. a.~25-700 kPa (formulation and temperature dependent), b. ~0.1</td>
<td>[113]</td>
</tr>
<tr>
<td></td>
<td>2. Poly(vinyl alcohol)</td>
<td>b. Friction coefficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c. Transparency</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>d. Pulsatility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endovascular blood vessel</td>
<td>Poly(vinyl alcohol)</td>
<td>a. Storage modulus</td>
<td>a. ~20-150 kPa (formulation dependent) b. ~1-14 kPa (formulation dependent)</td>
<td>Yes; dog</td>
</tr>
<tr>
<td>Blood vessel, carotid artery</td>
<td>Poly(vinyl alcohol)</td>
<td>a. Friction coefficient</td>
<td>Referenced other studies’ values</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b. Storage and loss modulus</td>
<td></td>
<td>[115]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c. Transparency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Chest phantom</td>
<td>3% agarose gel</td>
<td>a. Elastic modulus b. Poisson’s ratio c. Shear modulus</td>
<td>[116]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>a. 120 kPa b. 0.43</td>
<td>No</td>
</tr>
<tr>
<td>Pathological Visualization</td>
<td>Infant heart model</td>
<td>1. Photosensitive liquid-plastic epoxy material</td>
<td>a. Tensile strength 1. a. 78 MPa, b. 2.8 GPa</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[117]</td>
</tr>
</tbody>
</table>
II.5.2.1. Endoscopy & Laparoscopy

Endoscopic procedures involve intricate maneuvers and thus require extensive training. A simulation training box is available to allow for such maneuvers to be practiced and learned [107]. The endoscopy part task training box is a rigid structure (Fig. 12) in which the correct hand motions can be learned to ensure minimal patient discomfort. The box is made of plastic, polystyrene, and cardboard and bears no resemblance to tissues encountered in endoscopic procedures [119]. It is used to assess the competence of medical professionals before they perform live procedures. Kinematics data of the maneuvers was used to develop the training box and assess its validity [107].

Haptic feedback is an important consideration in laparoscopic procedures in order to avoid the application of excessive force. A laparoscopic simulator was developed to include all anatomical structures contacted during the surgery [108]. Such structures include the stomach, esophagus, omentum, crura, spleen, liver, and phrenoesophageal ligament. The correct geometry of the organs was obtained from human computed tomography (CT) scans, while the materials for the organs were selected based on mechanical property comparison with porcine tissues. Although each organ in the simulator had a different material composition, many organs were made of a silicone or latex base. Silicone rubber displayed an elastic modulus similar to that of porcine tissue, and silicone and latex had coefficients of friction resembling that of human tissue. Materials that were considered and compared before selecting silicone as the most promising material for the simulator were not listed. Velvet rope was used to connect the organs, allowing for easy replacement of tissues. Many of the parts were able to be reused up to 30 times but a few parts need to be replaced after each mock procedure. For this reason, the synthetic model is more cost effective than previously used live animal tissues [108].
Additionally, laparoscopic simulator models are often used to train surgeons for procedures that are difficult or risky, such as pyeloplasty. An organosilicate material was used to construct patient-specific renal pelvis models [16]. Material selection was based on previously obtained tissue mechanical properties and with input from practicing urological surgeons. Urological surgeons filled out a questionnaire to assess the validity after performing the simulated laparoscopic pyeloplasty. Of all the questions reported, the rating for material behavior during cutting was the lowest (3.25/5), while the rating for anastomotic suturing skill reproduction was the highest (4.42/5). Although the ratings from the surgeons indicated that the material behaved like human tissue overall, a quantitative mechanical comparison is not mentioned [16].

II.5.2.2. Neurosurgery

Neurosurgery is another clinical technique that often makes use of medical simulators for portions of surgeon training. A simulator was developed to be able to reproduce pediatric neurosurgical procedures involving the lumbar spine [109]. Scans of lumbar spinal geometry were used to obtain an accurate anatomical shape and then a ZCorp Rapid Prototyper was used to 3D print the spine model using an elastomeric powder. Water was used to model the cerebrospinal fluid because of their similar rheological properties. Synthetic materials to be used for the skin, fat, muscle, spinal cord, and dura mater were tested and chosen based on the similarity of their haptic feedback to the native tissues. All components were assembled in a custom retail mannequin. To better understand the surgeons’ ability to perform the surgery, pressure transducers were placed on the simulated spinal cord to record the pressure experienced during manipulation. The pressure placed on the spinal cord is important to monitor because too much pressure could cause damage. Pilot studies showed that the model is a functional tool for training medical students but further testing must be done to fully validate the model [109].

Another example of neurosurgical simulation is brain aneurysm modeling. Stereolithography has been used to create biomodels of aneurysms out of a photosensitive polymeric liquid-plastic epoxy material [14]. The tensile strength and the elongation at break of the material were reported to be 50 MPa and 15% to 20% respectively. Angiographic data was used to direct the laser to construct the model in the correct geometry for the skull and vessels. The aneurysm itself is made in a two-part silicone mold from a less rigid resin material. In this model the aneurysm
connects to the surrounding vessels to allow for many clippings to be done on the same model without replacement. The aneurysm portion of the model deteriorated after 15 clippings, however, the model was judged by neurosurgeons to be a valuable tool in visualizing the pathoanatomy of the aneurysm [14].

A second brain aneurysm model was constructed by the same group using Polyjet Matrix 3D printing [14]. The Objet Connex500 3D printer was used to produce the aneurysm biomodel from angiographic data. The skull and vessels were made from similar opaque liquid photopolymers and a rubberlike flexible photopolymer was used for the aneurysm. The tensile strength and elongation at break of the skull and vessels materials were reported to be 50 MPa and 20% respectively. The material used in the aneurysm had an elongation at break of 200% and a Shore A hardness of 27. The aneurysm connected to the vessels to become a replaceable part for the model. The aneurysm could endure 100 clippings before deteriorating and being replaced, and like the model constructed via stereolithography, was reported to be useful in pathoanatomical visualization and determination of a case-specific procedure. In addition, surgeons reported that the tactile feedback of the clipping procedure was realistic in comparison to a real clipping [14].

**II.5.2.3. Orthopaedic Surgery**

Selective layer sintering (SLS) was used to construct a model of the temporal bone to be used as a simulator for inner ear surgery [110]. The material used was a powder material of polyamide nylon with added glass beads. CT images were used to ensure correct geometry of the bone and also to determine the material density from the extracted bone shadow. SLS was able to accurately construct the small structures of the inner ear, including the incus and the malleus, but not the stapes. Dissection of the model indicated that the model’s hardness was similar to real bone. Real bone forms a dust when shaved, and although shaving the simulator produced dust, the simulator dust was stickier and had to be removed with a pick instead of a suction irrigator [110].

Reconstructive surgery has also taken advantage of simulation to achieve better symmetry and greater functionality. Stereolithography was used to construct plastic biomodels using a patient’s
CT scans [111]. Biomodeling allowed for realistic looking models with exact patient dimensions that were unique to each case. These models allowed for the surgeons to have exact calculations on the contour, angulation, and morphology of the bone before starting the surgery. The models also provided for better preoperative planning. This study found that 3D biomodeling was geometrically accurate and gave improved results over previous methods [111].

II.5.2.4. Endovascular Intervention

Although human cadavers are quite expensive and have limited availability, they can provide realistic simulations for a handful of scenarios that are not affected by tissue death or preservation chemicals. Arteries can be used in cadaveric tissue if perfusion of the vessels is used to simulate blood flow. This allows for accurate practice of central line placement to decrease the complications faced with this task [112]. Before perfusion, the cadaver was cannulated, conditioned, and then filled with water and red and blue pigments. To mimic realistic environment, the vasculature was pressurized to an arterial pressure of 80 mmHg and venous pressure of 15 mmHg. Physicians tested on this model concluded that it was an accurate representation of the conditions in live patients and that it allowed for skill acquisition [112].

The material properties of silicone and a PVA hydrogel material were compared in order to determine their use as simulator materials for endovascular intervention [113]. The comparison metrics were the complex modulus, friction coefficient, transparency, compatibility with medical imaging devices, and pulsatility. Both silicone and PVA hydrogel were used to create vascular models from human data using the lost wax technique. Both material models were transparent and compatible with medical imaging devices, but PVA had a lower friction coefficient than silicone and its complex modulus allowed for pulsation during flow simulation. The PVA hydrogel exhibited a lower coefficient of friction than silicone because it contained water and silicone did not. These properties of the PVA hydrogel make it a suitable material for research and development of endovascular medical devices [113].

Another study used PVA to form a blood vessel model for use as an endovascular training device [114]. The mechanical properties of blood vessels were considered in order to obtain a more accurate simulation environment. A variety of types, concentrations, and blends of PVA were
made to determine the composition that most resembled the viscoelastic properties of a dog blood vessel. The study found that the mechanical properties were able to be tuned based on the concentration, degree of polymerization, and saponification of the PVA hydrogels. A dynamic mechanical analyzer was used to measure the storage and loss moduli to be compared with real tissue. Temperature dependence was also examined to determine if the properties could be maintained in varying environments. PVA hydrogels were found to maintain their dynamic viscoelasticity between 0°C and 40°C. Overall the authors noted the importance of the blood vessel model to not only simulate tissue as a viscoelastic material but to simulate the dynamic viscoelastic behavior resulting from pulsatile flow in blood vessels [114].

A blood vessel model was made to numerically analyze and observe the motion of a guidewire used in catheterizations [115]. The goal of this experiment was to compare the needle trajectory in the model to a computer simulation of the same procedure. In order to ensure realistic experimental simulation results, the material used to form the blood vessel model was carefully selected. PVA was chosen because its transparency could allow for easy visualization of the guidewire. Additionally, it has lower surface friction than a silicone elastomer used in previous studies, and it has been shown to have a similar dynamic viscoelasticity as blood vessels. [114]. The PVA hydrogel was molded into a torus-shaped vessel with a 4mm diameter, which is characteristic of an internal carotid artery. An 80/20 wt% solution of dimethyl sulfoxide and water was used as the solvent in which PVA was dissolved to make a 12 wt% solution. Gelation was achieved by freezing. The PVA blood vessel model was able to validate the results obtained with the computer simulation, and the authors concluded that the model could also be used in medical simulation because the motions used are characteristic of those used by surgeons [115].

**II.5.2.5. Diagnosis**

The measurement of acoustic shear waves in the chest is a noninvasive diagnosis method for congenital heart disease. Acoustic properties of agarose gel have been studied to determine its usability as an anthropomorphic chest phantom for diagnosing congenital heart disease. The elastic modulus and Poisson’s ratio were quantified for 3% agar water-based gels [116]. This study was preliminary and did not make conclusions about how the properties of the agarose
compared to live tissue but it was able to show that the acoustic and elastic properties of the gel were able to be properly determined [116].

II.5.2.6. Pathological Visualization

Preoperative planning for congenital heart disease procedures in infants and neonates is aided by the biomodeling of the heart with epoxy resin (Fig. 13. a) [117]. Stereolithography was used to make models from multislice CT images. Through use of the epoxy models, surgeons were able to visualize the complexity of the case and determine the intended procedural approach before starting the surgery. To be able to practice the procedure, similar models were made of rubber-like urethane (Fig. 13. b). The urethane models were used to perform patient-specific mock procedures involving both cutting and suturing. Cutting and suturing of the urethane was achieved, however, neither the urethane model nor the epoxy model accounted for the mechanical properties of the tissue compared with live heart tissue. Regardless of the mechanical feel, surgeries following these visualization and practice techniques were successful. It was determined that this technique would be useful in training pediatric cardiac surgeons, but CT image quality could be improved in order to increase the accuracy of the intricate details in the simulated models of juvenile hearts [117].
Silicone balloons have been used to model the lungs and the pleural space in a mechanical respiration simulator [118]. This simulator was intended to mimic normal and pathological breathing conditions, and different balloon thicknesses were used to change the stiffness of the model lungs in order to mimic the different conditions. The purpose of this simulator was to allow for a better biomechanical and physical understanding of lung function under pathological conditions in order to optimize treatment. Many treatments for lung pathologies require the drainage of excess fluid from the pleural space. An important consideration in lung drainage treatments is the pressure in the pleural space, so pressure sensors were incorporated in the simulator in order to monitor pleural pressure under different physiological conditions. The authors state that this simulator could be used to improve drainage treatments because it can mimic the pleural pressure for specific pathologies. Because this simulator was only used to
replicate respiratory conditions of human lungs, no effort was focused on mimicking the mechanical properties of native lung tissue [118].

II.6. Challenges and Future Directions
Of the fourteen studies summarized in Table 4, only six specifically stated the mechanical properties of the simulator materials, and only two attempted a quantitative comparison between mechanical behavior of the simulant to that of native tissues. One challenge underlying this trend is that the mechanical properties of biological tissues are extremely complex and resist reduction to a single, easily computed metric. It is tempting to simply quantify Young’s modulus for a simulant material and compare that to reported values in literature for the target tissue. While there is some value in this approach it is severely limited by the fact that the Young’s modulus of a viscoelastic material will vary depending on the rate at which it is deformed. Moreover, for a strongly nonlinear material, as many tissues are, there is little meaning in computing the slope of the linear portion of a nonlinear stress-strain curve, so more sophisticated techniques must be used. Lastly, the reported values for Young’s modulus for a given tissue can vary widely depending on the exact conditions of the study.

It is worthwhile to try to address these challenges. As noted in the Introduction, developing medical simulators with realistic mechanical and geometrical properties has been shown to increase the safety of the patient, and to maintain and develop confidence and good practice for trainees [5, 6, 9, 10, 14, 16]. For procedures in which the mechanical response or “feel” of the simulated tissues is important for the trainee, simulator developers should seek to demonstrate that the simulated tissues accurately mimic the mechanical response of native biological tissue. As discussed above, this is not a trivial task, but some reasonable minimum recommendations may include visual comparison of stress-strain plots for simulant versus native tissue materials for mechanical test data collected under comparable conditions. It would also be advisable to choose the mechanical test conditions based on the loading regimes (tension, compression, shear, indentation, etc.) that are most relevant for the simulated procedure.

Due to the numerous challenges associated with developing life-like tissue phantoms for medical simulation, this field benefits strongly from interdisciplinary collaborations between clinicians,
engineers, and material scientists. Two areas of potential future growth include application of 3D printing methods and development of more advanced materials. These will be discussed in turn below.

3D printing has potential to reduce costs, speed simulator manufacturing, and make advanced simulation models more widely available. Though medical simulation currently takes advantage of 3D printing in order to construct patient-specific biological geometries, the recent studies cited in this work [14, 109–111, 117] were not focused on creating biomechanically accurate models. If mechanical properties are considered in 3D printing studies, typically the 3D printing techniques are utilized in order to make molds to cast biomechanically relevant materials into desired geometries. In the future, medical simulation could benefit from directly 3D printing biomechanically relevant materials into patient-specific geometries.

Because of their unique toughness properties [69] and ability to simultaneously mimic elastic and viscoelastic mechanical properties of biological tissues [66], alginate-polyacrylamide IPN hydrogels show promise as a material that can be 3D printed into components for medical simulation devices. Bakarich et al [120] also reported the 3D printing of a tough alginate-polyacrylamide IPN hydrogel. A similar IPN that has been 3D printed into biologically relevant structures, including a nose and an ear, is a poly(ethylene glycol)-alginate hydrogel with nanoclay particles [121]. This PEG-alginate IPN reported a high fracture toughness and because the materials were biocompatible, it was able to encapsulate living cells.

Future directions for the field include the improvement of 3D printed shape fidelity and mechanical integrity for 3D printed hydrogels and soft polymeric structures, as well as the incorporation of anisotropy. Additional considerations in the development of these materials should include the post-printing shelf life of the simulator and the reuse of the simulator for multiple practice procedures before it is rendered unreliable. If attention is given to these aspects, 3D printing of biomechanically accurate medical simulation devices can become a reality.
II.7. Acknowledgments
The authors would like to acknowledge John Kromer of the Miami University Libraries for his assistance with conducting the initial literature review.

II.8. References


43. Macosko CW (1994) Rheology: principles, measurements, and applications. VCH


45. Moore KL, Dalley AF, Agur AMR (2013) Clinically Oriented Anatomy. Lippincott Williams & Wilkins


CHAPTER III - 3D Printing of an Interpenetrating Network Hydrogel Material with Tunable Viscoelastic Properties†

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Keywords: 3d printing, additive manufacturing, hydrogel, IPN, stress relaxation, viscoelasticity

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†This is the postprint version of:

Supplemental information published with this work is included as APPENDIX I of this thesis.
°References are formatted as required by the Journal of the Mechanical Behavior of Biomedical Materials.
III.1. Abbreviations
APS – Ammonium persulfate
CaCO₃ – Calcium carbonate
GDL – D-glucono-δ-lactone
HEA – 2-hydroxyethyl acrylate
IPN – Interpenetrating polymer network
MBAA – N, N- methylenebisacrylamide
NIPAm – N-isopropylacrylamide
TEMED – tetramethylethlenediamine

III.2. Abstract
Interpenetrating network (IPN) hydrogel materials are recognized for their unique mechanical properties. While IPN elasticity and toughness properties have been explored in previous studies, the factors that impact the time-dependent stress relaxation behavior of IPN materials are not well understood. Time-dependent (i.e. viscoelastic) mechanical behavior is a critical design parameter in the development of materials for a variety of applications, such as medical simulation devices, flexible substrate materials, cellular mechanobiology substrates, or regenerative medicine applications. This study reports a novel technique for 3D printing alginate-polyacrylamide IPN gels with tunable elastic and viscoelastic properties. The viscoelastic stress relaxation behavior of the 3D printed alginate-polyacrylamide IPN hydrogels was influenced most strongly by varying the concentration of the acrylamide cross-linker (MBAA), while the elastic modulus was affected most by varying the concentration of total monomer material. The material properties of our 3D printed IPN constructs were consistent with those reported in the biomechanics literature for soft tissues such as skeletal muscle, cardiac muscle, skin and subcutaneous tissue.

III.3. Introduction
Hydrogels are hydrophilic, three-dimensional networks of crosslinked polymers (Hoffman, 2012). These materials are used for a wide variety of applications in biology and medicine, such as tissue engineering scaffolds (Lee and Mooney, 2001; Peppas et al., 2006) and drug delivery (Peppas et al., 2006, 2000), due to their high water content, structural similarity to biological
tissue, and biocompatibility (Lee and Mooney, 2001; Peppas et al., 2006, 2000). However, some studies (Choi and Kim, 2015; Low et al., 2015; Myung et al., 2007; Sun et al., 2012) cite the insufficient mechanical strength of hydrogels as reasoning for developing tougher hydrogels. A common approach to strengthening hydrogels is the incorporation of a second polymer network to form an interpenetrating network (IPN) hydrogel (Choi and Kim, 2015; Darnell et al., 2013; Gong et al., 2003; Low et al., 2015; Myung et al., 2007; Sun et al., 2012; Zhao, 2014). IPN hydrogel materials are of great interest due to their unique toughness properties. The toughness of the IPN arises from the combination of an ionically crosslinked polymer network and a covalently crosslinked polymer network (Sun et al., 2012). The ionically crosslinked network serves to dissipate energy from an applied load by breaking the ionic crosslinks to form damage zones. Damage accumulates in these zones before crack propagation can occur (Chen et al., 2014). The covalently crosslinked network maintains the elasticity of the hydrogel (Fitzgerald et al., 2015; Sun et al., 2012).

Tough IPN hydrogels have been used in numerous biomedical applications including drug releasing medical devices (Steffensen et al., 2015), actuators in soft robotics (Zheng et al., 2015), and the fabrication of an artificial cornea (Parke-Houben et al., 2015). IPN materials composed of polypyrrole and polycaprolactone, where the polypyrrole component is electrically conductive, have even been found to be a suitable material for the growth of functional cardiac myocytes (Spearman et al., 2015). In spite of these advances, investigators continue to seek new ways to control and enhance the mechanical behavior of IPNs. Yang et al. (2013) found that the mechanical properties of an alginate-polyacrylamide IPN can be significantly improved via secondary crosslinking of the alginate network by multivalent cations such as aluminum, iron, barium, and strontium (Yang et al., 2013). Fitzgerald et al. reported that the viscoelastic behavior of an IPN could be tuned to mimic that of porcine muscle tissue (Fitzgerald et al., 2015), and Myung et al. achieved an elastic modulus similar to that of the cornea for multiple species (Myung et al., 2007).

There is growing interest to develop methods to 3D print IPN hydrogels for ease and speed of manufacturing for their various uses in biology and medicine; however, this process presents significant technical challenges. Only a few groups have reported successful 3D printing of
IPNs. Bakarich et al. were able to 3D print alginate-polyacrylamide IPN hydrogels with high toughness (Bakarich et al., 2013). Another recent study reported 3D printing of a biocompatible poly(ethylene glycol)-alginate IPN gel with notable toughness properties (Hong et al., 2015). Currently little is known about the viscoelastic stress relaxation behavior of 3D printed IPN materials or how the viscoelastic properties may be controlled via the 3D printing process. This deficit has important implications for IPNs used in biomedical applications, since viscoelastic behavior is commonly observed in a wide range of biological and bio-mimetic materials (Kemper et al., 2013, 2011; Cheng and Bilston, 2007; Vito and Dixon, 2003; Jor et al., 2013; Evans et al., 2013). The objectives of the present study were (1) to assess the effects of different functional groups on the mechanical properties of IPNs in a bulk (cast molded) state; (2) to develop an IPN 3D printing approach that allows for near-simultaneous cross-linking of the two networks; and (3) to determine which network components affect the elastic modulus and stress relaxation properties of the 3D printed IPN. Our approach was to synthesize four different cast-molded IPNs, each containing alginate with varying covalent monomers including acrylamide (Darnell et al., 2013; Sun et al., 2012; Yang et al., 2013), N-isopropylacrylamide (NIPAm) (Zheng et al., 2015), and 2-hydroxyethyl acrylate (HEA) (Cheng et al., 2015) in order to compare their mechanical and swelling behaviors. Subsequently, alginate-polyacrylamide IPN was selected for 3D printing and characterization since this IPN formulation showed the greatest stress relaxation in the cast molded state.

III.4. Materials and Methods

III.4.1. Cast Molded IPNs

III.4.1.1. Materials
Alginate (type Manugel GHB) was donated by FMC Biopolymer. Calcium carbonate (CaCO₃), D-glucono-δ-lactone (GDL), acrylamide, and NIPAm were purchased from Acros Organics. N, N-methylenebisacrylamide (MBAA), ammonium persulfate (APS), and tetramethylethylenediamine (TEMED) were purchased from Fisher. HEA was purchased from Aldrich. All materials were used without further purification.
III.4.1.2. Material Preparation

Alginate-polyacrylamide IPN hydrogels (Scheme 1.B.1.) were synthesized using a previously reported procedure (Fitzgerald et al., 2015), described briefly here. Ionic crosslinking of the alginate was achieved by using a system of CaCO$_3$ paired in a 1:2 molar ratio with GDL to slowly release the calcium ions to control gelling time (Kuo and Ma, 2001). Acrylamide monomer was covalently crosslinked by MBAA. The polymerization of acrylamide was initiated by the initiator and accelerator pair of APS and TEMED which were held constant at a weight ratio of 25:17. Stock solutions of 4 wt% alginate, 200 mM GDL, 100 mM CaCO$_3$, 1.0 wt% MBAA, and 20 wt% APS were prepared with deionized (DI) water. From here, two solutions were prepared in separate conical mixing tubes: one containing APS, GDL, and CaCO$_3$ stock solutions in weights corresponding to the desired formulation, and the other solution containing the alginate and MBAA stock solutions plus the TEMED, acrylamide monomer, and DI water in formulation-specific weights. The former of the two solutions was prepared immediately before mixing the two to minimize the release of calcium ions from CaCO$_3$ by GDL. Both solutions were vortexed for 60 seconds to ensure thorough mixing. The solution containing APS, GDL, and CaCO$_3$ was poured into the solution containing the alginate, vortexed for 60 seconds, and the final solution was poured into Delrin molds (17 mm dia. x 12 mm height). The UV initiation occurred in a UV initiator box (Fisher Scientific, model 13-245-221, 115 VAC, 175 W, 1.6 A) under 254 nm light with an exposure time of 20 min.
Scheme 1. Interpenetrating network of alginate ionically crosslinked by calcium ions and a covalently crosslinked (MBAA) polymer network (A). The covalently crosslinked network (red) was varied in four different compositions throughout this study and the chemical structures of the repeating monomer unit(s) along with the name of the entire IPN (and abbreviations) are shown in (B.1-4.). The covalent network in the alginate-polyacrylamide IPN (B.1.) is entirely made up of polyacrylamide. The covalent network in the alginate-pNIPAm IPN (B.2.) is entirely made up of poly(N-isopropylacrylamide). The covalent network in the alginate-pHEA IPN (B.3.) is entirely made up of poly(hydroxyethyl acrylate). The covalent network in the alginate-poly(N-isopropylacrylamide-co-hydroxyethyl acrylate) IPN (B.4.) is composed of a random copolymer of NIPAm and HEA monomers.
To alter the overall chemical composition of the IPN (Scheme 1.A.), different monomers were selected to form the covalently crosslinked network (shown in Scheme 1.A. in red). NIPAm and HEA monomers were chosen because their polymerization can be carried out by the same initiator and accelerator pair as acrylamide monomer and crosslinking can occur via MBAA. The resultant polymers have the same carbon backbone as polyacrylamide, but the functional groups in the side chains are more hydrophobic. NIPAm has an isopropyl functional group and HEA has a carbon-carbon chain terminated by a hydroxyl functional group, whereas acrylamide has an amide functional group (Scheme 1.B.1-3.).

The alginate-pNIPAm IPN (Scheme 1.B.2.) and the alginate-pHEA IPN (Scheme 1.B.3.) were synthesized using the same method as described for the alginate-polyacrylamide IPN (Scheme 1.B.1.), except NIPAm or HEA monomers were used instead of the acrylamide monomer. Random copolymers of NIPAm and HEA were also prepared (Scheme 1.B.4.). Numerous formulations were prepared by varying the weight percent of the total monomer (alginate plus covalent monomer) in the solution, the weight percent MBAA to the weight of the covalent monomer, and the weight ratio of alginate to the covalent monomer. For the formulations containing both NIPAm and HEA monomers, the mole ratio of the two was varied. Compositions were based on a previously reported formulation (Fitzgerald et al., 2015).

**III.4.1.3. Mechanical Testing**

Ramp-hold unconfined compression stress relaxation testing was performed (Bose Electroforce 3200 Series III, Eden Prairie, MN) on the cylindrical samples with a ramp rate of 1% strain sec\(^{-1}\) to a final strain of 10%. The hold period at 10% strain was 500 seconds. The elastic modulus and stress relaxation were determined from this test. The elastic modulus for the ramp phase of the test, \(E_{\text{ramp}}\), was calculated as the slope of the engineering stress versus engineering strain curve for the ramp portion of the experiment. The long-term elastic modulus, \(E_{\text{inf}}\), was calculated based on the engineering stress and engineering strain results averaged over the last 10 seconds of the hold period. Stress relaxation was quantified as a percent relaxation, or how much the stress in the IPN decreased at the end of the hold phase versus the end of the ramp phase.
**III.4.1.4. Swelling**

Hydrogel samples were weighed immediately post polymerization and placed in beakers containing ~150 mL DI water. DI water was used over PBS because it has been found that for this IPN hydrogel network, PBS and DI water have the same effect on the mechanical properties of the hydrogels when swollen (Fitzgerald et al., 2015). The beakers were covered with Parafilm. The hydrogels were removed from the beaker, patted dry, and weighed approximately every 24 hours until their weight did not change. The increase in hydrogel weight with time was assumed to only be due to swelling. Swelling was quantified as weight percent water uptake, or how much the weight of the hydrogel had increased due to water.

**III.4.2. 3D Printing of Alginate-Polyacrylamide IPN**

**III.4.2.1. Initiator Selection**

In order to dissolve the dry weights of the network components (acrylamide, MBAA, CaCO$_3$, GDL) in the alginate stock solution for printing, it was necessary to heat the solution. Thus APS was not a suitable choice for a radical initiator for the 3D printed alginate-polyacrylamide IPN hydrogel, since APS is a thermal initiator in addition to a photo initiator. Accordingly, Irgacure 1173 (BASF, Greenville, OH) was selected as the initiator instead of the APS/TEMED pair, since it suspended well in the monomer solutions and was effectively initiated using the 365 nm lamp used by the 3D printer.

**III.4.2.2. Material Preparation**

For the dual syringe IPN 3D printing system developed in this work (Scheme 2), two separate solutions were made in which the GDL and CaCO$_3$ were separated to avoid premature release of calcium ions and subsequent crosslinking of the alginate, and the Irgacure 1173 was separated from the acrylamide and MBAA to prevent premature polymerization. Because the 3D printer uses one motor (Snap Motors 62:1) to depress the two syringes, the solutions must have similar viscosities (±20%, determined using a viscometer) to ensure a 1:1 mixing ratio. Alginate had the largest impact on viscosity and was distributed into both solutions to achieve this balance. For the materials investigated in this work, the viscosities of the two solutions were ~3000 cP, which was below the limit (~10,000 cP) of the motor. To prepare the two solutions, a stock solution of 4% alginate was made with DI water. One solution contained the alginate stock solution, the
acrylamide monomer, the MBAA, and the GDL. The other solution contained the alginate stock solution, the Irgacure 1173, and the CaCO$_3$ (see Scheme 2.B.1. for details). The separate solutions were warmed so that the components would dissolve in the alginate stock solution. Both solutions were vortexed until thoroughly mixed. The solutions were then transferred to syringes for printing. Since the reactive components that initiate the formation of each network are separated, reactive mixing only occurs immediately before extrusion, allowing increased control of reaction kinetics.

### III.4.2.3. 3D Printing Methodology

The hardware and software of an inexpensive (~$6000) commercially available 3D printer platform (Fab@Home Model 3 Research Platform, Seraph Robotics Inc.) were modified to accommodate IPN synthesis. An external extrusion tower (Scheme 2.A.1.) was designed to hold up to four 60 cm$^3$ syringes. Interchangeable parts provide the option of using smaller syringe volumes, and each pair of syringes is depressed using one motor so that a 1:1 ratio is maintained. Half-inch inner diameter flexible tubing runs from each syringe to a new 149 mm long static mixing head (Fisnar, 5.0 mm ID, 24 mixing elements) (Scheme 2.A.2., 2.B.2.). To accommodate the mixing head, the original printer carriage was removed and replaced with a custom carriage (Scheme 2.A.2.). The custom carriage houses four UV LED lights organized in an array around the print nozzle (Scheme 2.A.3, 2.B.3). The new hardware design facilitates printing 120 cm$^3$ constructs, compared to 10 cm$^3$ for the stock printer, within the 23 cm x 12.8 cm x 20 cm (x/y/z) build space.

Scheme 2.B. depicts the synthesis reactions during printing and how they relate to the printer hardware. The alginate-polyacrylamide IPN hydrogel material requires mixing of two solutions prior to extrusion (Scheme 2.B.1.). Therefore, a mixing head was employed to combine the materials directly preceding extrusion (Scheme 2.B.2.). During the residence time (~10 min) in the mixing head, calcium ions are released from CaCO$_3$ as a result of GDL hydrolysis and begin to ionically crosslink the alginate network. Upon extrusion, the 365 nm UV lights (intensity ~20mW/cm$^2$) decomposes the Irgacure 1173, which starts the free radical polymerization of the covalently crosslinked polyacrylamide network (Scheme 2.B.3.). The extrusion nozzle is polyethylene with a UV light-blocking additive (Nordson EFD, Westlake, OH).
Scheme 2. A) AutoCAD rendering of the adapted printer set-up, Seraph Printer files acquired as open source artifacts. B.1) Schematic representation of the two syringes with the reactive components separated to prevent early gelling. B.2) Static mixing head schematic showing that the reaction between GDL and CaCO$_3$ begins forming ionic crosslinks between the alginate chains during mixing. B.3) Schematic of the final hydrogel network with covalent crosslinks formed from UV-induced radical initiation of Irgacure 1173.

It was necessary to modify the printer control software and to develop custom code to accommodate the hardware modifications. Figure S.1 outlines the software processing procedure. Figure S.2 illustrates the fill pattern (the path of the print head) as well as a cross-sectional view of the internal structure generated by the fill pattern. Printer resolution ranged
from 780 to 1200 µm (Figure S.3.). Typical print speed and syringe fluid viscosity are also discussed in Supporting Information.

**III.4.2.4. Formulation Optimization**

In order to have appropriate viscosity immediately upon extrusion from the mixing head, it was necessary to optimize the concentration of CaCO$_3$ plus GDL because these components begin crosslinking the alginate network in the mixing head. At high concentrations, the formulation would be too viscous to be extruded. Likewise, if the concentration was too low, the resultant viscosity would be too low to create accurate geometries upon extrusion due to the increased flow compared to the crosslinking rate.

The optimized material formulation consisted of 2.5 wt% alginate, 17.5 wt% acrylamide, 15 wt% GDL plus CaCO$_3$ with respect to the weight of alginate, 0.6 wt% MBAA and 2.3 wt% Irgacure 1173 with respect to the weight of acrylamide, and the remainder deionized water. Based on the rate at which the motor depresses the syringes (0.026 cm min$^{-1}$) and the cross-sectional area of each syringe (~5.4 cm$^2$), the flow rate of material through the mixing head was determined to be 0.27 cm$^3$ min$^{-1}$ for the dual syringe system. Dividing the mixing head volume (between 2.5 and 3.0 cm$^3$, determined by filling with water) by the flow rate yielded a mixing head residence time between 9 and 10.8 minutes.

To examine the relation between the gelling time of the optimized material formulation and the residence time in the mixing head, separate gelling time experiments were performed on cast-molded gels by varying the concentration of GDL plus CaCO$_3$ with respect to the weight of alginate. Solution preparation was performed as described above, however the two solutions were combined in a conical mixing tube and vortexed, effectively mimicking the conditions of the mixing head. The mixed solution was quickly poured into new conical tubes to monitor the gel time. The tubes were flipped at 90° every minute until the solution no longer flowed.
III.4.2.5. Mechanical Testing
Cylindrical IPN hydrogel specimens were cut from a 3D printed rectangular bar (10 mm height x 10 mm width x 25 mm length) and mechanical testing was performed as described above to determine elastic modulus ($E_{\text{ramp}}, E_{\text{inf}}$) and stress relaxation.

III.4.2.6. Shape Fidelity
The shape fidelity of the printed constructs was quantified by printing a cube and a cylinder with the optimal formula. A cube of side length 20 mm was printed, then analyzed using open source image processing software called ImageJ to determine its deviance from the expected geometry. To do this, photographs of each of the six sides of the cube were taken. A ruler was included in the photographs for length scale reference in ImageJ. In ImageJ, two masks were created for each side of the cube in the expected dimensions (20 mm x 20 mm). One mask was used to calculate overfill and the other was used to calculate undershoot. Once the photographs were imported into ImageJ a length scale was set using the software’s analysis tools. A straight line was drawn on the ruler in the imported photograph and the known length was set. The software equated this length to the distance in pixels so that actual distance and area measurements could be made. From here, lines were carefully drawn around the perimeters of the printed cube faces, and the areas of the six sides were calculated. Next, the masks were applied to the images, each image was converted to binary, and the resulting overfill and undershoot areas were measured. The same procedure was followed to analyze the optimized cylinder (25 mm dia. x 20 mm height). An average of the percent deviances from expected area on each face of each construct was calculated to report the overall percent deviance.

III.5. Results
III.5.1. Cast Molded IPNs
III.5.1.1. Mechanical Properties
The effects of varying the functional groups on the polymer backbone by using different monomers (acrylamide, NIPAm, and HEA) on the elastic modulus and stress relaxation were quantified and compared (Figure 1). Alginate-pNIPAm IPN hydrogels were less stiff (6.86 ± 0.26 kPa) and maintained a moderate amount of stress relaxation (60.5 ± 8.8 %) while an alginate-pHEA IPN hydrogel was stiffer (24.9 ± 0.65 kPa) but exhibited very little stress...
relaxation (3.3 ± 1.9 %). The stiffness of the alginate-polyacrylamide IPN hydrogels (18.4 ± 2.1 kPa) fell in between these two while allowing for the most stress relaxation (74.6 ± 2.8 %) of the three. Stress relaxation is shown by a large difference between $E_{\text{ramp}}$ and $E_{\text{inf}}$, where $E_{\text{ramp}}$ is the elastic modulus during the ramp phase of the mechanical test and $E_{\text{inf}}$ is the average stress divided by strain value during the last 10 seconds of the hold phase. In general, it was observed that increasing the wt % monomer (alginate plus covalent monomer) for all formulations led to an increase in the elastic modulus while increasing the wt % MBAA decreased the percent relaxation (data not shown).

![Graph showing stress relaxation for different monomers](image)

**Figure 1.** The effect of monomer type on the elastic modulus ($E_{\text{ramp}}$) and the modulus at the end of the hold period ($E_{\text{inf}}$). The large difference between $E_{\text{ramp}}$ and $E_{\text{inf}}$ observed with NIPAm and acrylamide polymers indicates a large percent relaxation during the ramp-hold compression tests. The IPN prepared using HEA exhibited minimal stress relaxation. Percent relaxation shown above each monomer type. Error bars represent ± standard deviation.

### III.5.1.2. Swelling

For an alginate-poly(NIPAm-co-HEA) IPN, it was found that increasing the mol % of NIPAm monomer (and thus decreasing the mol % of HEA monomer) decreased the weight percent water uptake from 194.4 ± 2.5 % to 156.5 ± 3.6 % for the hydrogels swollen to equilibrium. An alginate-polyacrylamide IPN swelled to 399.7 ± 20.0 % (Figure 2.A.). Additionally, increasing the mol % of NIPAm from 20% to 50% increased the amount of stress relaxation of the swollen hydrogel from 11.4 ± 2.2 % to 24.0 ± 6.5 %. The swollen alginate-polyacrylamide IPN only relaxed 2.4 ± 0.4 % (Figure 2.B.).
Figure 2. A) Swelling profile of alginate-poly(NIPAm-co-HEA) IPNs for increasing mole percentages of NIPAm monomer in the covalently crosslinked network of the IPN and B) the stress relaxation behavior (% relaxation shown) when swollen for the same IPNs as in A). Weight percent water uptake at swollen equilibrium decreases and percent relaxation increases when the mole percent of NIPAm in the network is increased. Error bars represent ± standard deviation for measurements taken in triplicate.

III.5.2. 3D Printed IPNs

III.5.2.1. Gel Time Experiments

The gelling time of the alginate network can be controlled by varying the amount of GDL added to the reaction mixture. At low concentrations of GDL plus CaCO₃ (2%, 5%), gelation of the alginate network takes longer than 24 hours. Increasing the concentration of GDL plus CaCO₃ decreases the gel time to the minute scale. At high concentrations (25%), gelation occurs in 6 minutes, which is shorter than the residence time (~10 min) of the solution in the mixing head. A gel time of 10 minutes was achieved with a GDL plus CaCO₃ concentration of 15% (Table 1), and this concentration was selected for all of the alginate-polyacrylamide IPN material formulations used for 3D printing.
Table 1. Gel times for increasing concentrations of GDL plus CaCO₃. Concentrations are given as a weight percent to the total weight of alginate in the formulation.

<table>
<thead>
<tr>
<th>(GDL + CaCO₃) wt%</th>
<th>Gel Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>2%</td>
<td>&gt;24 hr</td>
</tr>
<tr>
<td>5%</td>
<td>&gt;24 hr</td>
</tr>
<tr>
<td>8%</td>
<td>155 min</td>
</tr>
<tr>
<td>9%</td>
<td>33 min</td>
</tr>
<tr>
<td>10%</td>
<td>25 min</td>
</tr>
<tr>
<td>15%</td>
<td>10 min</td>
</tr>
<tr>
<td>25%</td>
<td>6 min</td>
</tr>
</tbody>
</table>

III.5.2.2. Mechanical Properties

The viscoelastic stress relaxation of the 3D printed IPN hydrogels can be tuned by changing the concentration of MBAA in the solution. Decreasing the concentration of MBAA to 0.1 wt% with respect to acrylamide increases its relaxation dramatically so that it mimics the viscoelasticity of porcine muscle tissue (87 ± 2.4 %) (Fitzgerald et al., 2015) (Figure 3.A.). The elastic modulus of the 3D printed construct can be tuned through varying the total percent monomer (Figure 3.B.). At 15% monomer, the elastic modulus of the 3D printed IPN is 9.5 ± 1.6 kPa, and increasing to 23% monomer, the elastic modulus increases to 19.4 ± 1.3 kPa.

Figure 3. Unconfined compression stress relaxation tests show that (A) changing the concentration of MBAA can tune the percent of stress relaxation and (B) changing the weight percent monomer can tune the elastic modulus. Results are shown for the optimized formula, with changes only in MBAA concentration (A) or total monomer concentration (B) as shown. Results in (A) compared against porcine muscle (Fitzgerald et al., 2015). Results in (B) are compared against selected elastic moduli from
literature for skin/subcutaneous tissue (Zahouani et al., 2009), skeletal muscle (Lacourpaille et al., 2012), and cardiac muscle (Berry, 2006). Error bars indicate standard deviation for measurements in triplicate.

III.5.2.3. Shape Fidelity

3D printing of the optimized formulation (given in section 2.2.4.) into a cube shows the highest percent deviance from the expected area on the bottom face (Figure 4.A.). The high deviance on the bottom face is in excess (overshoot) of the ideal geometry, meaning there is more material present than should be. The top face of the cube shows the next highest percent deviance, however, the deviance is due to an undershoot of the expected area, or not enough material is present. The four sides of the cube show undershoot and three of the four sides show overshoot of the expected area. Likewise, 3D printing of the optimized formula into a cylinder (Figure 4.B.), shows the highest percent deviance in overshoot on the bottom face. Deviance due to undershooting the ideal geometry is displayed on both the top and the side of the printed cylinder. The top face of the cylinder also shows deviance from the expected area due to overshoot. For the optimized formulation, the technique developed in this study was able to create printed geometries with an average deviance of only 7.9% across the entire shape.

Figure 4. ImageJ shape fidelity analysis of the optimized prints of A) cube and B) cylinder shapes. Percent deviance from the expected area of each side of both shapes is shown. Overall average deviance was 7.9% from the desired geometry.
III.6. Discussion

III.6.1. Cast Molded IPNs

III.6.1.1. Mechanical Properties

Varying the functional groups of the monomers used to synthesize the covalent network of the hydrogel can impact the mechanical properties of the IPN hydrogel network. The IPN hydrogel made with NIPAm is likely less stiff than the IPN hydrogels prepared using acrylamide or HEA because the isopropyl functional group is not able to hydrogen bond with the acid groups in the alginate. As noted by Low et al., the presence of hydrogen bonds significantly impacts the elastic modulus and toughness of interpenetrating alginate-based hybrid networks. They found that changing the monomer from acrylamide to dimethylacrylamide reduced opportunities for hydrogen bonding in the network and led to a decrease in the elastic modulus of the alginate-poly(acrylamide-co-dimethylacrylamide) IPN hydrogel (Low et al., 2015). Acrylamide and HEA, which are able to hydrogen bond, resulted in IPN hydrogels with higher elastic moduli (Figure 1).

We hypothesize that the decrease in stress relaxation observed when switching covalent monomers from acrylamide to NIPAm to HEA could be due to the increase in size of their respective functional groups, making it more difficult for polymer chains to rearrange. Larger functional groups reduce chain mobility, thus limiting chain rearrangement and stress relaxation. The trends in tuning the elastic modulus and stress relaxation behavior for varying formulations of alginate-poly(NIPAm-co-HEA) IPNs were consistent with the trends described in our previous work (Fitzgerald et al., 2015) with alginate-polyacrylamide cast molded IPNs. This shows promise for the ability to eventually extend the 3D printing method developed here to the alginate-poly(NIPAm-co-HEA) IPN or similar systems.

III.6.1.2. Swelling

Using NIPAm and HEA monomers to form the covalent network of the IPN instead of polyacrylamide reduced the weight percent water uptake (swelling). Alginate-polyacrylamide IPNs almost identical in composition to those made with HEA and NIPAm monomers were found to swell to at least 400 weight percent water uptake, so all three of these formulations show less swelling. This is important because previous studies (Fitzgerald et al., 2015; Kamata et
al., 2014; Zhao, 2014) showed that mechanical properties were diminished when swollen. NIPAm and HEA are more hydrophobic than acrylamide (as discussed above), and using them in a hydrogel formulation allows for the hydrogel to absorb less water. It is understood that when a hydrogel absorbs water, the polymer chains are stretched as water moves inside its pores. Stress relaxation behavior is dependent on the ability of the polymer chains to realign themselves under the application of a stress, and stretched chains are less mobile than unstretched chains (Fitzgerald et al., 2015). For this reason, more stress relaxation is observed in the IPN hydrogels that swell the least (Figure 2). Additionally, the stiffness of the swollen hydrogel can be inferred from the peak stress, the highest value of stress for each IPN shown in Figure 2.B. Because of the way the elastic modulus is calculated, the trend in peak stress always mirrors the trend in elastic modulus. The stiffness trend among the alginate-poly(NIPAm-co-HEA) IPNs are consistent with the results shown in Figure 1. Increasing the mole percent of NIPAm (and decreasing the mole percent of HEA) causes a decrease in peak stress, which translates into a decrease in elastic modulus. The stiffness of a swollen alginate-polyacrylamide hydrogel can almost be matched by an alginate-poly(NIPAm-co-HEA) IPN in which the covalent network is composed of 25 mol% NIPAm monomer and 75 mol% HEA monomer (Figure 2.B., blue and red lines).

III.6.2. 3D Printed IPN Materials

III.6.2.1. Tunable Mechanical Properties

Compared with the baseline alginate-polyacrylamide formulation reported in our previous work for cast molded IPN gels (Fitzgerald et al., 2015), the development of the IPN formulation for use in the 3D printing process required the introduction of higher concentrations of initiator, monomer, and crosslinker to ensure sufficient gelling immediately upon extrusion to maintain shape fidelity. Previous studies of cast-molded alginate-polyacrylamide IPN hydrogels (Fitzgerald et al., 2015) indicate that increasing the monomer concentration leads to an increase in the elastic modulus, and increasing the initiator (MBAA) concentration leads to a decrease in viscoelasticity (stress relaxation behavior). These trends held true in the present work as well. Using the optimized formulation as a baseline and varying the monomer concentration from 15% to 23%, an increase in elastic modulus was observed in the range of biological tissues (Figure 3.B.), including human skin/subcutaneous tissue (8.3 ± 2.1 kPa), human skeletal muscle (9.78
kPa), and rat cardiac muscle (18 ± 2 kPa). A summary of the elastic moduli achievable by our materials is presented in Table 2. Various biological tissues whose elastic moduli match those of our materials are included for comparison. Decreasing the weight percent of MBAA from the optimal value (0.6 wt% with respect to acrylamide) in order to obtain more stress relaxation (Figure 3.A.), causes the shape fidelity of the print to decline due to fewer covalent crosslinks holding the material together.

<table>
<thead>
<tr>
<th>Tissue or IPN Type</th>
<th>Elastic Modulus, E (kPa)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroadenoma breast tissue, human</td>
<td>6.41 ± 2.86</td>
<td>(Samani et al., 2007)</td>
</tr>
<tr>
<td>Cast molded alginate-pNIPAm</td>
<td>6.86 ± 0.26</td>
<td>This work</td>
</tr>
<tr>
<td>Skin/subcutaneous tissue, human</td>
<td>8.3 ± 2.1</td>
<td>(Zahouani et al., 2009)</td>
</tr>
<tr>
<td>Thyroid gland, human</td>
<td>9.0 ± 4.0</td>
<td>(Lyshchik et al., 2005)</td>
</tr>
<tr>
<td>3D Printed alginate-polyacrylamide (15% monomer)</td>
<td>9.5 ± 1.6</td>
<td>This work</td>
</tr>
<tr>
<td>Passive skeletal muscle (vastus lateralis), human</td>
<td>9.78*</td>
<td>(Lacourpaille et al., 2012)</td>
</tr>
<tr>
<td>3D Printed alginate-polyacrylamide (17% monomer)</td>
<td>14.2 ± 1.4</td>
<td>This work</td>
</tr>
<tr>
<td>3D Printed alginate-polyacrylamide (20% monomer)</td>
<td>16.2 ± 2.5</td>
<td>This work</td>
</tr>
<tr>
<td>Passive cardiac muscle, rat</td>
<td>18 ± 2</td>
<td>(Berry, 2006)</td>
</tr>
<tr>
<td>Cast molded alginate-polyacrylamide</td>
<td>18.4 ± 2.1</td>
<td>This work</td>
</tr>
<tr>
<td>3D Printed alginate-polyacrylamide (23% monomer)</td>
<td>19.4 ± 1.3</td>
<td>This work</td>
</tr>
<tr>
<td>Liver, human</td>
<td>20</td>
<td>(Nava et al., 2008)</td>
</tr>
<tr>
<td>Normal breast tissue, human</td>
<td>22</td>
<td>(Evans et al., 2010)</td>
</tr>
<tr>
<td>Cast molded alginate-pHEA</td>
<td>24.9 ± 0.65</td>
<td>This work</td>
</tr>
</tbody>
</table>

**III.6.2.2. Gel Time**

Matching the gel time to the residence time was essential in determining the concentration of GDL plus CaCO$_3$ to use in the optimized formulation. When the solution is extruded from the mixing head it is desired to have a high viscosity in order to better maintain shape fidelity of the printed constructs. With a gel time and a residence time of 10 minutes, the calcium ions have begun to significantly crosslink the alginate network into a gel, thus increasing the viscosity of the solution upon extrusion.
III.6.2.3. Shape Fidelity
The large percent deviance from the expected area on the bottom faces of both the cube and cylinder constructs is likely due to the dripping of some of the formulation down the sides of the shape before polymerization can occur. This results in more material being on the bottom faces of the printed constructs versus their respective ideal geometries. Subsequently, this causes the large percent deviance on the top faces, because not enough material remains to achieve the ideal geometry. Overall, the 7.9% average shape deviance shows promise because previous studies using similar printing platforms with hydrogel materials have reported average deviances ranging from approximately 11 to 25% (calculated from reported shape accuracy data) (Duan et al., 2014; Hockaday et al., 2012).

III.7. Conclusion
This work describes the fabrication of complex and tunable IPN hydrogel materials possessing tissue mimetic properties. The stiffness of the hydrogels in this study range from 6 to 25 kPa, which is on the order of many biological soft tissues (Table 2). Stress relaxation, a mechanical behavior characteristic of biological soft tissues, was observed in the IPNs reported here. Further, it was found that incorporating more hydrophobic covalent network components decreased the swelling of the IPNs and increased the stress relaxation that was observed in the swollen state from only 2.4 % to about 24%.

We present an approach using a dual syringe technique for 3D printing of centimeter-scale constructs from complex, tunable IPN hydrogel materials. The technique is compatible with low cost hardware and software components, allowing for increased availability compared to higher cost printer models. It can produce tissue-mimetic structures with good shape fidelity, averaging only 7.9% deviance from the expected shape. These constructs not only have stiffness properties similar to biological soft tissue, but can also be tuned to display increased amounts of stress relaxation behavior by decreasing the concentration of covalent network crosslinker. The print speed is an additional attribute to the system as it exceeds traditional bioplotting systems and is competitive with other groups using the Fab@Home printer. The dual syringe technique reported here will allow materials with increasing complexity and mechanical relevance to be printed.
rapidly, accurately, and affordably, thus offering significant potential for advancing biomedical applications.

**III.8. Acknowledgements**

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**III.9. References**


Berry, M.F., 2006. Mesenchymal stem cell injection after myocardial infarction improves myocardial compliance. AJP Heart Circ. Physiol. 290, H2196–H2203. doi:10.1152/ajpheart.01017.2005


CHAPTER IV - Modification of a Flexible Lithium Ion Battery Separator to Enhance Sequential 3D Printability*

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*References formatted according to the National Science Foundation’s guidelines for grant proposals.
IV.1. Acknowledgements
The authors would like to acknowledge Ryan R. Kohlmeyer, Aaron J. Blake, James O. Hardin, and J. Daniel Berrigan at the Air Force Research Laboratory for initially presenting us with their difficulties in achieving sequential 3D printing of a full cell flexible battery. They provided incredible expertise and were patient with our progress. They also provided us with Figures 1 and 3 for this chapter.

IV.2. Abstract
3D printing is a popular manufacturing technique used to achieve unique product geometries. The sequential 3D printing of an entire flexible lithium ion battery, however, proves to be difficult because the inks used in the distinct battery components seep into each other and the different components of the battery are printed using the same solvent, which leads to layer dissolution. This study reports improvements to a flexible separator membrane for a lithium ion battery that could lead to eventual sequential 3D printing of the entire battery cell. A second polymer network was added to the existing separator formulation to create a semi-IPN. The improved separator membrane was found to lose less of its mass when submerged in the printing solvent, NMP, and it displayed a higher stress at failure under uniaxial tension. Additionally, the separator membrane solution, which represents the ink used to 3D print, was found to have shear thinning behavior.

IV.3. Introduction
Flexible energy storage is becoming increasingly popular for wearable electronics and other flexible devices, but typical materials used in standard lithium ion batteries are rigid and inflexible. A lithium ion battery generally consists of five parts: an anode and its metal foil current collector, a cathode and its metal foil current collector, and a separator to keep the two electrodes electrically separate. The separator consists of a liquid electrolyte component and a solid film to provide physical separation of the electrodes. The electrolyte is generally a lithium salt dissolved in a carbonate solvent. Lithium salts have the chemical formula LiXF₆, where X can be phosphorus, arsenic, or antimony [1]. Carbonate solvents are typically ethylene carbonate, ethyl methyl carbonate, diethyl carbonate, dimethyl carbonate, or a combination of two or more of these [2, 3]. The separator layer needs to permit ionic conductivity, be an electronic insulator, and provide mechanical stability [1]. Typical materials used for anode fabrication are carbon or
graphite and lithium titanate ($\text{Li}_4\text{Ti}_5\text{O}_{12}$) [4], and typical materials used for cathode fabrication are lithium metal oxides, such as lithium iron phosphate and lithium cobalt oxide. These electrode materials are not flexible, owing to the need for metal foil current collectors, which are made of copper and aluminum [4].

Recently, Blake et al. (2016) demonstrated the ability of flexible composite electrode layers to sustain electrochemical performance under mechanical abuse. They used carbon nanotube mats as current collectors for the lithium titanate anode and lithium iron phosphate cathode. Despite these recent advancements in making the anode and cathode flexible and functional, three problems still remain, all arising from the desire to sequentially print the electrodes and the separator layer. The first setback is that printing the separator layer on top of the printed electrode results in the separator ink seeping into the anode layer. This reduces porosity and the overall electrochemical performance of the cell. The second setback is that printing the second electrode layer on top of the printed separator (and bottom electrode) results in the electrode ink seeping through the separator layer and the bottom electrode. Due to this seepage, the two electrodes are in physical contact, and this electrically shorts the electrochemical cell. The third problem is that all three layers for the battery use a common solvent to print. Because all polymer components comprising the battery layers are soluble in this solvent, sequential printing could result in partial dissolution of the previous layer when printing on top of it.

To combat these problems three hypotheses were formed. To address the first problem, it was believed that by creating a more viscous separator ink with a faster crosslinking time would reduce the seeping of the separator ink into the electrode. To address the second problem, we proposed creating a semi-interpenetrating network (semi-IPN) by incorporating a second polymer network that contains crosslinks into the existing [5] separator layer. On the microscopic level, it was believed that this tighter entanglement of polymer chains would prevent the electrode ink from seeping into it. It was also hypothesized that this semi-IPN would aid in preventing layer dissolution in the common solvent owing to a tighter entanglement and crosslinking.
The semi-IPN hypothesis targeted two out of the three problems, so we proceeded down this avenue, but also kept the first hypothesis in mind. The existing separator formulation contained polyvinylidene fluoride (PVDF), alumina (Al₂O₃) nanoparticles, N-methyl-2-pyrrolidone (NMP), and glycerol, and it was desired to make as few changes to this as possible. Glycerol has three hydroxyl groups and these can crosslink with epoxides, so we chose to make the second polymer network out of glycidyl methacrylate (GMA) and methyl methacrylate (MMA) monomers, where the GMA monomer contains an epoxide. This way, the amount of crosslinking in the final separator membrane could be tuned by the amount of GMA monomer present in the second polymer.

**IV.4. Materials and Methods**

**IV.4.1. Copolymer Fabrication**
The random copolymerization of glycidyl methacrylate (GMA; TCI America) and methyl methacrylate (MMA; Acros Organics) was thermally initiated by azobisisobutyronitrile (AIBN; Aldrich) and carried out in tetrahydrofuran (THF; Fisher Scientific) or N-methyl-2-pyrrolidone (NMP; Aldrich). Monomer weights corresponding to the desired GMA to MMA mole ratio were added to a round bottom flask. 1 wt% (to monomer weight) AIBN was added. The solvent was added in a 3:1 weight ratio of solvent to monomers. The round bottom was sealed with a septum and purged with nitrogen at room temperature for 10 minutes. The flask was then submerged in an oil bath at 60 °C and allowed to polymerize overnight. If the solvent used was THF, the resulting polymer was precipitated in cold hexanes. If the solvent used was NMP, the resulting polymer was not precipitated. NMR runs indicated a conversion of nearly 100%, and from this information and the weights of monomers added, the concentration of polymer in NMP was determined in order to use it to fabricate the membranes.

**IV.4.2. Gel Permeation Chromatography (GPC)**
GPC was performed in order to determine the molecular weight (MW) and polydispersity (PD) of the GMA/MMA copolymers. For those polymerized in NMP, a small drop of the resulting polymer and NMP solution was added to a small GPC sample vial. One drop of toluene and about 2 mL of THF were added. This solution was filtered and then run on the GPC (Agilent 1260 equipped with an autosampler, a guard and two Mixed B analytical columns, and a
refractive index detector) with THF as the eluent running at 1 mL/min at 25 °C. From here, MW and PD were calculated by the software based on the baseline, peak, and flow marker of the resulting profile. For the polymers that were precipitated from THF, a small piece was re-dissolved in about 2 mL THF, and the same procedure described above was followed.

**IV.4.3. Separator Membrane Fabrication**

The weight ratio of polyvinylidene fluoride (PVDF) to the GMA/MMA copolymer was 90:10 or 75:25. The total weight of polymer (PVDF + GMA/MMA copolymer) was combined in a 5:95 weight ratio to the solvent. The solvent was a 5:95 weight ratio of glycerol to NMP. First, the GMA/MMA copolymer in NMP was weighed into a 17mm diameter glass sample vial. Next, the remaining volume of NMP was added as was the weight of glycerol. This mixture was vortex mixed (Fisher Scientific) for ~30 seconds. The PVDF was weighed and added to the sample vial. This was vortex mixed by hand in order to start the dissolving process, then the vial was clamped into the vortex mixer (Fisher Scientific, ampule tube holder) and was allowed to mix for 1-2 hours or until all PVDF was sufficiently dissolved. The mixed solution was set on the benchtop to allow the air bubbles to rise out. Alumina (Al₂O₃; Alfa Aesar) nanoparticles were added in a 70:30 weight ratio to the total weight of polymer. This mixture was again vortex mixed by hand for 1-2 minutes, and then it was clamped to the vortex mixer to mix for an hour. Once it was finished vortex mixing, it was placed in a foam float and bath sonicated for an hour. From here, the just enough of the mixture was poured into circular Teflon dishes (diameter: 63mm; Lab Depot) and allowed to sit uncovered in a 90 °C oven under nitrogen flow overnight. The dishes were removed and transferred to a vacuum oven at 120 °C and again allowed to sit overnight. From here, the membranes were removed and tests were performed.

**IV.4.4. Solvent Soaking**

Small pieces of the membranes were soaked in NMP in order to quantify how much the membrane dissolves. This was investigated in order to be able to print sequential layers that also use NMP as a solvent.
IV.4.4.1. Absorbance of Residual Solvent

A small piece of membrane was cut off and allowed to soak for 24 hours in 1 mL NMP. After 24 hours, the turbidity of the solvent was quantified with an absorbance measurement at 600 nm. Five different membrane compositions were used, including a membrane containing no GMA/MMA copolymer (only PVDF). The compositions are given in Table 1.

Table 1. Membrane compositions used in absorbance tests.

<table>
<thead>
<tr>
<th>Membrane Sample</th>
<th>GMA in GMA/MMA copolymer (mol%)</th>
<th>GMA/MMA copolymer : PVDF (wt ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>PVDF only</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>25%</td>
<td>25:75</td>
</tr>
<tr>
<td>C</td>
<td>25%</td>
<td>10:90</td>
</tr>
<tr>
<td>D</td>
<td>5%</td>
<td>25:75</td>
</tr>
<tr>
<td>E</td>
<td>5%</td>
<td>10:90</td>
</tr>
</tbody>
</table>

IV.4.4.2. Separator Membrane Mass Loss

A small piece of membrane (1 cm x 1 cm) was cut and weighed. It was then allowed to soak in 2 mL NMP for 24 hours. After 24 hours, the pieces were removed from the NMP, and placed in Teflon dishes in a 90°C oven for 3 hours. The pieces were then re weighed, and any loss in mass was attributed to dissolving in the NMP. The turbidity of the residual NMP solution was quantified with an absorbance reading at 600 nm.

IV.4.5. Tensile Mechanical Testing

Uniaxial tensile testing was performed with an Instron 3344 mechanical testing machine (Norwood, MA) using a 100 N load cell. Rectangular strips were cut from the membrane to have dimensions of 20 mm x 4 mm. 5 mm on each end of the strip was used to clamp in the Instron’s tensile grips, resulting in a gauge length of 10 mm. The thickness of each strip was measured with a micrometer at three locations along the gauge length. An average was computed to make the necessary calculations. The width of each strip was measured with a digital calipers and was also taken at three locations along the gauge length in order to compute an average. For the test, each specimen was extended at 5% strain per minute, which equates to 0.5 mm/min for a 10 mm long specimen. Each test contained a 0.01 N preload. Load, time, and extension data was
acquired by the computer every 0.1 seconds until specimen failure. Five trials for each membrane formulation were run.

**IV.4.5.1. Characteristic Average**

To account for the slight differences in strain at failure within a sample, methods for determining a characteristic average were adopted from Lessley et al [6] and are briefly described here. Stress and strain were calculated for each specimen. To account for the uptake in slack in the specimen before the preload was reached, the strain values had to be corrected. This was done by subtracting the value of strain at time zero from all strain values. The value for strain at failure was manually obtained from this data from where the stress started to decrease from its maximum value. Next, the corrected strain values were normalized as a percentage of the strain at failure for each specimen. At strain percentages 0%, 10%, 20%, through 100%, stress values were isolated. The average stress at each percentage was calculated from all specimens tested. This yielded the characteristic average stress. The characteristic average strain values were computed by calculating the average strain at failure and multiplying it by each strain percentage (0% through 100%).

**IV.4.6. Rheological Characterization**

A TA Instruments ARES-G2 Rheometer equipped with a stainless steel 20 mm flat plate geometry was used at 25 °C. A flow sweep was run on the separator membrane solution containing PVDF, GMA/MMA copolymer, NMP, Al₂O₃, and glycerol. This is representative of the ink that would be used in 3D printing the membrane. The flow sweep varied the shear rates from 0.1 to 100 s⁻¹, the step equilibration time was 30 seconds, 10 points per decade were recorded, and the gap was set to be constant at 950 μm. Three separate trials were run.

**IV.5. Results**

**IV.5.1. Molecular Weight and Polydispersity**

It was found that polymerizations carried out in NMP yielded polymers with higher MW and PD than polymerizations carried out in THF. These results are tabulated in Table 2.
Table 2. Molecular weight and polydispersity values for various polymerization conditions.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>GMA (mol%)</th>
<th>MW (g mol⁻¹)</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>THF</td>
<td>50</td>
<td>87,958</td>
<td>2.421</td>
</tr>
<tr>
<td>THF</td>
<td>25</td>
<td>80,730</td>
<td>2.297</td>
</tr>
<tr>
<td>THF</td>
<td>13</td>
<td>110,036</td>
<td>2.097</td>
</tr>
<tr>
<td>THF</td>
<td>5</td>
<td>77,969</td>
<td>2.013</td>
</tr>
<tr>
<td>NMP</td>
<td>50</td>
<td>201,434</td>
<td>3.108</td>
</tr>
<tr>
<td>NMP</td>
<td>25</td>
<td>128,984</td>
<td>3.089</td>
</tr>
<tr>
<td>NMP</td>
<td>10</td>
<td>140,591</td>
<td>2.392</td>
</tr>
<tr>
<td>NMP</td>
<td>5</td>
<td>148,568</td>
<td>2.023</td>
</tr>
</tbody>
</table>

For polymerizations carried out in NMP, increasing the mole percentage of GMA monomer making up the GMA/MMA copolymer (and thus decreasing the mole percentage of MMA monomer) led to a clear increase in PD. This is shown in Figure 1. A trend is not as clear for MW when increasing the mole percentage of GMA, but it can be inferred that a drastic increase in mol% GMA leads to an increase in MW. The MW values for mol% GMA of 5%, 10%, and 25% are all less than the MW for 50 mol% GMA at 201,434 g mol⁻¹.

Figure 1. MW and PD trends for polymerizations in NMP.
**IV.5.2. Resultant Separator Membranes**

Separator membrane formulations B through E from Table 1 were fabricated and photographed as shown below. Membrane E was the easiest to handle as it pulled up from the Teflon dish easily, was not sticky, and did not curl like the others did. They are shown in Figure 2.

![Figure 2. Resultant membranes B through E from Table 1.](image)

**IV.5.3. Dissolution**

It was found that increasing the mole percentage of GMA in the GMA/MMA copolymer component decreased the absorbance reading, meaning it did not dissolve as much. Additionally, there was not a significant difference in the absorbance values for samples B and C (both had readings of 0) or samples D (absorbance=0.1145) and E (absorbance=0.11). This indicates that the weight ratio of the GMA/MMA copolymer to PVDF does not affect the absorbance reading as much as mole percentage of GMA in the copolymer, at least at the ratios investigated here. All membrane formulations that contained the GMA/MMA copolymer displayed reduced dissolution in NMP (absorbances range from 0 to 0.1145) compared to a PVDF only membrane (absorbance=0.578). Figure 3 displays these results and gives a visual of the turbidity of the NMP for each membrane formulation.
Figure 3. Absorbances quantifying solvent turbidity for varying membrane compositions.

Further investigation of increasing the mole percentage of GMA in the copolymer found that in addition to decreasing the absorbance readings (Figure 4.A.), the percent mass lost from the membrane also decreased (Figure 4.B.). Compared to the membrane containing only PVDF, which lost almost 40% of its mass, all membranes containing the second network resulted in losses of less than 6% of their original weights.
Figure 4. Membrane dissolution in NMP as a function of mole percentage of GMA in the second network quantified by A) absorbance readings and B) percentage of weight lost from the original separator membrane piece.

From the results discussed thus far, it was determined that separator membrane sample E was the best formulation because it dissolved less than the PVDF only membrane and it was the easiest to handle. The remaining tests (mechanical, rheology) were carried out on only sample E for this reason.
IV.5.4. Stress-Strain Profiles

Mechanical test results show that separator membrane sample E had a higher stress at failure (3.69 ± 0.28 MPa) than a membrane made with only PVDF (3.61 ± 0.27 MPa), but that it could not endure as much strain at failure. The PVDF-only membrane strained to 22.88 ± 3.64% whereas sample E only strained to 8.79 ± 1.72%.

![Stress-strain profile for uniaxial tensile tests on membrane formulation E and a membrane containing only PVDF. Error bars represent one standard deviation for five measurements.](image)

**Figure 5.** Stress-strain profile for uniaxial tensile tests on membrane formulation E and a membrane containing only PVDF. Error bars represent one standard deviation for five measurements.

IV.5.5. Viscosity as a Function of Shear Rate

Rheological characterization of separator membrane sample E showed that the viscosity of the membrane ink decreased with an increasing shear rate. This behavior is known as shear thinning, and is shown in Figure 6.
IV.6. Discussion

IV.6.1. Molecular Weight and Polydispersity
Molecular weights for polymerizations carried out in NMP are likely larger because THF is more prone to chain transfer, and this results in smaller polymer chains. The increase in molecular weight that is seen with a drastic increase in mole percentage GMA in the copolymer could be due to impurities (likely water) in the polymerization solvent (NMP). The water could open up the epoxide ring on the GMA monomer, and this could lead to crosslinking with other growing polymer chains via the open epoxide ring, thus increasing the molecular weight. As discussed above, a high molecular weight polymer was desired for this application, in order to address one of the setbacks encountered in 3D printing. Additionally, carrying out the polymerization in NMP reduced the amount of time necessary to fabricate the membranes. It no longer needed to be precipitated immediately post polymerization, and then it did not need to be re-dissolved in NMP to make the membrane.

IV.6.2. Compatibility with NMP
The soaking and dissolution tests were important to perform in order to determine whether 3D printing of the electrode layers and the separator membrane could be carried out sequentially. The electrode layers also use NMP as the solvent, so it is possible that printing on top of the
separator with NMP could result in the dissolution of the separator layer. This study found that increasing the mole percentage of GMA reduces the amount of dissolution in NMP and this can be attributed to the increased number of crosslinking sites present in the polymer. Additionally, incorporating a second polymer network, regardless of its composition, was found to decrease the membranes’ dissolution in NMP.

**IV.6.3. Mechanical Properties**

The incorporation of a crosslinked polymer network into the already existing PVDF polymer matrix caused an increase in stress at failure but a decrease in strain at failure. Crosslinking a polymer makes it stiffer [7], as demonstrated by the increase in the slope of the stress-strain curve before yield in Figure 5. Crosslinking also makes a polymer more brittle [8], resulting in failure at smaller deformations.

**IV.6.4. Rheological Properties**

The shear thinning behavior exhibited by formulation E is desirable for eventual 3D printing purposes [9]. The results shown here are on the order of what we previously found for the existing formulation [5]. At any given shear rate, it appears that the viscosity values reported here were slightly less than what was reported by Blake, et al. This is expected because the higher molecular weight PVDF (1.1x10⁶ g mol⁻¹) component was partially replaced by a smaller molecular weight GMA/MMA copolymer. A smaller molecular weight results in a less viscous solution, thus giving smaller viscosity values at all shear rates.

**IV.7. Conclusion**

The goal of this work was to improve upon the shortcomings of an existing separator layer for a flexible lithium ion battery so that sequential 3D printing of the entire battery cell could be achieved. The shortcomings were focused on the seepage of electrode ink into the separator layer and the potential for dissolution of sequentially printed layers printed using the same solvent. A semi-IPN was created to combat these, and it was found that the new membrane formulation lost as little as 1.25% of its mass when exposed to NMP for 24 hours, compared with the original formulation which lost almost 40% of its mass. As expected, mechanical testing of the membrane showed improved stress at failure but a decreased strain at failure compared to the
original membrane formulation. Though the focus of this work was not the actual 3D printing of the separator membrane, rheological characterization demonstrated shear thinning behavior which is necessary for 3D printing applications.

The next step in this work is electrochemical characterization. Preliminary electrochemical cycling of the membranes proved to be promising, but further characterization and 3D printing experiments are needed to explore the suitability of this approach for 3D printed flexible batteries.

IV.8. References


CHAPTER V - Conclusions & Future Work

Katherine Bootsma
V.1. Conclusions
The goal of this research was to develop 3D printable IPN materials for medical simulation and flexible lithium ion battery applications. A biomimetic IPN hydrogel composed of alginate and polyacrylamide was used as a basis for the formulation that was eventually 3D printed. The composition of the covalently crosslinked network was altered to control the adverse swelling behavior observed when the hydrogels were placed in water for storage purposes, but ultimately it was desired to 3D print the formulation that displayed the most stress relaxation. This led to the use of an alginate-polyacrylamide IPN hydrogel formulation for 3D printing and characterization. From here, the formulation was chemically altered so that 3D printing with good shape fidelity could be achieved. The resultant 3D printed hydrogels were tuned to mimic the elastic and viscoelastic behavior of numerous biological tissues. Finally, shape fidelity of the 3D printed hydrogels was calculated to be competitive with reported values for similar soft materials.

It was found that using a semi-IPN instead of a single polymer in the separator membrane of a flexible lithium ion battery improved upon the shortcomings encountered in sequential 3D printing of the full battery cell. The use of the IPN reduced separator membrane dissolution in the common solvent, NMP, increased the stress at failure of the membrane, and the ink demonstrated shear thinning behavior.

V.2. Future Work
There exist a few avenues of future work for the 3D printed IPN hydrogels. Acrylamide and bisacrylamide monomers are toxic. If polymerization does not run to completion, handling the 3D printed structures could be unsafe. Additionally, if it is ever desired to grow cells on the structures or to include cells in the ink, the covalently crosslinked network would need to be replaced by a nontoxic alternative. Another consideration in future work could be the further reduction of the observed swelling behavior which diminishes the tuned mechanical properties. It may be necessary, however, to invent other methods for storing the IPNs if their use in medical simulation devices is to be realized.
As stated in CHAPTER IV, additional electrochemical characterization tests need to be carried out on the full cell battery assembled with the improved separator membrane. It is necessary to determine if the battery can cycle with the new separator membrane before 3D printing experiments are run. If those tests are promising, then sequential 3D printing can be carried out, and these full cells can be cycled in order to assess electrochemical performance.
APPENDIX I - Supporting Information for CHAPTER III°

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http://dx.doi.org/10.1016/j.jmbbm.2016.07.020

°References are formatted as required by the \textit{Journal of the Mechanical Behavior of Biomedical Materials}.
**AI.1. Printer Software Workflow**

![Flow diagram showing the software processing that was performed. Programs are boxed, and file-types are indicated over the arrows, and manual processing is denoted M.P.](image)

Figure S.1. Flow diagram showing the software processing that was performed. Programs are boxed, and file-types are indicated over the arrows, and manual processing is denoted M.P.

Three-dimensional geometries, such as those obtained from computer aided design (CAD) software can be assembled and used to generate Standard Tessellation Language (.stl) files, which provide control over scaling and printable geometry. The .stl files are imported to the program Slic3r (developed by Alessandro Ranellucci, GNU Affero General Public License, version 3), which generates print paths as a string of $x$, $y$, and $z$ coordinates based on the print parameters that are specified by the user from knowledge of the print material’s properties. Several new variables are introduced into the coding with the SeraphPrint software and are set in Slic3r using similar parameters. The effects of these variables on the printed result have been previously studied (Hockaday et al., 2012).

**AI.2. Fill Pattern**

![Slic3r software allows for the path used by the printer to be set. A) View of the print path on the z-axis. B) A side view of the print path when a cross-sectional cut is made.](image)

Figure S.2. Slic3r software allows for the path used by the printer to be set. A) View of the print path on the z-axis. B) A side view of the print path when a cross-sectional cut is made.

Print parameters available for manipulation in Slic3r include path height, path width, fill pattern, and fill density, among others. The fill pattern determines the way in which the material is laid down during printing. A rectilinear fill pattern was used for this material (Figure S.2.A).
Consecutive layers of the same pattern are extruded on top of one another while alternating starting points allowing for the consecutive layer patterns to be perpendicular to each other. A cross sectional cut of the layers gives a view of the internal structure of the printed object (Figure S.2.B).

**AI.3. Print Resolution**

Differences in the single-line print resolution for changing values of area constant (AC) were investigated via image analysis (Fig. S.3). AC is a Seraph-defined parameter that relates the depressor distance traveled to the volume that is extruded by the syringes. To quantify printer resolution for our IPN material, single line prints were deposited on a glass cover slip and allowed to remain under the UV light for approximately 30 seconds after printing for completion of polymerization. Images were obtained using a light microscope and hemocytometer grid, and line widths were quantified in ImageJ. Nozzle width was 0.41 mm for all trials. Results show that the AC printer parameter strongly impacts single-line print resolution, with optimal performance at AC=0.185 yielding a line width of 0.78 ± 0.04 mm.

**Figure S.3.** Variation in printed strand width for increasing values of Seraph parameter AC.

**AI.4. Print Speed**

Using the dual syringe method reported here for alginate-polyacrylamide IPN hydrogels, simple geometries were 3D printed with a high degree of accuracy (average of 7.9% deviance from
desired geometry) at a print speed of 258 mm$^3$min$^{-1}$, so that a 2 cm x 2 cm x 2 cm cube can be printed in 31 minutes. This is relatively fast, as compared to other reported IPN 3D printing methods (Bakarich et al., 2013) that may require up to 90 seconds of UV light exposure in between each layer.

**AI.5. Syringe Fluid Viscosity**

For the dual syringe method, the viscosity of the syringe fluids can be relatively low compared to other printing platforms. This set up allows for a single motor to push both syringes while maintaining control of gel formation. The ability to print with relatively low viscosity fluids (~3,000 cP) using the dual syringe method avoids the need for expensive pneumatic drivers and allows the use of more affordable printer platforms for rapid IPN hydrogel model fabrication.

**AI.6. Opacity**

Opacity of the 3D printed IPN gels was investigated as a function of the initiator concentration. An increase in opacity was observed with higher concentrations of the Irgacure 1173, making it a chemically tunable parameter (Figure S.4.). Since Irgacure 1173 is only sparingly soluble in water, this may be due to highly crosslinked or water insoluble domains formed within the network. The additional layers required for the larger constructs cause even the 2.3 wt% concentration, as in the optimal formulation, to appear completely opaque (Figure S.4).

Figure S.4. Opacity can be controlled by changing the concentration of Irgacure 1173 as shown by 5-layered prints and the gradient created by printing with varying amounts of initiator.
**AI.7. References**
