COMPRESSIVE MECHANICS OF A POLY( VINYL ALCOHOL)-BASED HYDROGEL SYSTEM FOR THE REPLACEMENT OF THE KNEE MENISCUS

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ABSTRACT

Osteoarthritis and cartilage deterioration are favored by meniscetomy, which is the ablation of the meniscus from the knee joint. Meniscectomy can be partial or total. This procedure is performed when meniscus lesions and tears or the degeneration of the meniscus caused by its natural dehydration occur. There is a peak of meniscal lesions observed between 20 and 29 years old. Alternative methods such as sutures fail in that they present a short term solution which is ideal for a less active, older generation. A long term solution is needed for a younger population to reduce the number of procedures over the lifetime of this active group. There is a crucial need for a functional implant designed in the image of the native meniscus.

Blends of poly (vinyl alcohol) PVA and poly (vinyl pyrrolidone) (PVP) present a potential solution. PVA has shown similar characteristics to soft tissues. PVP further stabilizes the hydrogel network. This work is the mechanical characterization of PVA/PVP (99:1) hydrogels under physiological conditions. Equilibrium swelling in a medium replicating the ionic and the osmotic content of the synovial fluid was investigated during 35 days. The mass retention of hydrogels was characterized using data obtained from the swelling study and was examined as a function of the cross link density and the polymer content. The modulus of hydrogels was obtained in unconfined compression, first at a strain rate slow enough to ignore fluid flow in and out of the gels, and subsequently at a physiological strain rate of walking.

Results indicate that PVA/PVP hydrogels volume swelling ratio and weight swelling ratio show no significant difference for most formulations by the 14th day of immersion. A few hydrogels would reach equilibrium by day 21. Additionally,
percentage polymer mass retention increases with the cross link density. However, there is no consistent trend with the polymer content. All formulations with 10\% wt of polymer show the highest mass retention while 15\% wt show the lowest. Interestingly, the mechanical characterization of hydrogels at 100\%/min strain rate shows that 15\% wt is the only formulation whose compressive modulus falls within the targeted range whereas 10\% wt proves to not be stiff enough. 20\% wt and 25\% wt are always too stiff. Results obtained from unconfined compression at the physiological strain rate, that is 1920\%/min, are rather inconclusive. There is not enough consistency in the literature to narrow the results down to one successful candidate formulation.

The modulus range obtained at physiological strain rate encompasses the range obtained at 100\%/min strain rate. The highest modulus value obtained is 10 times higher at physiological strain rate than the modulus of a real human meniscus obtained at 100\%/min strain rate. It is not reasonable at this time to make a choice of a formulation at physiological strain rate due to highly varying modulus of a human meniscus as a result of its intrinsic anisotropy. All formulations tested would be considered successful candidates, which is irrational considering the difference in their stiffness.
ACKNOWLEDGMENTS

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CHAPTER 1
PROJECT SUMMARY AND SPECIFIC AIMS

In the United States, meniscal lesions are the primary reason for surgical procedures undertaken by orthopedic surgeons [1]. In a year, 66 per 100,000 inhabitants on average suffer meniscal lesions among which 61 result in meniscectomy [2]. Injury to the meniscus can occur rapidly from athletic efforts or gradually from daily activities as a result of its nonuniform loading. Rapid meniscal injuries are caused by twisting, hyperextension or other actions of great force [3].

The anatomical, biomechanical and functional importance of the meniscus has been recognized and established. The meniscus’s main functions are: redistributing the load across the knee, from the femur to the tibia, stabilizing the motion around the knee, and absorbing shocks [4]. It was not until the 1950s that it was discovered that total meniscectomy, which is the excision of the meniscus from the knee joint, favored articular cartilage deterioration in the knee and the expansion of osteoarthritis [5]. Meniscectomy reduces the contact area on the tibial plateau thus increasing the average stresses resulting from axial loading [6]. Novel treatments have shifted from meniscectomy to partial excision to repair. Two main surgical solutions have been proposed: menisci allograft transplantation after total meniscectomy or partial meniscectomy replacement with collagen scaffolds. Meniscus allograft can survive two to seven years [7]. Efforts to regrow the entire meniscus after complete meniscectomy have failed in animal models [8]. Wound infections or failures of allograft healing other than at the periphery of the meniscus are recurrent complications in humans [9]. Artificial meniscal replacement seems to be the best solution for patients who choose to not live with pain.
A. Lowman and other researchers have suggested the development of a biomaterial made of synthetic polymers whose mechanical properties can be tailored to match those of the native meniscus [10]. Ideally, this meniscal implant should fulfill these requirements: satisfactory compressive and tensile properties, biocompatibility, and translational aspect from the bench top to the bedside. With appropriate selection of materials with established biocompatibility and the potential for adequate mechanical function, we can focus the aims on this project on investigating and optimizing mechanical performance.

Hydrogels, which are three-dimensional hydrophilic polymer networks highly swollen in water, are a potential solution to the failing previous approaches. Their material properties can be tailored to match those of native tissue by varying polymer composition, cross linking density and network morphology [11]. The objective of this project is to characterize the mechanical and material properties of a hydrogel made of poly (vinyl-alcohol) (PVA) and poly (vinyl-pyrrolidone) (PVP). This hydrogel is biocompatible, non-degradable and physically cross-linked through freeze-thaw cycles, thus forming cryogel [12]. It is necessary to evaluate its load-bearing ability under various loading modes such as tension, compression or shear as the meniscus is subject to heterogeneous loading. The cross-linking density and the change in water content of hydrogel in environmental conditions matching the knee joint must be accounted for as well. The final result would be to provide a substitute that can replace a damaged meniscus and restore the biomechanical function of the knee.

To achieve these goals the following specific aims were proposed:
Specific aim 1: Characterize the swelling behavior in an osmotically controlled environment, thus replicating in vivo conditions and determine when candidate hydrogels reach equilibrium.

Hydrogels samples were immersed in osmotic solution to replicate the physiological environment. Their volume swelling ratio was statistically analyzed throughout the duration of the swelling study to define when equilibrium swelling is reached.

Specific Aim 2: Characterize the % mass retention throughout the length of the swelling study.

The percentage of total polymer mass retention was computed from the theoretical dry mass before swelling and the dry mass after swelling, once equilibrium was reached. Mass retention was characterized with respect to polymer content and cross linking density.

Specific Aim 3: Characterize the structure-property relationship of the hydrogel system during unconfined compression at 100%/min strain rate and at physiological strain rate.

Cylindrical hydrogel samples were compressed to 15% strain during static unconfined testing at a 100%/min strain rate and at physiological strain rate. Moduli values were compared with respect to the strain rate and to other moduli values of real menisci.
CHAPTER 2

PROJECT BACKGROUND

2.1 The Meniscus

2.1.1 Anatomy

The menisci, one lateral and one medial, are located between the femur and the tibia. The lateral menisci are about 32.4-35.7 mm in length and 26.6-29.3 mm wide, whereas the medial menisci are 40.5-45.5 mm in length and 27 mm wide [12]. Both menisci have a semi-lunar shape. Lateral menisci tend to vary greatly in shape and thickness and cover a larger portion of the tibial plateau (75 % to 93%) than the medial menisci (51-74%) [13]. They are glossy white tissue with a complex arrangement of fibers and appear smooth at the surface. A meniscus is composed of cells and an extracellular matrix (ECM). The menisci are held in place by ligaments: the medial collateral ligament, the transverse ligament, the meniscofemoral ligaments, and other attachments. Only 46% of people have all of these ligaments. However, 100% of people have at least one of them. Until birth the menisci are entirely vascularized. As the body evolves, the vascularization becomes partial. At about 10 years, only 10-30% of the menisci are vascularized and by maturity, the innervation is reduced to 10-25% in the periphery of the tissue [13]. For this reason there are three distinct parts of the meniscus: the red-red zone that is vascularized, the white-white zone that is avascular and the red-white zone that lies in between [14]. The less innervated a region the more its regeneration ability is limited to selfheal after tears.
2.1.2 Biochemical Content and Meniscus Cells

The meniscus contains 72% water by weight and 28% organic matter (ECM and cells) [15]. The organic matter comprises of collagen (75%), glycoaminoglycan (GAG)(17%), adhesion glycoproteins and elastin [16]. The biochemical content of the meniscus varies with age and preexisting conditions [17]. In the periphery of a mature meniscus, cells have an oval, fusiform shape, and the ECM is mostly made of collagen type I. Within the meniscus, cells tend to have a round shape and are embedded in an ECM largely composed of collagen type II and GAGs. Generally, near the surface, the meniscus is like fibrocartilage and, within the tissue, like articular cartilage [18]. Figure 2 displays the fiber arrangement in the meniscus.
2.1.3 Biomechanical And Functional Properties

The meniscus is responsible for many functions: load bearing and transmission, shock absorption, and lubrication [4]. During daily activities, axial tibiofemoral forces compress on the menisci. These vertical forces are then converted into horizontal hoop stresses. Simultaneously, there are shear forces between the collagen fibers in the menisci and the ECM [17]. The meniscus can withstand axial compression up to 100-150 KPa in aggregate modulus [19]. There is tension radially and circumferentially. Moduli are respectively 10-30MPa and 100-300 MPa. Within the meniscus the shear modulus can reach up to 120 KPa [20].

Load transmission heavily depends on the menisci health and the contact area between the menisci and the tibial plateau. When the knee is at 90° flexion the axial force is 85% greater than when there is no flexion at all.
2.2 Current Methods Of Treatment

Subsequent to the discovery of the degenerative effects of total or subtotal meniscectomy on articular cartilage, solutions have been geared towards total replacement or meniscal repair, including sutures and arthroscopic methods.

2.2.1 Meniscal Repair

Often times, surgeons choose preserving the meniscus instead of meniscectomy, in case of a meniscal tear. Meniscal repair methods are either traditional sutures or arthroscopic techniques. The all-inside technique has gained popularity because of decreased risk of neurovascular complications associated with traditional suture techniques [25]. A limiting factor to appreciate the success rate of meniscal repair is the need for a second arthroscopy because no conclusion can be made based on the absence of symptoms only. Furthermore, the success rates although positive, tend to be subjective, and highly dependent on the evaluation method. Another common consequence is aseptic synovitis, which is a reaction to a foreign body. This is usually followed by swelling, caused by synovial fluid collection [26].

2.2.2 Tissue Engineering For Total Replacement

Tissue engineering aims at either reproducing a tissue identical to the native tissue or just developing a tissue that satisfies the functions of the original tissue. This is possible using a scaffold and cells.

*Autologous cells*

Designing an implant using autologous cells involves two surgical procedures: a biopsy to collect autologous meniscal cells and a surgery to implant the engineered meniscus. However, there are several limitations. Techniques used nowadays only allow
the collection of a limited number of cells [27]. Moreover, only the cells that produce enough GAGs are of interest. There is advancement in the development of tissue engineered cells but several limitations persist. It is still impossible to get a significant number of cells at once. Some of these cells may dedifferentiate or may already be degenerated or not healthy for other reasons. For this reason other cells sources have been considered [27].

**Stem cells**

There has been an increasing interest in stem cells to regenerate degenerated menisci. Stem cells have the ability to differentiate into the host’s cells, to produce cytokines and growth factors. Human Embryonic stem cells (hESCs) are pluripotent and differentiate indefinitely, which solves the problem of isolation of cells. Hoben et al. monitored the differentiation of hESCs into fibrochondrocytes-like cells for 3 weeks. Results showed the production of collagen type I, II and III and GAGs [28]. However, hESCs present an ethical challenge in the scientific community.

Adults’ stem cells also known as mesenchymal stem cells (MSCs) are also multipotent and can be found in the adult bone marrow. These can differentiate into several terminal cells than can synthesize mesenchymal tissue and thus mesenchymal secreted tissue. Also MSCs, secrete immune regulatory cells which are useful for healing lesions [29].

**Scaffolds**

Scaffolds for the tissue engineering of menisci prosthesis can be categorized into 4 groups: synthetic polymers, ECM components and tissue-derived materials. Hydrogels will be addressed thoroughly later in this discussion, as they are the subject of this
project. Synthetic polymers present several advantages such as the possibility to vary the pore size, the fiber diameter and length, the geometry, and the mechanical properties [11]. The hydrogel system that is the subject of this study is made of synthetic polymer.

**Scaffoldless implants**

Recently, the need for a scaffold has been substituted by cell seeding in very high density, thus encouraging cell-cell adhesion, cell-matrix adhesion, and cell-cell signaling. The main advantage for a scaffoldless implant made of autologous cells would be no immune response as there would be no foreign body, greater integration within the body, and excellent bioactivity. Additionally, a scaffoldless implant will not generate degradation products that might be toxic. Although these implants are scaffoldless, agarose hydrogel serve as a mold to prevent cell-substrate adhesion. However, these molds interfere with required mechanical properties for a successful knee meniscus implant [30].

**Hydrogels**

Hydrogels are usually made of polymer and water. The polymers can either be synthetic or natural. Their properties heavily depend on their water content. They can be physically or chemically crosslinked via several methods. Hydrogels and their materials properties will change with respect to their environment. Temperature, pH, electric field, ultrasound or salt concentration can all affect their swellability [11]. For this reason, hydrogels are often referred to as smart biomaterials because they can be tailored to take a specific form in a specific environment.

In this project, we use a polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) blend that is mixed with dionized water. PVP improves the chemical stability of
the PVA/PVP blend through the hydrogen bonding [31]. The polymer content and the water content vary. The hydrogel is later reinforced with fibers, in a following study. Hydrogels are physically cross-linked through freeze-thaw cycles.
CHAPTER 3

PROPOSAL

The objective of this project is to synthesize a physically cross-linked hydrogel made of PVA and PVP, characterize its mass retention throughout the duration of the swelling study with respect to the cross link density and the polymer content and characterize its resistance to compressive loading at a 100%/min and at physiological strain rate of walking. The physiological strain rate is calculated from the duration of the single-leg stance of a healthy adult.

Polyvinyl alcohol is produced by polymerization of vinyl acetate to polyvinyl acetate (PVAc) followed by hydrolysis of PVAc to PVA [32]. This reaction is always uncomplete and as a result PVA is always a blend of PVA and PVAc. The hydrophilicity and the crystallinity of PVA are affected by their acetate group content [33]. Highly hydrolysed PVA (above 90%) needs to be heated above 80°C to dissolve in water [34]. PVA can be crosslinked chemically using glutaraldehyde, acetaldehyde and other monoaldehydes. However, chemical crosslinking is not ideal for biomedical applications because of the toxic residual monomers and the costly process to remove these [35]. PVA can also be physically crosslinked using γ-irradiation or freeze thaw cycling to name few methods. γ-irradiation causes bubbles that do not allow for control of the mechanical properties of hydrogels [36].

PVA is a semicrystalline polymer with a layered structure [37]. Hydrogen bonds hold double layers together. These double layers in turn are held together by Van der Waals [38]. The melting temperature of PVA is between 220 and 240°C. This range is
due to the variation of crystallite size and the hydrogen bonds in the crystalline regions [39]. PVA glass-transition temperature is approximately at 80°C [40].

PVA has been thoroughly studied and used for biomedical applications. It is non-toxic and non-carcinogenic. Its biocompatibility has led several researchers to investigate its properties. Oka et al. found that PVA mechanical properties are most similar to those of the articular cartilage which justifies its use for the design of a hydrogel for meniscal replacement [41]. Also a PVA implant does not cause nearly as much damage as aluminum and titanium that are used in other body implant because of its compliance and its viscoelastic properties that are comparable to those of human’s tissue by adjusting the water content.

Poly (vinyl pyrrolidone) (PVP) is a highly amorphous polymer which glass-transition is around 150°C [42]. PVP is synthesized through polymerization of the monomer N-vinyl-2-pyrrolidone and is highly hydrophilic due to the hydrogen bonds between its carbonyl group and the water molecules. It is often used to improve the dissolution rates of hydrophobic drugs [43]. PVP is non-carcinogenic, non-toxic and pharmacologically inert.

The hydrogel system designed here is of a polymer blend. It is usually cost effective to mix polymers rather than investigate the development of a new monomer or study a new polymerization method. Not all polymers can blend. Their miscibility is measured by the free energy of mixing. To obtain a homogenous blend, in which each polymer loses some of its independent properties, the free energy of mixing has to be negative. See equation [3.1]

\[ \Delta G_{mix} = \Delta H_{mix} - T \cdot \Delta S_{mix} \]  

[3.1]
ΔG is the free energy of mixing, ΔH is the enthalpy change of mixing, T is the
climate and ΔS is the entropy change of mixing.

PVA and PVP are miscible because of the hydrogen bonding between the PVA
hydroxyl group and the PVP carbonyl group. The miscibility of two polymers is
confirmed by a single glass-transition temperature that is between the glass-transition
temperatures of the polymers used. Cassu and other researchers have reported 1 single
glass-transition temperature for this blend [42]. PVP decreases the degree of crystallinity
in PVA with up to no crystallinity with more than 60% wt PVP as reported by Nishio et
al [44]. Also, the melting temperature of the blend of PVA, a semi crystalline polymer
and PVP, an amorphous polymer is depressed because of the presence of the amorphous
polymer. Liu et al. have studied the stability of PVA/PVP blends [31].
CHAPTER 4

EXPERIMENTAL: MATERIALS SYNTHESIS AND TESTING METHODS

4.1 Hydrogel synthesis

To synthesize the hydrogel system many formulations are blended: 10% wt, 15% wt, 20% wt, and 25% wt. PVA (99 % hydrolized from Sigma Aldrich, 89,000-98,000 g/mol) and PVP (from Sigma Aldrich 40,000 g/mol) are blended with a ratio of 99:1 in this study. These polymeric solutions are autoclaved for 2 hours at 121° C. Weight proportions of each reagent are indicated in the Table 1.

Table 1.Formulations for 100-125 ml of hydrogel composites

<table>
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<tr>
<th>Formulation</th>
<th>PVA (g)</th>
<th>PVP (g)</th>
<th>Water (ml)</th>
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<tr>
<td>10%</td>
<td>7.9244</td>
<td>0.0838</td>
<td>72</td>
</tr>
<tr>
<td>15%</td>
<td>11.8823</td>
<td>0.1259</td>
<td>76</td>
</tr>
<tr>
<td>20%</td>
<td>15.8428</td>
<td>0.1765</td>
<td>64</td>
</tr>
<tr>
<td>25%</td>
<td>23.7612</td>
<td>0.2489</td>
<td>72</td>
</tr>
</tbody>
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4.2 Physical Cross-Linking

When the PVA/PVP/H₂O blend is exposed to a temperature below 0°C, water freezes and drives PVA/PVP out. Separate zones of water and of PVA/PVP blend are formed. The PVA chains then form crystallites via hydrogen bonding with each other and these crystals persist through thawing. This process is called freeze-thaw cycling and it is a method of physical cross linking which is ideal for prosthesis as it requires no additional monomer that may be leaked in the body. The crystallinity [45], the polymer phase separation [46] and the hydrogen bonding [47] increase with the number of freeze
thaw cycles. Holloway et al. reported no significant increase in crystallinity beyond 6 freeze-thaw cycles [10].

Following 2 hours in the autoclave oven, PVA/PVP is allowed to cool and release pressure. The blend is then poured into cylindrical test tubes (Becton Dickinson, 15 mm by 120 mm) which are frozen for 21 hours at –20 °C and thawed for 3 hours at 25° C for a complete cycle of 24 hours, up to 6 freeze-thaw cycles.

4.3 In Vitro Swelling Analysis

Hydrogels are often characterized by their degree of swelling, which is the amount of space inside the hydrogel network available for water or any other solvent to occupy. There are three forces that contribute to expand a hydrogel network during swelling: polymer-water interactions, electrostatic, and osmosis. Hydrogels are able to swell and not dissolve because cross-links hold the network together [11].

This step is necessary to mimic the in vivo behavior of the PVA/PVP hydrogel implant as mechanical properties of the hydrogel are highly dependent on its water content. Prior to using osmotic solutions, most in vitro studies have used Phosphate Buffer Saline (PBS). While PBS may replicate the electrolyte content of the synovial fluid it does not mimic its swelling pressure, which is the driving force behind hydrogel swelling and deswelling. Solution based on macromolecules can permanently replicate the osmotic pressure encountered in the knee. Previous studies have been done to observe the swelling behavior of cartilage using solutions made of macromolecules [48]. Yet, few have investigated the impact of these osmotic solutions on biomaterials. Spiller et al. studied the influence of osmotic solution at 0.95 atm versus a PBS solution with an osmotic pressure of 0 atm on a 10 wt % PVA hydrogel at 6FT [49]. This study used
hydrogels in cartilage defects *ex vivo* as the control. It was noted that hydrogel samples behaved similarly at 0.95 atm and in the cartilage defects *ex vivo* yet, differently at 0 atm in PBS. Subsequently, Holloway et al. pursued this study for a wider range of hydrogels (10% wt, 20% wt, 30% wt and 35% wt) with varying polymer weight content and number of freeze thaw cycles (2, 4, 6,8,10) [10].

Osmotic solution (0.95 atm) is prepared separately using polyethyleneglycol (PEG) (20,000 g/mol) and saline at 0.15M prepared with sodium chloride (NaCl) and deionized water. This is prepared following the equation below:

\[
\pi = RT \left( \frac{c_2}{M_2} + B c_2^2 + C c_2^3 + \cdots \right) \tag{4.1}
\]

where \(M_2\) is the polymer molecular weight, \(R\) is the universal gas constant, and \(T\) is the absolute temperature [48]. \(B\) which is equal to \(2.59 \times 10^{-3}\) and \(C\) which is \(13.5 \times 10^{-3}\) are viral coefficients for 20,000 g mol\(^{-1}\) PEG. For 1L of saline at 0.15M, 94.04g PEG is used.

Thermo resistant jars are filled with 1 L each of osmotic solution. Dialysis tubing, with a 3500 g mol\(^{-1}\) molecular weight cut off (Fisher Scientific, Pittsburgh, PA), containing the gels are inserted in these jars. The dialysis tubing is used to prevent PEG uptake by hydrogels. Each dialysis tubing contains hydrogels cylindrical samples with approximately 7.0 mm thickness. The fluid pressure outside the bags can drive water into or out of the hydrogel samples to attain equilibrium. Finally, the jars are seated in a water chamber at 37 \(^\circ\) C for the duration of the swelling study. In this project, swelling studies span over 5 weeks or 35 days.
4.4 Hydrogel Characteristics

In this study we investigate the volume swelling ratio of the hydrogel to determine when equilibrium is attained. This is necessary to determine when the implant is stable with no significant variation in water content as its mechanical properties highly depend on it. For this purpose, the weight swelling ratio, which is the difference between the swollen mass and the relaxed mass divided by the relaxed mass, is a measure of the percentage of water gained/lost by the hydrogel compared to its relaxed mass, before immersion [11]. See equation [4.2]

\[ Q_w = \text{weight swelling ratio} = \frac{w_s}{w_r} - 1 \quad [4.2] \]

Swollen hydrogel samples are weighed in air and in heptane. These numbers are used to calculate the density of the swollen samples following equation [4.3]. The density of heptane is 0.69 g/ml.

\[ d_2 = \text{density of swollen hydrogel} = \frac{\rho_{\text{heptane}} \cdot w_{\text{sinair}}}{w_{\text{sinair}} - w_{\text{sinheptane}}} \quad [4.3] \]

The weight fraction of the polymer \( w_2 \) and the weight fraction of the water \( w_1 \) are then computed using equation 4.4 and 4.5.

\[ w_2 = \text{weight fraction of polymer} = \frac{1}{1 + Q_w} \quad [4.4] \]

\[ w_1 = \text{weight fraction of water} = 1 - w_2 \quad [4.5] \]

Finally the volume fraction of the polymer is calculated using equation [4.6] and the volume swelling ratio which is the inverse of the volume fraction is deduced. See equation [4.7]. The density of water is 0.997g/ml.

\[ \phi = \text{volume fraction of polymer} = \frac{\frac{w_2}{d_2}}{\frac{w_2}{d_2} + \frac{w_1}{d_1}} \quad [4.6] \]
\[ Q_v = \text{volume swelling ratio} = \phi^{-1} \]  

4.5 Compressive Modulus at 100%/min strain rate

The goal of this aim is to identify the hydrogel formulation for which the instantaneous modulus falls within 0.1-0.15 MPa at equilibrium when compressed to 15\% of its original strain. All hydrogel samples are compressed to 15\% strain of their original strain. All compression testing is performed with a testing machine Instron Materials Testing System Series 4442 (Norwood, MA). A 50N load cell is used to test cylindrical specimen in unconfined static compression.

Testing is conducted immediately after the gel has been taken out of the swelling jars and weighed. A complete compression test consists of loading and unloading. However, only data recorded during loading are used to compute the modulus. The modulus is calculated as the derivative at 15\% strain of a 2\textsuperscript{nd} degree polynomial. The strain rate is 100\% per min and all samples are compressed to 15\% of their original strain. Holloway et al. found there was no correlation between the strain rate from 10\% to 100\% and the Young’s modulus [10]. Load and displacement are recorded every 0.1 s for the duration of the test. The tare load is -0.01 N for all compressive testing. The engineering axial strain is calculated as the variation in extension divided by the original thickness. Due to the fact that it is a compressive test the new axial length is always subtracted from the original axial length. See equations [4.8] and [4.9].

\[ \sigma_i = \frac{-F_i}{\pi r^2} \]  

\[ r = \text{cylindrical sample radius}; F_i = \text{load} \]
Hydrogels are viscoelastic in their nature and do not deform linearly. For this reason, a polynomial of order 2 was used to fit the stress vs strain curve. All samples used were deemed satisfactory for $R^2 > 0.99$. The derivative of this polynomial was calculated for the strain at 15% from which the Young’s modulus was obtained. See equation [4.10]. Each Young’s modulus was calculated as an average of 4 values. Some formulations have 3 values instead. Standard deviation is indicated.

$$\sigma = y = ax^2 + bx + c; x = \varepsilon; \frac{d\sigma}{d\varepsilon} = \frac{dy}{dx} = 2ax + b$$  \[4.10\]

The compressive modulus of a viscoelastic material varies significantly with the strain at which it is extended as well as the strain rate. Here the strain rate was held constant, 100% strain per min. The goal of this aim is to identify what formulation falls within the 0.1 MPa-0.15MPa range at equilibrium at 15% strain. Data from hydrogels that have reached equilibrium will be used for obtaining reasonable results.

### 4.6 Compressive Modulus at physiological strain rate

At 100%/min strain rate, there is only one realistic situation illustrated, which is standing. It is necessary to investigate the compressive modulus at the physiological strain rate of walking to observe the change in stiffness of the modulus as compared with standing at equilibrium. It is also crucial to compare the modulus obtained at physiological strain rate to values of real human meniscus subjected to similar testing conditions. Hydrogels samples will be tested at the average strain rate illustrating a single
leg stance, which is 32%/s or 1920%/min strain rate. Data from hydrogels that have reached equilibrium will be used for obtaining reasonable results.

4.7 Statistical Analysis

Samples tested at 100%/min strain rate at 2 freeze thaw-cycles are n=4. The number of samples was reduced to n=3 for 4 and 6 freeze-thaw cycles later in the study for ease of processing. There are 3 samples (n=3) for each calculated mean and standard deviation at physiological strain rate. The results presented here are compared statistically using one way Analysis of Variance (ANOVA) with 95% confidence level.
CHAPTER 5

RESULTS

5.1 Swelling Characterization

Two separate swelling studies of 35 days each were used to establish swelling equilibrium of candidate hydrogels in osmotic solution. The volume swelling ratio was calculated for the first swelling study and the weight swelling ratio was calculated for the second swelling study, as previously described. Each ratio shows no significant change when equilibrium swelling is reached. ANOVA results for swelling characterization were followed by a Tukey test to establish the time point at which equilibrium is reached.

Each hydrogel sample is used once and weighed 3 times: before the swelling study in its relaxed state, immediately after the study in its swollen state (in air and in heptane) and after drying in an oven for at least 14 consecutive days in its dry state. Each value contributes to computing the weight and volume swelling ratios.

The formulations studied in this work are 10% wt, 15% wt, 20% wt and 25% wt. This percentage represents the polymer content. The swelling equilibrium of each formulation is established individually. The objective is to determine the time point at which all hydrogels irrespective of their polymer content and cross linking density have reached swelling equilibrium. Figure 5.1 shows the volume swelling ratio of a selected formulation from the first swelling study and Figure 5.2 shows the weight swelling ratio of a selected formulation from the second swelling study as a function of time of immersion.

According to the Tukey test from either swelling studies most gels have reached equilibrium by day 14. However there are instances when a hydrogel does not reach
equilibrium until day 21 such as 15% wt at 2 freeze-thaw cycles from the first welling study. Additionally, there are some hydrogels samples that never seem to reach equilibrium such as 20% wt at 2 freeze thaw cycles from the first study. However, during the second swelling study equilibrium is reached for this formulation by day 14.

From the second swelling study, 25% wt at 2 freeze thaw cycles and 15% wt and 25% wt at 6 freeze-thaw cycles never seem to reach equilibrium. However, from the preceding study, these formulations all reach equilibrium by day 7. This suggests that there may have been exceptional circumstances that caused instability of these gels during immersion. It is safe to say that all formulations have reached equilibrium by day 21, most of them reaching equilibrium by day 14.

![Figure 5.1](image_url)

**Figure 5.1:** Volume swelling ratio as a function of time of immersion in osmotic solution. Data is reported as mean + SD (n=3). This figure represents all formulations at four freeze-thaw cycles during the first swelling study. Blends with higher polymer content deswelled to a smaller extent as compared to blend with lower polymer content, thus higher water content.
Figure 5.2: Weight swelling ratio as a function of time of immersion in osmotic solution. Data is reported as mean + SD (n=3). This figure represents all formulations at four freeze-thaw cycles during the second swelling study. Blends with higher polymer content deswelled to a smaller extent as compared to blends with lower polymer content, thus higher water content.

5.2 Mass Retention Of Hydrogels Characterization

Mass retention is calculated to evaluate the amount of polymer loss or retained during the swelling study. Ideally, there should be no polymer loss to maintain the integrity of the hydrogels and the expected mechanical properties. The mass retention is characterized with respect to the cross link density and the polymer content. The mass retention analysis is presented in Figures 5 and 6 as the results of immersion *in vitro* over 35 days at 37°C. These figures illustrate the mass retention of hydrogels at a time point when equilibrium swelling has been reached.
5.2.1 Mass retention Characterization with respect to Cross Link Density

The retention is expected to increase with the number of freeze thaw cycles because the more cross links are present in the hydrogel network, the less the hydrogel is likely to dissolve. The mass retention for 10 wt % at all freeze thaw cycles is above 100%. This suggests that there may be ion or PEG uptake during the time of immersion.

Figure 5.3: Mass Retention as a function of the cross link density (freeze-thaw cycles). Data is reported as mean + SD (n=3). The percent mass retention significantly improves (p<0.05) with the increasing number of freeze thaw cycles. This trend is also observed at day 28 and day 35 for the same formulation. Figures are available in appendices.

5.2.2 Mass retention Characterization with respect to Polymer Content

Figure 5.4 shows the variation of mass retention with the increasing polymer content. The sample chosen is at 4 freeze-thaw cycles from the second swelling study
because all formulations reach equilibrium. It is not possible to assume that a higher polymer mass content will absolutely result in a higher mass retention.

**Figure 5.4:** Mass Retention as a function of the total polymer. Data is reported as mean + SD (n=3). 10 wt % shows the highest mass retention while 15 wt% shows the lowest mass retention. This trend is also observed for day 28 and day 35. 20 wt % and 25wt % are always in between. Figures are available in appendices.

### 5.3 Mechanical Evaluation Of The Compressive Modulus At 100%/ Min And At Physiological Strain Rate

#### 5.3.1 Compressive Modulus at 100%/min strain rate

The goal of the study is to identify a hydrogel system that can behave like the meniscus once it is at equilibrium. Most studies publish the modulus of the meniscus obtained in tension and in compression at a strain rate slow enough to ignore flow of synovial fluid in and out of the tested sample. This is experimental set-up is commonly referred to as equilibrium. For this reason candidate hydrogels were tested at 100%/ min strain rate to compare results to published values. In a previous work, samples have been
tested at 10%/min strain rate and it was shown that there is no significant difference observed in the compressive modulus between 10%/min and 100%/min, yet it takes 1/10 of the time to the run the test at 100%/min than it originally did. The aggregate modulus of the meniscus is within the range of 0.1 to 0.15 MPa [19]. In this aim, the compressive modulus obtained at a slow strain rate is compared to the reported aggregate modulus.

![Figure 5.5: Compressive modulus of hydrogel formulations (10 wt %, 15 wt%, 20 wt% and 25 wt%) at 2, 4 and 6 freeze-thaw cycles tested at 15% strain and 100%/min strain rate. Data is reported as mean + SD (n=3). 15 wt % at any freeze thaw cycle falls within the targeted range. While all 10 wt % samples at equilibrium seem to never be stiff enough, all samples at 15 wt % seem to fall within the targeted range of 0.1 to 0.15 MPa. 20 wt % and 25 wt% are always too stiff.](image-url)
Often times the modulus of the meniscus is reported without any indication of the strain and the strain rate used to obtain it. It is assumed that the strain is 12% to 15% and that the strain rate is slow enough. We have previously shown that the modulus is strain-dependent and strain rate-dependent for a viscoelastic material so it is crucial to understand how the modulus was obtained to be able to compare values and draw a conclusion. Despite the fact that 25 wt% and 20 wt % formulations seem to have a stiffer modulus than required for a meniscal implant, all tests were done at 100% strain rate which is much slower than a realistic strain rate. So it is impossible to dismiss these formulations at this time.

Each complete compression test lasts 16 to 19 s at a strain rate of 100% per min, which is about 10 s to fully compress the stiffest hydrogel sample to 15% of its strain. This is not a realistic representation of the average walking rate. For this reason, the strain rate will be increased to reduce the loading portion of the test cycle to the average duration of a single-leg stance (0.38s) [50].

Consequently, hydrogel samples will be compressed at physiological strain rate and moduli will be compared to reported values of human meniscus obtained at physiological strain rate as well, in unconfined compression. The values obtained will be compared to reported compressive moduli of human meniscus or articular cartilage obtained at a physiological strain rate. The formulation with the least significant difference in modulus with a human meniscus will be identified as a good candidate for the meniscus prosthesis.
5.3.2 Compressive Modulus at Physiological Strain Rate

Candidate hydrogels were tested at the physiological strain rate of walking in unconfined compression testing up to 15% peak strain. Each compression lasted by 0.5 to 0.55 s. Most samples were compressed at 12% strain by 0.4 s. The objective is to compare experimental values to published values of the knee meniscus at physiological strain rate.

Results show that at 3%, 6%, 9% and 12% strain, at all freeze thaw cycles, 25wt % has the highest modulus followed by 20 wt %. There is an exception at 12% strain for 20 wt % at four freeze thaw cycles where the compressive modulus is higher than 25 wt %. 15 wt % is less stiff than 20 and 25 wt % yet stiffer than 10 wt % at all strains but only at four and six freeze thaw cycles. At two freeze thaw cycles, 10 wt % is stiffer than 15 wt % at all strains. Generally the compressive modulus increases with the polymer content. The same trend is observed with the mechanical analysis at 100%/min.

At physiological strain rate the modulus is expected to be higher than at 100%/min strain rate.
Figure 5.6: Compressive modulus obtained at 15% strain and at physiological strain rate on day 21. Data is reported as mean + SD (n=3). All hydrogel specimen have reached equilibrium. 10 wt % is generally the least stiff formulation while 20 and 25 wt % are the stiffer formulations.
Figure 5.7: Compressive modulus obtained at 15% strain and at physiological strain rate on day 28. Data is reported as mean + SD (n=3). All hydrogel specimen have reached equilibrium. 10 wt % is generally the least stiff formulation while 20 and 25 wt % are the stiffer formulations.
CHAPTER 6
DISCUSSION

6.1 Equilibrium Swelling

Swelling studies are necessary when working with hydrogels. In previous studies, the swelling medium has only replicated the electrolyte content of the synovial fluid. In this work, extra efforts were made to replicate the ionic and the osmotic contents of a realistic swelling medium surrounding the meniscus of the knee. PEG contributed to provide an osmotic solution at 0.95 atm, which is the average swelling pressure in the knee. Interestingly, few samples never reached equilibrium as their volume swelling ratio or weight swelling ratio never reached a range within which values became insignificantly different. The majority of hydrogels samples reached equilibrium by the 14\textsuperscript{th} day regardless of the formulation. It is possible that some gels never reached equilibrium because of the lack of homogeneity in the gel formulation. It is almost impossible to homogeneously mix these gel solutions after they have been autoclaved, especially those with higher polymer content because they are very viscous.

Every mean is based on 3 hydrogel samples. However for 10\% wt during the first swelling study, n=4. To have more statistically significant results it may be necessary in the future to obtain data from more hydrogels samples such that despite removing an outlier, data will remain statistically significant. Dialysis tubing with a larger flat width will ease the processing for swelling studies.
6.2 Mass Retention Analysis

The limitations that impact the swelling ratio will inevitably impact the mass retention as well. Generally, the mass retention increases with the cross link density. This was foreseeable because the more cross links there are the less monomers are likely to leach from the hydrogel network.

The mass retention does not show a similar trend with and increasing polymer content however. 10% wt always shows the highest mass retention regardless of the crosslink density whereas 15% wt always shows the lowest mass retention. 20% wt and 25% wt are always in between. The absence of a trend makes it difficult to give a reasonable explanation for the aforementioned observations. It is certain that there is ionic and PEG uptake by each of these gels samples. However, it is impossible to explain why the formulation with the lowest polymer content and the highest water content has the highest mass retention overall.

6.3 Mechanical Analysis

It is crucial to understand how soft tissues are tested and modeled to eventually compare candidate hydrogels menisci values to published values. Soft tissues such as the meniscus or articular cartilage are inherently anisotropic. Additionally, their integrity is significantly altered by the preservation method between harvesting and preconditioning for testing. It has been shown that beyond 96 hours post-mortem, rabbit ligaments show a significant difference in their tensile modulus [51]. Soft tissues can be preserved by freezing at -20°C or by cryopreservation at -80°C. It has also been shown that storage at 4°C in culture medium for 28 days or less does not affect material properties [51]. Soft
tissue can also be preserved in saline at 40⁰C and significant differences in materials properties are not seen until after 40 days [52].

Prior to testing, soft tissues samples need to be preconditioned to relieve the soft tissues samples from the effects of storage. Preconditioning methods vary and may affect samples differently. Often times this step is described superficially.

The environmental conditions in which the soft tissues samples are tested matter just as much. Ideally, tissue should be tested at 37⁰C in a fluid that can replicate the osmotic and the ionic content of the synovial fluid. In this work we do the mechanical testing in air. This is possible because the duration of one test is not long enough so that the absence of synovial fluid will make a significant difference in the materials properties. The swelling studies, however, are done in osmotic solutions that replicate the swelling pressure and the ionic content of the synovial fluid.

There are various techniques used to cut specimen that will be tested and these too, have an impact on the materials properties of the soft tissue to be tested. Soft tissues are generally heterogeneous and to obtain consistent samples it is necessary to cut thin samples to limit variation among samples. However, thin samples cannot realistically replicate the fiber content of the soft tissue and thus, moduli values may be overestimated. Cutting samples accurately is crucial because the size of the specimen matters. Finally, the design of the protocol used for testing the samples can also cause some variation in results. Soft tissues material properties depend on their loading history, time of experiment and strain rate. This represents a major challenge because protocols vary significantly from one researcher to another and it becomes difficult to compare results among themselves and design prosthesis at the image of the native meniscus. As
part of a parameter of the testing protocol, the tare load can induce a lot of variation in results obtained. This load is usually introduced to make sure that samples to be compressed are indeed in contact with the plunger. However, the contact can never be perfect because the sample will have to be perfectly flat which is impossible. This issue is also encountered with synthetic samples. It is also important to observe how the tare load compares to the error limits of the apparatus.

Hydrogel candidate samples were tested in unconfined compression at 32%/s calculated from a compression up to 12% strain in 0.38s. The physiological strain rate is 32%/s. This protocol followed the format of a study with real human medial menisci by Chia et al [50].

Chia, H et al [50] studied the compressive moduli of human medial menisci obtained from cadaveric knees ranging from 23 to 57 years with a mean of 40.4 years. There is no indication of the standard deviation of the age of cadaveric knees. Unconfined compressive testing was conducted at physiological strain rate, which is 32%/s here. Samples were cut as 2 mm cubes. The capacity of the load cell used was 10N. The peak strain was 12% and the modulus was calculated as the derivative of Fung’s exponential model. Moduli were obtained at 3%, 6%, 9% and 12%. The modulus values varied from 0.041 MPa at 3% to 1.130 MPa at 12%. Standard deviation were, in most samples, as high as the mean and in some cases higher. It was concluded that the material properties of the meniscus are highly dependent of the activity of interest: standing, running or walking. Chia, H L et al. obtained data that show standard deviation higher than the mean for most samples and for this reason cannot serve as a basis to
determine which hydrogel formulation is the best candidate. Several other studies were observed.

Denewth et al. [53] studied the compressive mechanics of human articular cartilage in the tibial plateau at physiological strain rate. The physiological strain rate was defined as 100%/s strain rate for cylindrical samples of 4 mm diameter with a varying thickness of 0.9 mm to 4 mm. These specimen were obtained from non-osteoarthritic and non-osteoporotic human-tibial plateau from eight females. The age varied from 18 to 55 years with a mean of 49 years and a standard deviation of plus or minus 4 years. The tangent modulus was calculated at 10% strain and the peak strain was 20%. The modulus obtained varied from 1.0 to 80.0 MPa. There is no indication of the capacity of the load cell yet the sensitivity is mentioned to at 120mV/N. It was concluded that articular cartilage response to physiological strain rate is non-uniform and region specific.

Leslie et al. [54] claim to have tested human meniscus at physiological relevant levels yet perform all experiments at 0.5 mm/min on rectangular samples with varying thickness from 0.89 to 2.69 mm. The menisci were obtained from human cadaveric knees varying from 52 to 89 years old with an average of 71 years. Fung’s model was used to fit data and calculate the Young’s modulus which varies from 19 MPa in the axial direction at 20% strain to 299 MPa at 80 % strain. Menisci were tested at physiologically relevant levels of load that vary between 0-1500N. Standard deviation for parameter A was as high as the value of the parameter at times yet small for parameter B in the Fung’s model.

These studies show that unconfined compression testing has been the standard for testing soft tissues. However, the physiological strain rate is a term that seems to be
randomly used and there is no consistency among researchers. The term physiological strain rate should be standardized as well as the expression physiological relevant level. There should be a universal protocol written to test soft tissues with a clear indication on the mean of the samples of human menisci to be used, the capacity of the load cell, the peak strain and most importantly the model and the method used to calculate the compressive modulus.
CHAPTER 7

CONCLUSIONS

PVA/PVP hydrogels can potentially become the long term solution for the replacement of the knee meniscus. PVA has been widely studied, is biocompatible and shows a good wear resistance. PVP is pharmacologically inert and acts as a great stabilizer for the PVA/PVP hydrogel system. It takes 14 days for these hydrogels to reach equilibrium and be stable in their environment, and this represents a reasonable amount of time for a patient to start walking after surgery and regain mobility. There is essentially very little polymer mass loss and for most gels, there is polymer mass gain instead. This can be attributed to ion uptake or PEG uptake. In this work, the integrity of the dialysis tubing, that is the membrane that separates the gels from the osmotic solution, has not been tested. It is likely that this membrane disintegrates and lets the higher molecular weight PEG through. Furthermore, there is no indication on the durability of this membrane in the product’s specifications. The proportions of PEG and ion responsible for the polymer mass gain should be investigated to validate or deny the hypothesis of the degeneration of the dialysis tubing.

The need to test candidate hydrogels at 32%/s strain rate arose from the fact that at 100%/min strain rate there is only one physiological situation that is illustrated, which is standing. However there exists at least 1 other situation: walking. Therefore, it was deemed necessary to investigate the compressive modulus of hydrogels at physiological strain rate of walking. The values obtained for the modulus at a physiological strain rate vary from 0.04 MPa to 1.36 MPa. This range is based on the compressive modulus obtained at 15% for all the samples that have reached equilibrium regardless of the cross
linking density and the polymer content. This range encompasses the modulus range found at equilibrium at 100%/min, 0.04 MPa to 1.04 MPa. Hydrogels samples tested at physiological strain rate are 10 times stiffer than the targeted range of 0.1-0.15 MPa at 100%/min strain rate.

The mechanical analysis was conclusive at 100%/min strain rate and 15% wt has been chosen as the best candidate formulation for the purpose of meniscal replacement. It is possible to select one formulation at this strain rate because the targeted range is small enough and generally agreed upon. However, the same analysis performed at physiological strain rate is not conclusive. The modulus range for the human meniscus at physiological strain rate is rather broad such that all formulations tested here will be a good fit. Real human menisci moduli values range from 0.3 to 1.13 MPa at 12% strain. In this work, hydrogels moduli range from 0.04 to 0.985 MPa at the same strain rate. However it is not reasonable to arrive at any conclusion because at this time standard deviation values for real human menisci are as high as mean values. At this stage, one formulation cannot be selected based on studies done at physiological strain rate due to inconsistency in testing methods.

There is a crucial need for more studies that will follow a standard for testing soft tissues such that results obtained can be reasonably compared and synthetic implants can be designed in the image of the native meniscus. Selecting one candidate formulation from results obtained at physiological strain rate is not feasible at this time because the range of the human meniscus modulus is wide enough for all formulations to be elected as successful candidates. A similar trend is observed with articular cartilage tested at physiological strain rate. The modulus varies greatly from one region to the other due to
the intrinsic anisotropy of the meniscus and it is not reasonable to select a single formulation for further processing. Instead, a single formulation should be matched to a specific region of the meniscus and a successful implant will be a patchwork of hydrogels formulations with varying polymers content.
CHAPTER 8
RECOMMENDATIONS FOR FUTURE RESEARCH

8.1 Hydrogels Processing

The mass retention of hydrogels calculated on the basis of their theoretical polymer mass prior to immersion and experimental dry mass after immersion is higher than 100% for several samples. It is possible that during immersion, the dialysis tubing made of cellulose may be degrading thus letting in higher molecular weight PEG. There is no information provided on the duration of the integrity of the dialysis tubing. As a consequence, it is possible that there may be some low molecular weight PEG or sodium chloride ions that travel through the nanopores of the dialysis tubing and stick on the gels. In the future, the permeability of the dialysis tubing should be tested for the duration of the swelling study.

8.2 Load Cell Capacity and Sensitivity

Several hydrogel formulations were used as candidates for the meniscus prosthesis. Their modulus vary significantly with the cross linking density and the polymer content. A 50 N load cell is used for all samples whereas some samples test below 1 N, which is below 2% of the capacity of the load cell. In the future, a smaller load cell of 10 N or 5 N should be used for formulations with a lower cross link density and polymer content while the 50 N load cell should be reserved for formulations with higher polymer content and cross linking density.
8.3 Standardization of testing procedures for human menisci

The objective of this work was to determine a hydrogel candidate formulation for the replacement of the knee meniscus. A compressive modulus was obtained at a slow enough strain rate to be compared to the standard aggregate modulus range for a knee meniscus. Additionally, a compressive modulus was obtained at physiological strain rate of walking to be compared to published values. There were several limitations encountered overall. Generally, testing methods are described superficially and it becomes challenging to repeat these experiments for candidate prosthesis and to compare data. It is necessary for the future that testing methods for soft tissues are standardized and this includes post-mortem preservation, preconditioning, specimen machining and testing protocol. Additionally, it is crucial that the physiological or clinical relevance of the testing protocol be clearly defined. Physiological conditions vary from immobility to walking to running and so does the strain rate, which greatly impacts the results obtained for the tensile or compressive modulus. Only then will it be possible to reasonably compare results between studies and to develop a realistic functional prosthesis based on standardized and widely accepted values of the meniscus modulus at various strain rates.

8.4 Alternative until standardization

Considering that it may take several years and a significant amount of discussion among researchers to agree on a standard protocol to process and test soft tissues, specifically the meniscus, a researcher can individually test post mortem menisci and follow the same protocol for a candidate hydrogel prosthesis. For this reason, menisci should be harvested in great amount to obtain statistically meaningful data. Additionally
menisci should be obtained from a population within the same age range. As humans get older, the menisci dehydrate and naturally degenerate despite a seemingly healthy state. Therefore, it is recommended that tensile and compressive modulus of real human menisci be obtained prior to developing meniscus prosthesis, following an identical testing protocol.
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