**Computational and Experimental Comparison on the Effects of Flow-Induced
Compression on the Permeability of Collagen Gels**

A Thesis

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Abstract

Collagen is a fibrous material and is ubiquitous throughout the human body. It is a biphasic material, consisting of a solid fibrous matrix and interstitial fluid. Collagen is one of the primary components within the extracellular matrix, which plays a vital role in the physiological, mechanical, and transport functions of various systems and processes of the body. Understanding the mechanical and transport properties of collagen can help us understand the roles and processes of the extracellular matrix. Additionally, understanding these properties may lead to more rational design choices in tissue engineering, where the permeability of the biomaterial in tissue engineering is critical for the transport of nutrients. ***The underlying goal of this study is to experimentally determine and to develop a finite element model showing the effect of concentration and compression of collagen gels on permeability.***

In this study, two methods to determine the permeability of collagen gels was developed. With both methods, the interstitial fluid of collagen gels was expelled under a pressure load, resulting in compressed collagen gels that were denser than the starting gels. The permeability of collagen gels was determined using Darcy's Law. In the vertical apparatus, a changing height of fluid pushed water through and compressed a collagen sample. In the horizontal apparatus, a syringe pump delivered water through three collagen concentrations (1.98 mg/mL, 3.5 mg/mL, 5 mg/mL) at a constant volumetric flow rate of 0.85 ml/min. Instantaneous permeability values were obtained at various points of compression and fitted to the α and M values of the strain-dependent Holmes-Mow permeability model where α and M are defined as intrinsic permeability parameters. A finite element model was developed to model the biphasic compression of the collagen gels using FEBio. A neo-Hookean material was used to model the solid matrix and Young's modulus was changed to match the degree of compression.

The vertical apparatus found a higher permeability compared to the horizontal apparatus. The vertical apparatus showed a permeability of $2.37 \times 10^{-11} \pm 2.4 \times 10^{-11} \text{ m}^2$. The initial permeability doubled as the collagen went from a starting concentration of 5 mg/mL to a starting concentration of 1.98 mg/mL. Each concentration compressed to a final concentration of about 12 mg/mL, resulting in no dependence on starting concentration for the permeability of compressed samples. The M values of the Holmes-Mow model increased from 2.4 to 5 with increasing concentration, while the α value decreased from 1.3 to 0.5 with increasing concentration. The Young's modulus found by the finite element model increased from 200 to 3700 Pa with increasing initial collagen concentration. The Young's modulus determined in the current study was similar to the short-time modulus of other published works.

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List of Abbreviations

Abbreviations	Description
A	Cross-sectional area
a	Fibril radius
b	External body force per mass
b^w	External body force per mass acting on the fluid
ECM	Extracellular matrix
FEBio	Finite Elements for Biomechanics
g	Gravitational acceleration constant
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
I	Identity matrix
ID	Inner diameter
L	Sample Length
M	Holmes-Mow exponential strain-dependence coefficient
NaOH	Sodium Hydroxide
p	Fluid pressure
p_d^w	Momentum exchange between the solid and the fluid
Q	Volumetric flow rate
R	Resistance
t	Time
TM	Trademark
V	Volume
v^s	Solid matrix velocity
w	fluid flux relative to the solid matrix
α	Holmes-Mow power-law exponent
Δh	Change in height
ΔP	Pressure difference
κ	Permeability
κ_0	Initial permeability
μ	Fluid viscosity
ρ	Fluid density
ρ^w	Apparent fluid density
ρ_T^w	True fluid density
σ	Cauchy stress
σ^c	Effective stress due to deformation
ϕ	Solid volume fraction
ϕ_0	Initial solid volume fraction
ϕ^w	Solid matrix porosity

Chapter 1: Introduction

Cells reside in the extra-cellular matrix (ECM), which provides mechanical support, determines mechanical properties, and transfers signals across the cells [1]. The specific composition of the ECM is highly dependent upon tissue type, which determines its resistance to fluid flow. Specifically, fibrous materials in the ECM create high resistance to fluid flow [2]. Collagen is one such fibrous material that is ubiquitous in the ECM, accounting for more than two-thirds of the protein content found in many soft tissues, as shown in Figure 1 [1]. Fluid flow through the ECM is called interstitial fluid flow, which helps the transport of nutrients and wastes between cells [3], helps lymphatic regeneration [4], and affects the production rates of vascular smooth muscle cells [5]. Understanding the roles and processes of interstitial fluid flow will aid in drug delivery and can be used in the advancement of tissue engineering, where the permeability of these tissue equivalents impacts the transport of nutrients through the tissues. Determining the permeability of collagen is one of the first steps necessary towards understanding the biotransport properties of interstitial flow.

Figure 1. A cell is shown in green, with common ECM components surrounding the cell and where collagen is represented by yellow cylinders. This visualizes the prevalence of collagen within the ECM and why understanding fluid flow through collagen is vital towards understanding interstitial flow [1].

Numerous computational methods estimate the permeability of collagen (e.g. Scheidegger [6]), as well as a few experimental methods. Some studies have fit the permeability under finite deformation to the Holmes-Mow model including one study that analyzed the permeability of bovine articular cartilage [7] and another study that analyzed the permeability of human medial collateral ligament [8]. However, purified collagen is most readily available in the form of collagen soft gels, which have a vastly different microstructure.

Collagen gels are a biphasic material, composed of an insoluble network of fibers and an interstitial fluid. The lattice structure created by the fiber network makes collagen soft gels an attractive scaffold for tissue engineering [9]–[11]. The properties of collagen gels are dictated by the interactions between the movement of the interstitial fluid and the collagen fibers. Many studies have analyzed changes within the interstitial fluid and how these changes impact the overall properties of collagen. For instance, one study showed that under a compressive load, the interstitial fluid is expelled from the biphasic material, changing the mechanical properties of the collagen [12]. Another study analyzed the strain-induced alignment of collagen fibers [13]. A study by Yang, et al., showed that Young's modulus increases as the concentration of collagen gels increases [14]. Knapp et al., showed that collagen displays an aggregate modulus over long periods when the collagen can relax under a constant strain, and a much higher short-time modulus when the collagen does not have time to relax [15]. In looking at the transport properties of collagen gels, Serpooshan, et al. examined the permeability of collagen gel scaffolds under self-compression but did not account for external fluid forces [16]. Ramanujan et al. showed the permeability of collagen gels is inversely proportional to the concentration of collagen but did not account for any compression of collagen gels [17]. To our knowledge, there are no studies that combine computational and experimental studies of the transport properties of collagen gels under flow-induced compression.

The overall objective of this study is to experimentally determine the permeability of collagen gels as a function of compression, to evaluate the effect of concentration on permeability, and to develop a computational method to model the collagen under a compressive load. In this study, the permeability of collagen soft gels undergoing fluid-induced compression is modeled using the Holmes-Mow strain-dependent permeability model. A computational model is then developed to model this compression using FEBio, a free finite element software developed for the biomechanics community. To account for the biphasic nature of collagen gels consisting of a solid matrix and an interstitial fluid, a finite element model is often used with simplified models. The collagen solid is often modeled using a Neo-Hookean material [18]–[20]. The experimental data can then be compared with the Neo-Hookean material in the finite element modeling software, FEBio, and to evaluate the material’s capabilities in estimating the collagen gel’s transport properties.

Chapter 2: Experimental Characterization and Computational Modeling of Collagen Gel Permeability

2.1 Vertical Apparatus

2.1.1 Collagen Preparation

1.98 mg/mL of collagen type I was made using: 660 μ L 3 mg/mL collagen I rat tail (ThermoFischer Scientific, Waltham, MA), 204 μ L 1M HEPES buffer (Sigma-Aldrich, St. Louis, MO), 26 μ L 1M NaOH (Sigma-Aldrich, St. Louis, MO), and 10 μ L 200 mM L-Glutamine (Life Technologies, Carlsbad, CA). After mixing, the solution is incubated at 37°C for 30 minutes to allow the gel to set.

2.1.2 Theory

Darcy's Law (eq. 1) is commonly used to describe low-velocity fluid flow through a porous medium. It is used to describe the flow of groundwater through sediment and for fluid flow through a filter. Since collagen is a porous polymeric material that is permeable to fluid flow, collagen is often modeled using Darcy's Law where the volumetric flow rate (Q) is proportional to the permeability of the sample (κ), the cross-sectional area of the sample (A), fluid viscosity (μ), length of the sample (L), and the pressure difference across the sample (ΔP). This equation can be written in terms of an overall hydraulic resistance (R):

$$Q = -\frac{\kappa A}{\mu L} \Delta P = -\frac{1}{R} \Delta P \quad \text{Eq. (1)}$$

2.1.3 Apparatus

To test the permeability of collagen, a vertical apparatus was developed (Figure 2). This apparatus consisted of a 30" long, 1/2" ID PEX tubing that was marked vertically every 5 cm. A separate piece of PEX tubing with the same diameter held the sample at the bottom of the apparatus. Collagen was prepared in this smaller PEX tubing and a piece of Scotch-Brite™ was rolled up and placed below the collagen to hold it in place. With the valve closed and the sample in place, the PEX tubing was filled with water to the top to create a pressure difference. The valve

was opened, and the height of the fluid (Δh) was measured over time (t) as the water permeated the sample.

Figure 2. Vertical apparatus used for testing. Full length of the PEX tubing is not shown for clarity purposes

The volumetric flow rate, Q , can be rewritten in terms of the volume of water in the tube, V (eq. 2):

$$Q = \frac{dV}{dt} \quad \text{Eq. (2)}$$

The volume of the tube is the product of the cross-sectional area, A , and the height of the fluid. Since the cross-sectional area of the tube is constant, equation 2 can be rewritten as:

$$\frac{dV}{dt} = A \frac{d(\Delta h)}{dt} \quad \text{Eq. (3)}$$

Combining equation 1 and equation 3 yields a manipulated form of Darcy's Law, which can be used to find the hydraulic resistance of the sample, R :

$$A \frac{d(\Delta h)}{dt} = -\frac{1}{R} \Delta P \quad \text{Eq. (4)}$$

Since the pressure difference is due to the height of fluid above the sample, equation 4 can be rewritten in terms of the density of water, ρ , the gravitational acceleration constant, g , and the change in height:

$$A \frac{d(\Delta h)}{dt} = -\frac{1}{R} \rho g \Delta h \quad \text{Eq. (5)}$$

Solving this differential equation yields:

$$\Delta h = \Delta h_0 \exp\left(-\frac{\rho g t}{RA}\right) \rightarrow \ln(\Delta h) = -\frac{\rho g}{RA} t + \ln(\Delta h_0) \quad \text{Eq. (6)}$$

where Δh_0 is the initial height of the water in the tube. Initially, experiments were run with Scotch-Brite only to find the hydraulic resistance of the Scotch-Brite ($R_{\text{Scotch-Brite}}$) itself. Since fluid flow resistance acts in series, the total resistance (R_{series}) could be used to find the resistance of the collagen (R_{collagen}), which could then be used to estimate the permeability of collagen using:

$$R_{\text{series}} = R_{\text{Scotch Brite}} + R_{\text{Collagen}} \quad \text{Eq. (7)}$$

2.2 Horizontal Apparatus

To compare with the vertical apparatus, a horizontal apparatus was developed as seen in Figure 3. The inlet was connected to 1 cm ID tubing. The outlet was connected to 0.35 cm ID tubing, corresponding to a cross-sectional area, A , of 0.096 cm². A pressure transducer was connected to the inlet and the corresponding pressure drop, ΔP , across the sample was monitored

Figure 3. Horizontal apparatus used for determining the permeability of collagen. The syringe pump delivers fluid into the sample at a constant rate. The pressure transducer monitors the pressure gradient across the sample. The collagen is held with mesh and an adapter. The inlet tubing is larger than the outlet tubing to ensure fluid flows through the sample and not around the sample.

with LabVIEW (National Instruments, Austin, TX). A syringe pump delivered fluid at a constant volumetric flow rate, Q . This pump was calibrated by measuring the volume of fluid collected in a graduated cylinder after one minute.

Collagen was loaded into an adapter shown in Figure 4 lined with 250 mesh. The inlet of this adapter was larger than the outlet to ensure fluid flow through the sample. The collagen was prepared using the same technique as described in Section 2.1.1. Water was dyed with blue food coloring to track compression. The viscosity of the water, μ , was taken to be 8.90×10^{-4} Pa s. A Nikon D7500 digital camera recorded the experiments. Screenshots of the video were used to measure the length of the collagen sample, L , using the free software, ImageJ (National Institutes of Health, Bethesda, MA).

Figure 4. Adapter used to hold collagen in the horizontal apparatus

The instantaneous permeability, κ , was found using Darcy's Law (eq. 1). Experimental data were fit to the power-law exponent, α , and the exponential strain-dependence coefficient, M , of the strain-dependent Holmes-Mow permeability model:

$$\kappa = \kappa_0 \left(\frac{J - \varphi_0}{1 - \varphi_0} \right)^\alpha \exp \left(\frac{1}{2} M (J^2 - 1) \right) \quad \text{Eq. (8)}$$

where κ_0 is the initial permeability, J is the Jacobian of the deformation gradient, and φ_0 is the initial solid volume fraction. The deformation gradient is a second order tensor that describes the motion of a material. J is a measure of how the volume of a material has changed with the deformation, which can be measured with the volume ratio. In this experiment, the cross-sectional area of the collagen is constant, so the volume ratio of the collagen undergoing compression is simply the ratio of the compressed collagen length to the original collagen length, i.e. the compression stretch ratio, λ . In the Holmes-Mow model, α measures how quickly the permeability approaches zero as φ approaches 1, while M represents the sensitivity of the permeability to strain [21].

2.3 Computational Models

2.3.1 Happel's Model

The calculated permeability is compared to Happel's model for flow through a random array of long cylindrical fibers [22]. It uses a weighted average to account for the different orientation of the cylindrical fibers as shown in the following equations:

$$\kappa = \frac{2}{3}\kappa_{\perp} + \frac{1}{3}\kappa_{\parallel} \quad \text{Eq. (9)}$$

$$\kappa_{\perp} = \frac{a^2}{8} \left[\frac{1}{\varphi} \ln \left(\frac{1}{\varphi} \right) - \frac{1}{\varphi} \frac{1 - \varphi^2}{1 + \varphi^2} \right] \quad \text{Eq. (10)}$$

$$\kappa_{\parallel} = \frac{a^2}{8} \left[4 - \varphi + \frac{1}{\varphi} \left\{ 2 \ln \left(\frac{1}{\varphi} \right) - 3 \right\} \right] \quad \text{Eq. (11)}$$

Where $k_{\parallel}$ is the permeability contribution of fibers aligned parallel to fluid flow, $k_{\perp}$ is the permeability contribution of fibers aligned perpendicular to fluid flow, a is the collagen fibril radius of 50 nm and φ is the solid volume fraction. Based on a collagen density of 1.41 g cm⁻³ and the assumption of negligible collagen loss, a φ value of 0.007 was used for the compressed collagen [23].

2.3.2 *Biphasic Theory*

The finite element method was used to solve the permeability as a function of compression. A biphasic analysis was done via FEBio (FEBio, Salt Lake City, Utah). FEBio is a free, open-source finite element analysis software that was specifically designed for use in biomechanics and bioengineering. FEBio utilizes the biphasic theory to model a biphasic material such as collagen [24]. It assumes both the solid and fluid phases are intrinsically incompressible, but the mixture can change volume if fluid enters or leaves the porous solid matrix. Under quasi-static conditions, the conservation of momentum reduces to:

$$\text{div } \boldsymbol{\sigma} + \rho \mathbf{b} = 0 \quad \text{Eq. (12)}$$

where $\boldsymbol{\sigma}$ is the Cauchy stress for the mixture, ρ is the mixture density, and $\mathbf{b}$ is the external mixture body force per mass. For porous mixtures, the Cauchy stress can be rewritten as:

$$\boldsymbol{\sigma} = -p\mathbf{I} + \boldsymbol{\sigma}^e \quad \text{Eq. (13)}$$

where p is the fluid pressure, $\mathbf{I}$ is the identity matrix, and $\boldsymbol{\sigma}^e$ is the effective stress due to the deformation of the solid matrix. The conservation of mass for the mixture can be written as:

$$\text{div}(\mathbf{v}^s + \mathbf{w}) = 0 \quad \text{Eq. (14)}$$

where $\mathbf{v}^s$ is the solid matrix velocity and $\mathbf{w}$ is the flux of the fluid relative to the solid matrix. The conservation of linear momentum for the fluid can be reduced to:

$$-\boldsymbol{\phi}^w \text{grad } \mathbf{p} + \rho^w \mathbf{b}^w + \hat{p}_d^w = 0 \quad \text{Eq. (15)}$$

where $\boldsymbol{\phi}^w$ is the solid matrix porosity, $\rho^w = \boldsymbol{\phi}^w \rho_T^w$ is the apparent fluid density and ρ_T^w is the true fluid density, $\mathbf{b}^w$ is the external body force per mass acting on the fluid, and $\hat{p}_d^w$ is the momentum exchange between the solid and the fluid. Combining equations 9-12 produces:

$$\mathbf{w} = -\kappa * (\text{grad } \mathbf{p} - \rho_T^w \mathbf{b}^w) \quad \text{Eq. (16)}$$

2.3.3 Geometry and mesh

The collagen was modeled as a cylinder (Figure 5). A radius of 5 mm and a height of 9 mm was used to match the size of the experimental sample. A butterfly center mesh was used with 4 slices, 4 segments, and 8 stacks. A z-bias of 1.5 was applied to create a finer mesh at the bottom of the cylinder since the fluid flux changes rapidly here. The cylinder only changed in the z-direction (the direction of the compression seen experimentally), so the mesh only depended on the number of stacks used. A mesh analysis was performed until the solution was found to be independent of the mesh spacing.

Figure 5. Cylinder created in FEBio to model collagen. A butterfly center mesh with 4 slices, 4 segments, and 8 stacks was used.

2.3.4 Material Parameters

A biphasic material was prescribed to the cylinder to model the collagen. Like the Happel's model, assuming negligible collagen loss and a density of 1.41 g cm^{-3} , the initial solid volume fraction, φ_0 , was set to 0.0014. The fluid density was set to have the same density of water (1000 kg/m^3). A Poisson's ratio of 0 was used since the circumferential sides of the collagen was held in

place by the adapter, resulting in no lateral expansion. The Holmes-Mow parameters, κ_0 , α , and M were taken from the experimental results. The Young's Modulus was adjusted to best match the final length of the compressed collagen gel.

2.3.5 Boundary Conditions

The model is prescribed with the following boundary conditions and step sizes:

- The nodes of the bottom surface were constrained in the x, y, and z-directions. These fixed nodes represent the bottom of the sample in contact with the adapter support and the mesh, which does not move.
- The nodes of the top and circumferential surfaces were constrained in only the x and y-directions. The x and y-directions represent the lateral edges of the collagen held in place by the adapter. However, these surfaces could move in the z-direction to represent the compression of the collagen.
- A fluid flux of -0.475 mm/s was prescribed to the top surface based on the experimental volumetric flow rate of 0.014 mL s⁻¹ and the inlet cross-sectional area of 0.096 cm²
- The nodes equivalent to the outlet size (circle with a 3.5 mm radius) were prescribed at the bottom as a free-draining surface as seen by the red nodes within the black circle in Figure 6. This is to represent the outside edges of the collagen being in contact with an impermeable support and also to represent a smaller outlet compared to the inlet.
- Since the volumetric flow rate was constant but the outlet had a smaller cross-sectional area than the inlet, a nodal fluid flux of 1.47 mm/s was prescribed to the same bottom surface nodes selected for the free-draining surface

Figure 6. Red nodes within the black circle show the bottom surface nodes selected for the free-draining surface and fluid flux conditions

- The model ran for 200 time steps with a step size of 0.1 seconds
- A pressure load was applied to the top surface and adjusted to match the maximum pressure seen experimentally. The curve editor was used to have the pressure load reach its maximum value at the very end of the run.

2.4 Results and Discussion

2.4.1 Vertical Apparatus

A representative plot of $\ln(\Delta h)$ versus time is shown in Figure 7 below. The data showed a linear trend initially before becoming nonlinear, implying the resistance of the Scotch-Brite changes as the head of fluid changes. As the pressure decreases, the corresponding resistance of the Scotch-Brite™ also decreases. This could occur because at higher pressures, the volumetric flow rate was also higher, forcing some of the fluid through the solid portion which generated a larger resistance than fluid traveling through the pores of the material. To find the average

resistance of the Scotch-Brite™ over the changing pressure, a linear trendline was fit to the Darcy model seen in Eq. (6). The slope from Figure 7a. (left) was combined with equation 7 to calculate the flow resistance and the permeability of Scotch-Brite™. Its permeability was calculated to be $2.16 \times 10^{-10} \text{ m}^2$, which is on the same order of magnitude as the literature value of $5.03 \times 10^{-10} \text{ m}^2$ [25]. It is unknown how tightly wound the Scotch-Brite™ was while in the apparatus, which could lead to some variations with the permeability values. The slope from Figure 7b (right) was used to calculate the total hydraulic resistance. The resistance due to the collagen sample determined through equation 7 and the permeability of the collagen sample was found to be $9 \times 10^{-11} \pm 2 \times 10^{-11} \text{ m}^2$.

Figure 7. *ln(height) versus time for a sample of just Scotch-Brite™ (left) and of one containing both Scotch-Brite™ and collagen (right). The permeability of the collagen gel, $9.49 \times 10^{-11} \text{ m}^2$ is calculated from the slope of the right graph.*

2.4.2 Horizontal Apparatus

Five runs were fit to the Holmes-Mow model, resulting in an α value of 1.1 ± 0.5 , and an M value of 3.0 ± 0.5 . Instead of fitting the initial permeability, κ_0 , to the Holmes-Mow model, the initial value of κ for each experimental run was used. The graph in Figure 8 shows a representative plot of the experimental permeability values overlaid with the average Holmes-Mow permeability values. These fitted values resulted in a permeability that decreased from $3.18 \times 10^{-11} \text{ m}^2$ to $1.38 \times 10^{-12} \text{ m}^2$ as the collagen sample became denser under compression

Figure 8. Compressive stretch ratio vs the permeability. Example of experimental data overlaid with the average Holmes-Mow model parameters. This experiment had an initial κ value of $3.18 \times 10^{-11} \text{ m}^2$. The Holmes-Mow parameters had an α value of 1.1 ± 0.5 and a M value of 3 ± 0.5

2.4.3 Computational Models

The permeability calculated by Happel's Model had a range of $1.58 \times 10^{-12} - 2.21 \times 10^{-13} \text{ m}^2$ as ϕ increased with compression. Meanwhile, the FEBio model used the permeability determined experimentally. A Young's Modulus of 400 Pa resulted in a model with the closest

Figure 9. Pressure gradient vs the compressive stretch ratio. The experimental results show a linear relationship between the pressure and the compression level. The FEBio model showed three distinct regions, a quick increase in pressure initially followed by a more gradual, linear pressure increase at low-mid compression levels, and ending with an exponential rise at high compression levels.

amount of compression seen in the experimental section. As seen in Figure 9, the FEBio model had a different relationship between the pressure and the level of compression. The experimental result showed a more linear relationship between the pressure and the compression of the collagen. Meanwhile, the pressure of the FEBio model saw three distinct regions. The first region occurred immediately as the pressure increases quickly from 0-200 Pa. The second region occurred at low-mid compression levels and while it also resulted in a linear relationship, the FEBio pressure was lower than the experimental pressure. The third region showed an exponential rise in pressure at high compression levels. Both the experimental collagen sample and FEBio model compressed to a final length of about 1.2 mm.

Figure 10 shows a comparison between the computational model and the experimental data. At lower compression levels, the FEBio model showed lower pressure levels compared to the experimental pressures while at higher compression levels, the FEBio model pressure was very similar to the experimental pressures.

Figure 10. Experimental results (top) vs FEBio results (bottom). The experimental pressure was larger at low-mid compression levels, while the FEBio model showed similar pressure s at high compression levels.

other published works. The vertical apparatus had a permeability value more like the initial permeability found with the horizontal apparatus rather than the final permeability. However, the permeability determined with the vertical apparatus was for a compressed collagen sample. The vertical apparatus likely overestimated the permeability value due to near-wall flow, where water flows around the collagen rather than through the sample. The permeability values found in this study are still within the permeability values found by Varley [26] and Ramanujan [17], which varied between 4.80×10^{-10} and $1.00 \times 10^{-15} \text{ m}^2$. There is a large discrepancy seen between studies, likely due to different gel preparation techniques which may drastically alter the network morphology (e.g. connectivity, fiber diameter, etc.). Happel's model found permeability values on the same order of magnitude as the experimental data but have inherent limitations. Happel's model assumes the fibers are randomly oriented but do not change, even as the solid volume fraction changes. However, the collagen fibers likely align as the sample compresses, which the Happel's model does not account for. Additionally, Happel's model assumes that the fibers have a uniform fibrillar radius, which may not reflect the microstructure of the fibers within collagen gels.

Figure 11. Comparison between permeability values. The vertical apparatus had a higher permeability value than the horizontal apparatus, while Happel's model had a lower permeability

The final compression length of the FEBio model was within 1% of the fully compressed length of the experimental collagen, indicating that the neo-Hookean material mimicked the experimental data reliably. Interestingly, the bottom surface fluid flux is much higher than the prescribed value once the compression begins (Figure 12, a). As the sample compressed, the fluid flux fell to the prescribed value upon reaching its final compressed length (Figure 12, b). This is likely because interstitial fluid is expelled together with the water flowing through the sample. This additional fluid being expelled from the model results in a higher volumetric flow rate than expected, reaching a value of nearly double the prescribed value of 0.014 mL/s (Figure 12, c). Since the volumetric flow rate found experimentally is time-averaged, it did not account for the instantaneous increase in fluid flux seen in the model. Additionally, the fluid flux at the bottom surface of the model was not uniform. Instead, the edges of the outlet saw a larger fluid flux than the middle of the outlet. Since fluid could not exit through the outside edges of the model, the fluid in this area was forced to travel inwards towards the outlet edge.

Figure 12. Fluid flux is much larger than the prescribed value once the sample starts compressing (left). Fluid flux in the middle of the bottom surface is smaller than the edges of the outlet (right). The outlet reached a volumetric flow rate nearly double the prescribed value (bottom)

The FEBio model also showed the collagen sample compressed further in the middle compared to the edges, a phenomenon that could not be observed experimentally (Figure 13). This likely occurred because the edges of the model along the outlet is impermeable, preventing fluid from leaving as quickly as the middle of the cylinder. Both the experimental collagen sample and the center of the FEBio model had a total displacement of 6.74 mm, resulting in a final length of 1.24 mm. Meanwhile, the edge of the model only had a displacement of 6.15 mm, resulting in a final length of 1.84 mm.

Figure 13. FEBio model compressed further in the middle of the sample compared to the edges of the sample.

There are further limitations to the FEBio model. The FEBio model assumed the material is isotropic throughout. However, collagen is an anisotropic material. As the sample compresses, the fibers are likely to align perpendicular to the fluid flow, likely changing the transport and mechanical properties of the collagen gels. Since FEBio models assume an isotropic material, it cannot account for this phenomenon.

Chapter 3: Effect of Collagen Concentration on Gel Permeability

The permeability of three different collagen concentrations was found and fit to the Holmes-Mow model (eq. 8) using the horizontal apparatus. The corresponding parameters were changed in FEBio to model the collagen soft gel at various initial solid volume fractions.

3.1 Materials and Methods

A different source of collagen type I rat tail was used to allow for a wider concentration range. The collagen was prepared by mixing 204 μL 1M HEPES buffer, 26 μL NaOH, 100 μL 10x Minimum Essential Medium, 10 μL L-Glutamine, and the amounts given in Table 1 of collagen type I rat tail (Corning Life Sciences, Bedford, MA) and 20 mM acetic acid (Sigma-Aldrich, St. Louis, MO). The permeability of the collagen was determined using the horizontal apparatus as described in Section 2.2. The same FEBio model was used as detailed in Section 2.3.

Table 1. Amounts of acetic acid and collagen type I rat tail added to create three different concentrations

Concentration (mg/mL)	20 mM acetic acid (μL)	9.33 mg/mL collagen type I rat tail (μL)	Initial solid volume fraction, ϕ_0
1.98	448	212	0.0014
3.5	285	375	0.00248
5.0	124	536	0.00355

3.2 Results and Discussion

3.2.1 Experimental Results

The starting length for each run was approximately 0.9 cm. Five runs for each concentration were fit to the Holmes-Mow permeability model. The results for these runs are seen in Table 2.

Table 2. Average initial permeability values and the average Holmes-Mow permeability parameters for each collagen concentration. Maximum pressure for the experiments is also recorded

Concentration (mg/mL)	k_0 (m^2)	M	α	Maximum ΔP (in. H_2O)
1.98	6.10×10^{-11}	2.4 ± 0.9	1.29 ± 0.4	4.00
3.5	4.16×10^{-11}	3.8 ± 1	0.701 ± 0.4	4.42
5	2.94×10^{-11}	5 ± 1	0.047 ± 0.006	4.74

A representative plot of the experimental permeability values overlaid with the average Holmes-Mow permeability model values is seen in Figure 14. As expected, the higher starting collagen concentration resulted in a lower initial permeability. However, these higher concentrations underwent less overall compression, resulting in a smaller α value and a larger M value for the Holmes-Mow model. As the lower concentration collagen compressed further, more interstitial fluid was expelled, resulting in ϕ approaching 1 quicker than the higher concentration collagen. This effect resulted in larger α values for the lower concentration collagen gels. Meanwhile, the permeability of higher concentration collagen gels decreased exponentially without compressing as much as the lower concentration collagen gels. This suggests that the higher concentration collagen gels were more sensitive to compressive strains, resulting in larger M values. When fitting the permeability of bovine articular cartilage, Ateshian et al. found an α value of 2 and an M value of 2.2 [7]. Similarly, fitting the permeability of human medial cartilage ligaments, Weiss et al. also found an α value of 2 but found an M value of 7.988 [8]. Both studies focused on human tissue, which will have a quite different structure than collagen gels.

Figure 14. Example of experimental data overlaid with the average Holmes-Mow permeability model. Note that for the Holmes-Mow models, κ_0 was the initial permeability value for that specific run; this parameter was not fit, unlike the α and M values.

3.2.2 *Computational Results*

As shown in Table 3 below, a Young's Modulus of 200 Pa, 1550 Pa, and 3700 Pa best replicated the experimental results. As the concentration increased, a higher Young's Modulus is required to model the collagen gels using a Neo-Hookean material. The graphs in Figure 15 show the pressure difference vs the compressive strain. The 1.98 and 5 mg/mL collagen concentration showed a linear relationship between pressure and compression, while the 3.5 mg/mL showed a nonlinear relationship. Meanwhile, the 1.98 mg/mL and 3.5 mg/mL models showed similar pressure values to the experimental results, while the 5 mg/mL model resulted in pressure values slightly below the experimental collagen sample.

Table 3. FEBio results showing the Young's Modulus and the corresponding comparison to the experimental results for each collagen concentration

Concentration (mg/mL)	Young's Modulus (Pa)	Final Model Length (mm)	Experimental Length (mm)	Maximum ΔP (Pa)
1.98	200	1.26	1.32	1000
3.5	1550	3.72	3.46	1100
5	3700	3.93	3.88	1300

Figure 15. Graphs showing pressure vs compression. The left graph (a) shows 1.98 mg/mL collagen, the middle graph (b) shows 3.5 mg/mL collagen, and the right graph (c) shows 5 mg/mL collagen. While the 1.98 mg/mL and the 3.5 mg/mL models showed similar results for pressure vs compression, the 5 mg/mL had slightly lower pressures compared to the experimental results.

The figures shown below show that at specific compressive strain values, the experimental collagen and the FEBio model resulted in similar pressure values for the 1.98 mg/mL and 3.5 mg/mL collagen concentrations. The FEBio model resulted in lower pressure values than the 5 mg/mL collagen.

Figure 16. Experimental results (top rows) vs FEBio results (bottom rows) for 1.98 mg/mL collagen gels (top left, a), 3.5 mg/mL collagen gels (top right, b), and 5 mg/mL collagen gels (bottom middle, c)

Figure 17 shows a comparison of permeability values. The permeabilities for each collagen concentration had a similar order of magnitude. The concentration of the fully compressed collagen gels was about 12 mg/mL, regardless of the starting concentration. Therefore, we see minor differences in the final permeability values of each starting concentration. As collagen concentration increased, the corresponding initial permeability decreased, suggesting an inverse relationship between concentration and permeability. This supports the results found by Ramanujan, etc. which also showed this phenomenon [17]. The Ramanujan study found a permeability of about $1.00 \times 10^{-15} \text{ m}^2$ for 10 mg/mL collagen gels and a permeability of about $1.00 \times 10^{-16} \text{ m}^2$ for 45 mg/mL collagen gels. These collagen gels were much more concentrated than the ones used in the current study, resulting in lower permeability values. The Varley study used freeze-dried collagen scaffolds and had a corresponding permeability of

$4.80 \times 10^{-10} \text{ m}^2$ [26]. The discrepancy between these studies is likely due to the different casting techniques.

Figure 17. Effect of concentration on the permeability of collagen gels. The initial permeability decreases as concentration increases. The final permeabilities were on a similar order of magnitude

Previous studies have shown a wide range of the Young's Modulus of collagen gels. Lai, et al, performed mechanical tests on 1.98 mg/mL collagen rings by looping the gel rings over T-bar grips and uniaxially stretching the grips [27]. The force data was exported to calculate the first Piola-Kirchoff stress, which was used to find a tangent modulus of 7750 Pa. This value is much larger than the Young's modulus values found in the current study but underwent tensile testing rather than compression testing. A study by van Oosten et al. used a tensile tester which subjected 2.5 mg/mL collagen to both a compressive and a tensile stress-relaxation test, [28]. The tensile Young's modulus was 6520 Pa, which is much higher than the Young's modulus in compression, which was 29.1 Pa. The Young's modulus in the van Oosten study was much lower than the Young's modulus found in the current study. The collagen in the van Oosten study was only subjected to 10 μm steps of compression at 2 $\mu\text{m/s}$ and was then allowed to relax for 15

minutes between steps. The current study had no relaxation period and underwent much larger deformations than the ones seen in the van Oosten study. A study by Knapp et al., found values for the short-time modulus and the aggregate modulus for 2 mg/mL collagen [15]. The aggregate modulus in this study was 6.32 Pa and was determined using a creep test where a constant compressive stress was applied to the collagen for 5 hours. The short-time modulus was 318.3 Pa, which was found using a ramp test where the collagen was compressed for 120 seconds to a 10% strain. Both the experiment length and the corresponding Young's modulus are in good agreement with the current study for the 1.98 mg/mL collagen. This suggests that collagen under pressure-driven flow does not have time to relax, generating high resistance.

Figure 18. Comparison between Young's modulus values. As the collagen concentration increases, the corresponding Young's modulus also increases.

Chapter 4: Conclusions and Future Work

Two different apparatuses were developed to determine the permeability of collagen soft gels. The vertical apparatus resulted in higher permeability values than the horizontal apparatus. The horizontal apparatus found permeability values of three different collagen concentrations as a function of compression and the results were fit to the strain-dependent Holmes-Mow permeability model. The initial permeability decreased as the collagen concentration increased. The α value decreased as the initial concentration increased, while the M value increased as the initial concentration increased. The final concentration of the fully compressed collagen was similar, regardless of the initial concentration. A FEBio model was developed to model the compression of collagen gels. The Young's modulus required to match the compressed sample length increased with concentration. The modulus found in the current study was similar to the compressive short-time modulus found in other published studies.

Future work could expand upon compression studies. Notably, future work could analyze compression if the collagen had time to relax. For instance, the impact of different flow rates on the permeability and compression of collagen gels could be explored. Lower flow rates would likely decrease the pressure acting against the collagen, which would allow more time for the collagen to relax and potentially lead to a lower Young's Modulus. Another study would be to allow relaxation periods for the collagen. The syringe pump could cycle between delivering fluid at a constant rate and delivering no fluid. When the syringe pump is turned off, the pressure will decrease and may give the collagen gel time to relax.

Future work could also look to refine the finite element model. The model could be refined to have a more uniform compression at a high strain. Additionally, the model could be further refined to test for a lower Young's Modulus. The FEBio model shows the same limitations as the original model seen in section 2.5. Notably, it still is unable to account for the

anisotropic nature of the collagen gels. Future work could involve the development of a model that accounts for fiber alignment throughout the compression. One such study could look at how the fibers align under pressure-driven flow and how concentration impacts this alignment.

Chapter 5: Bibliography

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Chapter 6: Appendix

6A. Constitutive Equations

Taken from the FEBio Theory Manual version 2.9 [29]

6A.1 Neo-Hookean Material

A compressible neo-Hookean material has the following hyperelastic strain energy function:

$$W = \frac{\mu}{2}(I_1 - 3) - \mu \ln J + \frac{\lambda}{2}(\ln J)^2$$

where the parameters μ and λ are the Lamé parameters from linear elasticity. The Cauchy stress, $\boldsymbol{\sigma}$, is given by:

$$\boldsymbol{\sigma} = \frac{\mu}{J}(\mathbf{b} - \mathbf{I}) + \frac{\lambda}{J}(\ln J)\mathbf{I}$$

where $\mathbf{b}$ is the left Cauchy-Green deformation tensor and $\mathbf{I}$ is the right-Cauchy-Green tensor. The spatial elasticity tensor, $\mathbf{C}$, is given by:

$$\mathbf{C} = \frac{\lambda}{J} \mathbf{I} \otimes \mathbf{I} + \frac{2}{J}(\mu - \lambda \ln J)\mathbf{I} \underline{\otimes} \mathbf{I}$$

6A.2 Holmes-Mow Permeability

The coupled hyperelastic strain-energy function for a Holmes-Mow material is given by:

$$\Psi(I_1, I_2, J) = \frac{1}{2}c(e^Q - 1)$$

Where I_1 and I_2 are the first and second invariants of the right Cauchy-Green tensor, J is the Jacobian of the deformation, and Q and c are as follows:

$$Q = \frac{\beta}{\lambda + 2\mu}[(2\mu - \lambda)(I_1 - 3) + \lambda(I_2 - 3) - (\lambda + 2\mu) \ln(J^2)]$$

$$c = \frac{\lambda + 2\mu}{2\beta}$$

where λ and μ are Lamé parameters. The corresponding Cauchy stress tensor is:

$$\boldsymbol{\sigma} = \frac{1}{2J}e^Q([2\mu + \lambda(I_1 - 1)]\mathbf{b} - \lambda\mathbf{b}^2 - (\lambda + 2\mu)\mathbf{I})$$

where $\mathbf{b}$ is the left Cauchy-Green deformation tensor and $\mathbf{I}$ is the right-Cauchy-Green tensor. The spatial elasticity tensor, $\mathbf{C}$, is:

$$\mathbf{C} = \frac{4\beta}{\lambda + 2\mu} J e^{-Q} \boldsymbol{\sigma} \otimes \boldsymbol{\sigma} + J^{-1} e^Q [\lambda (\mathbf{b} \otimes \mathbf{b} - \mathbf{b} \underline{\otimes} \mathbf{b}) + (\lambda + 2\mu) \mathbf{I} \underline{\otimes} \mathbf{I}]$$

6B. FEBio Code

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        <max_ups>10</max_ups>

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        <etol>0.01</etol>

        <rtol>0</rtol>

        <ptol>0.01</ptol>

        <l stol>0.9</l stol>

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        <mixed_formulation>0</mixed_formulation>

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            <dtmax>0.1</dtmax>

            <max_retries>5</max_retries>

            <opt_iter>10</opt_iter>
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</Control>

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        <R>0</R>

        <Fc>0</Fc>

    </Constants>

</Globals>

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        </phi0>

        <fluid_density>0.001</fluid_density>

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            <density>1</density>

            <E>

                //Young's Modulus altered to match
                experimental results

            </E>

            <v>0</v>

        </solid>

        <permeability type="perm-Holmes-Mow">

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            // Initial permeability found
            experimentally

        </perm>

        <M>

            // M value found experimentally

        </M>

        <alpha>

            // Alpha value found
            experimentally

        </alpha>

    </permeability>

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</Material>

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    </Nodes>

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        <elem id="1

            // Element IDs of the cylinder

        </elem>

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</NodeSet>

<NodeSet name="FixedEffectiveFluidPressure1">

    // Node IDs of the outlet to make it a free-
    draining surface

</NodeSet>

<NodeSet name="FixedDisplacement3">

    //Node IDs of the top and side surfaces that
    could move in the z-direction

</NodeSet>

<Surface name="FluidVolumetricFlowRate1">

    // Top surface IDs assigned a fluid flux

</Surface>

<Surface name="PressureLoad1">

    // Top surface IDs assigned a pressure load

</Surface>

</Geometry>

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    <fix bc="p"
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    <fix bc="x,y" node_set="FixedDisplacement3"/>

</Boundary>

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        <point>1,1</point>
    </loadcurve>

    <loadcurve id="3" type="smooth">
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        <point>20,1</point>
    </loadcurve>

</LoadData>

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        <var type="stress"/>
        <var type="effective fluid pressure"/>
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    </plotfile>
</Output>

</febio_spec>

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