A Sustainable Additive Approach for the Achievement of Tunable Porosity

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Abstract

This study aims to design a green additive approach for the fabrication of controlled porosity on hydrogels. Although hydrogels have been of common use in tissue engineering, the generation of controllable porosity remains an issue due to their swelling and degradation properties. Hydrogels in this study were fabricated by physical cross-linking and the porosity was generated by casting the solution in a 3D printed mold prior to physical cross-linking. This approach eliminates the use of chemical cross-linking compounds which are often toxic and not environmentally friendly. Polyvinyl alcohol was selected to validate this technique due to its biocompatibility and adequate mechanical properties. The microstructure, mechanical properties and deformation of the porous hydrogels were characterized. Results revealed that the proposed bioplotting technique reduced variation of pore size and allotted for the realization of controlled and tunable pore structures.

Keywords: 3D printing, Pores, Polyvinyl alcohol (PVA) hydrogel, Cross-linking

1. Introduction

Hydrogels are networks of polymer chains, which possess the ability to swell and retain fluids within their structure. These can be derived from natural or synthetic polymers. Their application has been extended to include fields such as agriculture, medical drug delivery, and biosensors [1]. Hydrogels have become attractive particularly in vascular applications due to their favorable characteristics and wide spread use across the other fields of tissue engineering. They are often used in scaffolds due to their biocompatibility, degradability, and the resemblance to body tissues in terms of water content and mechanical properties. Polyvinyl alcohol (PVA) hydrogels have become widely used in the field of tissue engineering. Besides properties such as biocompatibility, biodegradability, and hydrophilicity, these hydrogels have similar mechanical properties to the porcine aorta, making them ideal for vascular applications [2]. In scaffold applications, porosity within the hydrogel is required for proper cell proliferation. Pores must exhibit regulated shapes and sizes, with the necessary interconnectivity to promote survival of the cells [3]. However, traditional fabrication methods are known to cause swelling problems, and irregularity in pore shape and size [4].

Previous studies have shown organic materials, such as collagen, to produce similar properties to the native vessel [5]. Yet, type I collagen by itself cannot withstand high pressures and collagen hydrogel mixtures are prone to thrombogenicity and insufficient tensile strength [6].The use of synthetic materials such as polycaprolactone (PCL) provide a durable solution due to their low degradation rate, but they are prone to calcification [6].

Hydrogels' similar morphological structure to human tissue matrix make them suitable for tissue scaffolding [7]. PVA hydrogels are used in vascular applications in particular due to their similar tensile properties to that of the porcine aorta [8]. Though its characteristics make PVA a viable material for vascular scaffolding, controlling the porosity remains a challenge. Interconnected and evenly shaped and distributed pores are necessary for cell survival and proliferation, vascularization, and nutrient circulation [3]. Conventional pore fabrication, however, leads to variable pore distribution, size, and interconnectivity. To combat this, common methods such as particle leaching limit the size and shape of the final hydrogel product, ultimately hindering the applications of the material [3].

Traditional hydrogel pore fabrication includes particle leaching, gas foaming, and freeze-drying [4]. For example, a porous structure can be obtained through particle leaching by adding salt or other particulates to act as a porogen, which is leached out using a soluble substance after the hydrogel has been cross-linked. Porogens located further from the outer surface of the hydrogel present a problem as they are difficult to leach due to the limited accessibility of the solvent which results in porogens to remain embedded in the hydrogel [9]. In gas foaming, bubbles are introduced into the hydrogel via a physical or chemical process and are stabilized by use of a surfactant [10]. The gas is then removed from the pockets leaving a porous final product. Freeze-drying is the freezing of a hydrogel so that ice crystals form. These are subsequently sublimated through freeze-drying in vacuum leaving pores [3]. These four traditional methods of pore fabrication result in limited pore design and substantial variation in pore distribution and geometry.

The previous methods of bioplotting use 50 percent infill to produce a lattice-like structure which allows for interconnected pores to be placed in the polymer [11]. Hydrogels use a variant of this method as hydrogels need to be printed into a medium that allows for their cross-linking [12]. Hydrogels require cross-linking to change from a liquid-like state to a semi-solid gel to be able to retain their structure. The main advantages of this method are the result of chemical cross-linking which produces stronger chemical bonds with improved mechanical properties [12]. However, one major issue with this commonly used bioplotting approach of hydrogels is the high toxicity of the mediums that enable chemical cross-linking are not environmentally friendly. Since the main application of the hydrogels in this study will be used for biomedical applications, a more sustainable method of fabrication has to be developed.

In this study, we propose a green 3-D printing approach for the fabrication of controlled porosity in hydrogels. This proposed method utilizes physically cross-linked PVA hydrogels to avoid the use of toxic chemical cross-linking agents [12]. Pores are fabricated by casting the PVA solution in a 3D printed mold prior to the freeze-thaw process. The casting process allows for controlled porosity which is capable of producing more precise interconnected pores that have shown to result in improved cell proliferation and vascularization [3]. By controlling porosity, we are able to tune the mechanical properties of the hydrogel [13]. Thus, with this sustainable approach, controlled porosity is achieved with improved repeatability in comparison to other processes such as porogen leaching. In addition, the 3D printing process is highly customizable

when compared to porogen leaching methods. For all methods of pore fabrication, a target of 30 % porosity was used based on past research which shows this percentage as an optimal[10]. The hydrogel strength was examined using tensile testing. The swell rate was used to inspect the hydrophilic properties, and degradation rates were also investigated. In addition, the size and arrangements of pores were analyzed using a scanning electron microscope (SEM).

2. Materials and Methods

2.1. Synthesis of PVA hydrogel

A solution was formed by mixing 10 g PVA (Fisher Scientific Ltd., Montreal, Quebec), 17 g distilled water, and the 80 g DMSO (Fisher Scientific Ltd., Montreal, Quebec) solvent at 140◦C for 2 hours using a magnetic stirrer. The homogeneous solution was then cast into 3mm x 3mm cube and tensile type v-shaped molds for solidifying. The molds used are as shown in Figure 1 below. The casted mixtures were then allowed to rest at room temperature for 20 hours to allow for the rise and dissolution of air bubbles. The samples were then placed in a freezer at -20◦C for 10 hours. After the freezing phase, the casted parts were left to thaw at room temperature for 5 hours, completing the freeze-thaw cycle. The hydrogels were then removed from the molds and placed in the fume hood to allow them to dry. This is the method used for the fabrication of control samples.

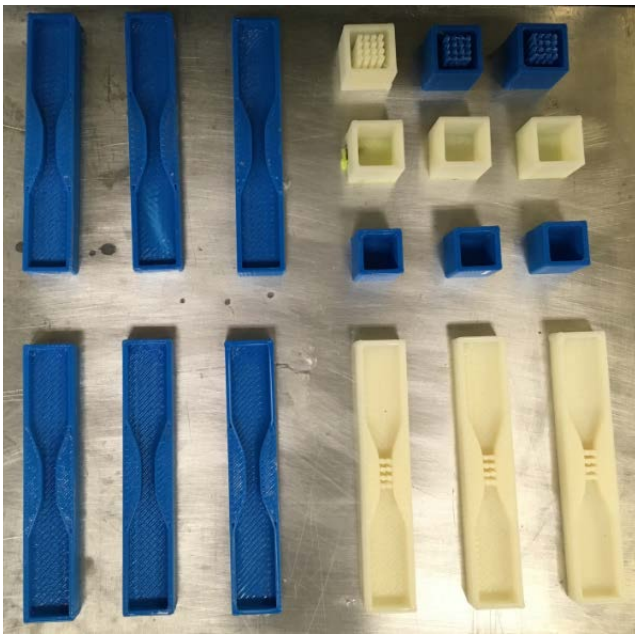


Figure 1: 3D printed cube and tensile type v-shaped molds.

2.2. Porogen leaching

Polycaprolactone (PCL) was chosen as the sacrificial porogen material. The morphology of the PCL powder was modified via mechanical cryomilling to yield 5 g powder to 15 mL of PVA hydrogel solution. PCL pellets were weighed and transferred to a vial, which underwent cryogenic freezing at –196 °C using liquid nitrogen. Repeated collisions and fractures during the grinding process. The duration of the grinding operation was five minutes, divided into four cycles

separated by a 5-minute dwell time. Cryogenic grinding was carried out using a freezer mill (SPEX, NJ, USA). PVA hydrogel solution synthesis is detailed in Section 2.1. PCL powder was added to the hydrogel solution at equal weight ratios and was homogeneously mixed with a lab spatula. It was then poured into a 10×10 mm hollow cube mold and submitted to physical cross-linking via freeze-thawing as discussed in Section 2.1. To leach the hydrogel of PCL, the hydrogel was removed from the mold and placed in acetone for 10 days within an incubator at 37 °C [9]. An identical process was used for porogen leaching hydrogels formed in ASTM D638-10 type-V tensile molds, rather than cube molds, for tensile testing.

2.3. 3D printed mold with pore design

PVA hydrogel solution was synthesized as discussed in Section 2.1. The resulting solution was then poured into two casting molds [3]. Figure 2 (a) shows the 10×10 mm hollow cube mold with 16 pins of 0.5 mm diameter and 1.5 mm separation. Figure 2 (b) shows the ASTM D638-10 tensile mold with4 pins of 0.5 mm diameter. Within the molds, the hydrogel solution was submitted to the freeze-thaw method detailed in Section 2.1 for physical cross-linking.

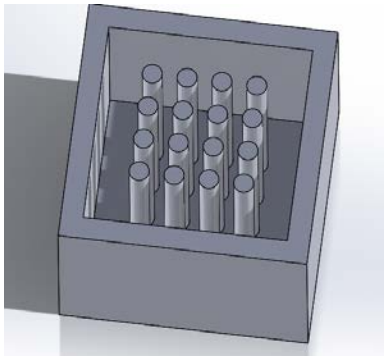
a.  b. 

Figure 2: CAD model of pinned 10×10 mm cube casting mold (a), CAD model of pinned ASTM D638-10 type v tensile casting mold (b).

2.4. Measurement of swelling of PVA hydrogel

Swelling rate of PVA hydrogels in distilled water was investigated by measuring the weight gained by the cube-shaped samples due to increase in water content. The swelling ratio is dependent on the strength of the cross-linking in the polymer. Three sets of each type of porosity were placed into a plastic tube containing 30 mL of distilled water and then into an incubator at 35 °C [14]. Samples were placed in the fume hood for 10 minutes before each weighing to ensure the sample was dry. Measurements were taken every 12 hours for 48 hours to compare the swelling characteristics between the different pore fabrication methods. The swelling percentage was captured using equation (1):

$$\text{Degree of swelling (\%)} = \frac{Ws - Wd}{Wd} \times 100 \quad (1)$$

Where Wd is the final dry weight of the hydrogel and Ws is the swollen weight of the hydrogel at time the (t) in distilled water.

2.5. Measurement of degradation of PVA hydrogel

The degradation rate of PVA hydrogels at room temperature in no solution was studied by measuring the percentage weight loss by the cube-shaped samples from degradation of the hydrogel. These degradation rates will give additional insight into cross-link strength and use inside the body as a cell scaffold. The rate of degradation can influence cell proliferation dependent on pH changes caused by the breakdown of the polymer [15]. Three sets of each type of porosity were placed in the petri dish and were measured every 12 hours for 48 hours. Samples were placed in the fume hood for 10 minutes before each weighing to ensure the sample was dry. The degradation rate was captured using equation (2):

$$\text{Weight loss (\%)} = \frac{Wo - Wt}{Wo} \times 100 \quad (2)$$

Where Wo is the initial weight of the hydrogel at time 0 and Wt is the weight of the hydrogel at the given measure time(t).

2.6. Tensile properties of PVA hydrogel

The three methods of fabrication were mechanically tested using a Universal Testing Machine (Test Resources 800LE2 Series, Shakopee, MN, USA) by performing tensile tests. Molds were printed with type V dog-bone coupon specifications in accordance to ASTM D638. Tensile strength was calculated using the measured cross-sectional area in the neck area of the coupon and the measured load. Strain was calculated using the original length of the coupon and the measure length at break to determine the change in length. Using both the stress and strain values, the modulus of elasticity was derived.

2.7. Statistical Analysis

The tensile properties were analyzed using RStudio (Boston, MA, USA) to determine statistical significance of measurements. One-way ANOVA was performed on the measured tensile strength, strain and modulus of elasticity using Type I error rate of 0.05. Consequently, Tukey's multiple comparison test was done to determine significant differences between the different methods of fabrication.

2.8. Micrographs of pores

The pores micrographs were taken using Scienscope (Chino, CA, USA) to inspect the resultant pore geometries obtained from porogen leaching and the casted fabrication methods. Magnification of the lens was set at two times the size of the object.

3. Results and Discussion

3.1. Water absorption rates

Water absorption rates were investigated by calculating the swelling ratio of PVA hydrogels in distilled water. Figure 3 below shows the swelling rate of the control, cast and porogen leached samples. The casted pores samples showed the highest swelling rates followed by the control sample and the porogen leached samples. The control and the casted samples approached a similar equilibrium value, which can be seen in Figure 3 from t = 36 hours to t = 48 hours. The porogen

leached samples showed significantly lower swelling rates when compared to the casted and control samples.

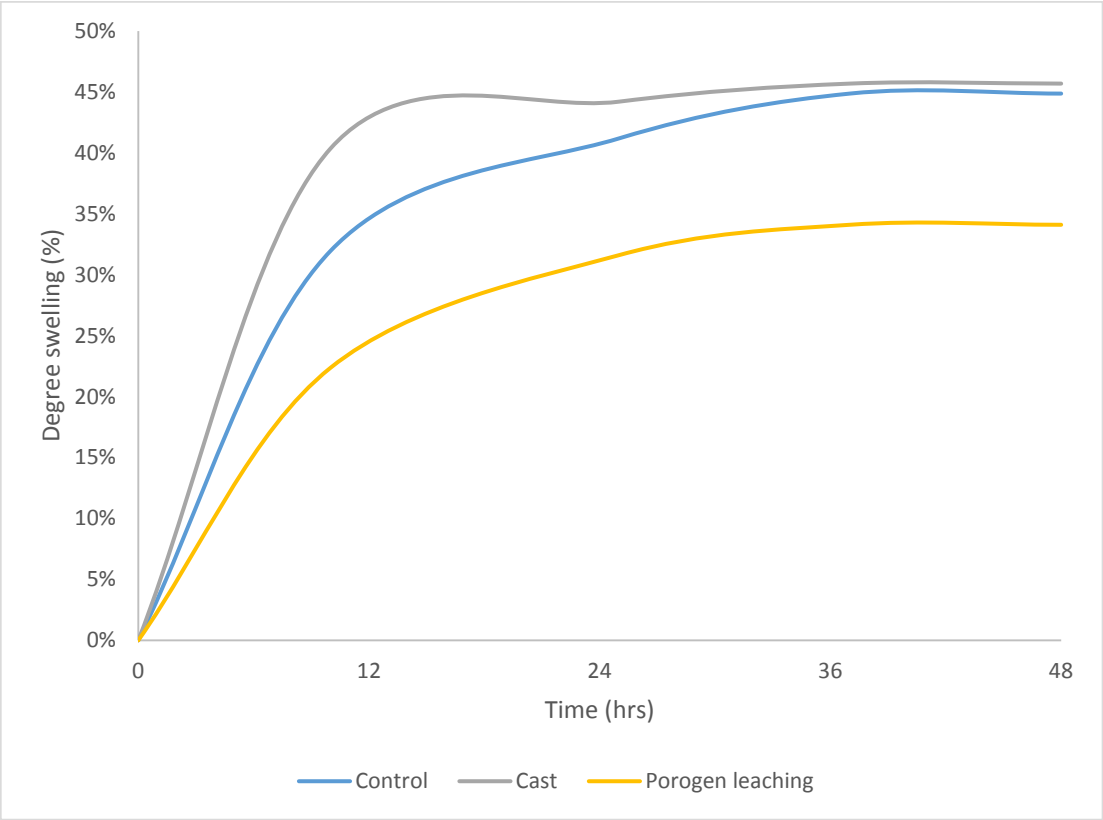


Figure 3: Swelling percentage of PVA hydrogels.

The casted samples had the highest swelling properties due to the presence of more interconnected pores since the mold was predesigned to ensure the formation of interconnected pores. The porogen leached samples contained pores on the outer surface of the scaffold, but visually, white particles of PCL were still embedded in the inner part of the scaffold. Interconnected pores were shown to lead to faster swelling rates due to its less dense structure [16]. The control sample required more compressive force initially to swell as a consequence of its denser structure [17] which resulted in a lower initial swelling percentage when compared to the casted pores. Once the force to swell for the control samples were overcome, the control and the casted pore then reached a similar swelling percentage equilibrium. The porogens were not completely leached from the hydrogel scaffold which is an issue shared with past researchers when using this method for pore fabrication [18]. This resulted in the porogen leached samples having embedded PCL particles which reduced the number of pores in the scaffold. The reduced amount of porosity combined with the hydrophobic nature of the PCL particles [19] led to the low rates of water absorption in the porogen leached samples.

3.2. Degradation rates

The degradation rates were studied by measuring the weight loss at room temperature in no solution. The graph in figure 4 below, shows casted pores had higher initial degradation but at t = 29 hours the casted pores and the control had similar degradation rate when t > 29 hours. While from t=0 to t=24 hours the control and porogen leached samples had similar degradation rates.

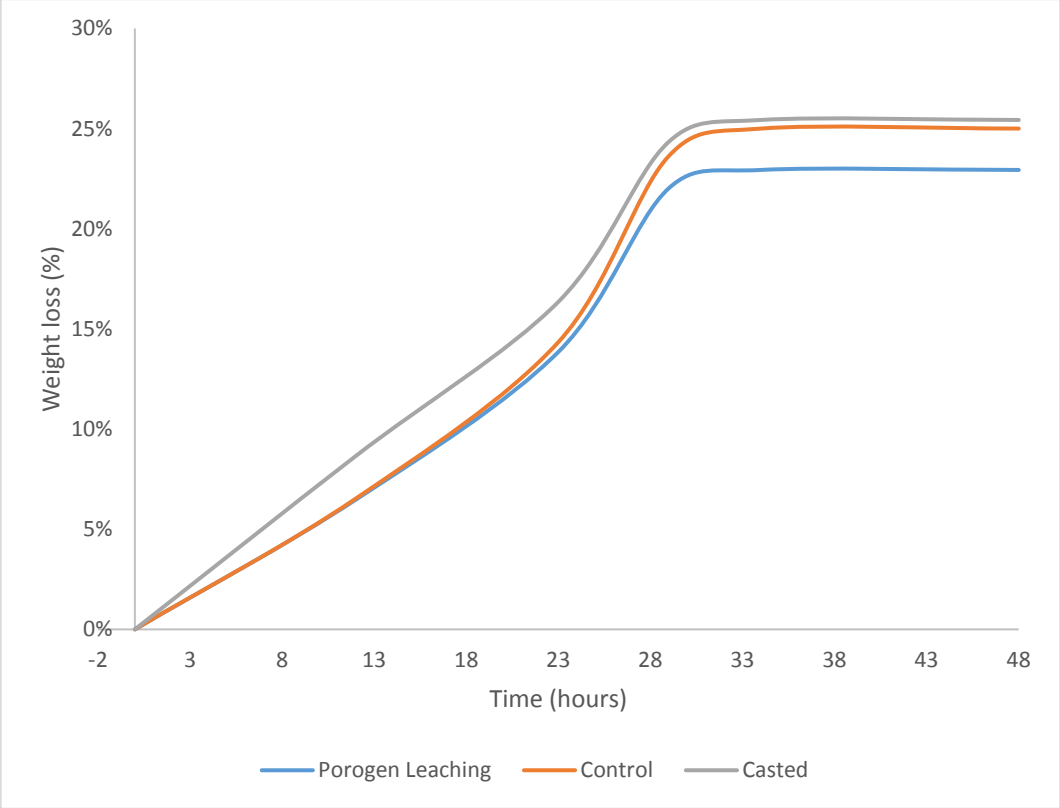


Figure 4: Weight loss percentage of PVA hydrogels.

Past researchers have shown that the increase in porosity increases the degradation rate of the polymer [20]. This explains why the casted pores displayed higher degradation rate from t=0 to t=29 hours when compared to the other two samples. Porogen leached samples as explained in section 3.1, had remaining PCL particles embedded inside the sample, therefore, mimics the control sample with similar degradation rates from t=0 to t=24 hours due to the similarities in densities between the two samples. Once the force to deform the control sample was overcome [17], the control sample showed degradation kinetics similar to the casted sample at t = 29 hours similar to the swelling behavior shown in section 2.1. Since the porogen leached sample had remaining PCL particles embedded in it, the degradation rate remains notably lower than the control and casted sample. PCL is known to have slower degradation rates [21] in comparison to PVA hydrogels hence the lower degradation equilibrium displayed in figure 4 from t= 29 to t= 48 hours.

3.3. Mechanical properties of PVA hydrogels

Tensile tests were performed following ASTM specifications to evaluate the mechanical properties of each pore fabrication method, Figure 5. The control sample resulted in the highest ultimate

tensile strength, just below 25 psi, followed by porogen leaching and casted samples. The control is expected to have the highest ultimate tensile strength due to the lack of pores. Porogen leached samples showed the highest strain values followed by the control. Casted samples had strain values less than half of either the control or the porogen leaching. The three methods resulted in similar modulus of elasticity values. Past research shows that the size and the distribution of pores affect the modulus of elasticity [22]. At lower porosity percentages, the effect of porosity on the modulus of elasticity is known to be negligible [23].

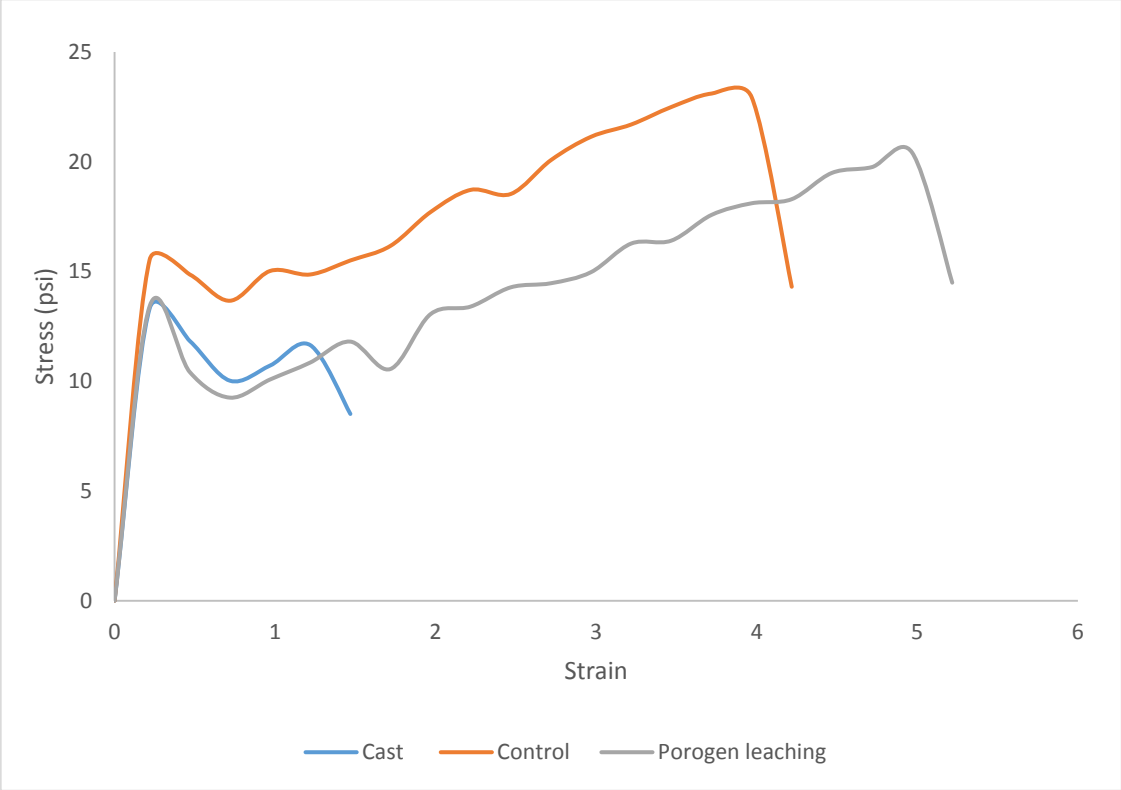


Figure 5: Stress-strain curve of various pore fabrication methods for PVA hydrogels.

Statistical analysis of the data was performed using a One-way ANOVA in RStudio (Boston, MA, USA) with a Type I error rate of 0.05. The results of the analysis are shown in Figure 6. Significance codes '***', '**' and '*' denote the alpha value of 0.001, 0.01 and 0.05 respectively. No statistical differences were found between tensile strengths of the control and porogen leached samples. Yet, casted samples had a significantly lower tensile strength when compared to the two. This is was the expected result due to the lack of pores in the control and the porogen leached samples only presented pores on the surface [24]. Strain rates for each hydrogel were significantly different with the casted sample being the lowest and porogen leaching having the highest as a result of the embedded PCL particles. No significant differences were found between samples when comparing the modulus of elasticity. One main concern for the application of hydrogel synthesis for vascular applications is the potential for rupturing

during testing. Yet, the typical stress arterial pressure is only 2 psi so even the casted hydrogel should suffice [25].

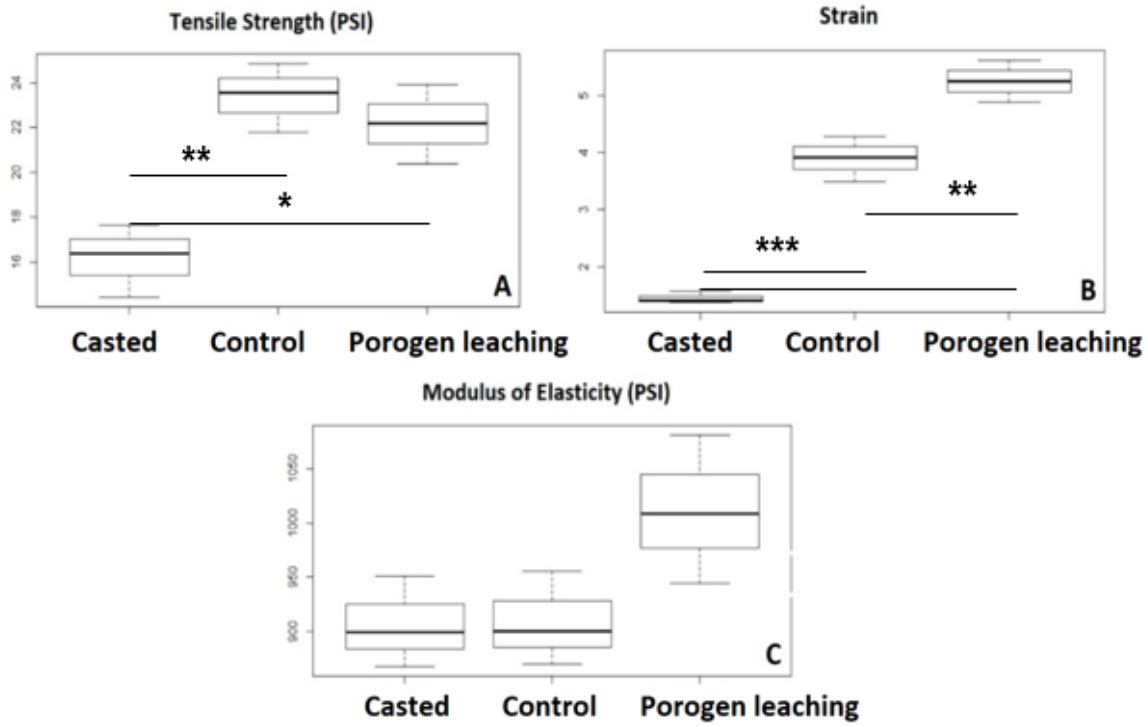


Figure 6: One-way Anova analysis.

3.4. Micrographs of pores

Micrographs of a porogen leached and a pore-casted sample were taken and observed to determine differences, as shown in Figure 7. Analysis of micrographs of the pores concluded that the pore-casted samples (Figure 7: A and C) displayed more consistent geometry and dimensions, possessing an average size of 0.52mm and a standard deviation of 0.02mm. Notably, the porogen-leached samples (Figure 7: B and D) varied significantly throughout in not only dimension but also in geometry, with an average size of 0.31mm and standard deviation of 0.47mm. The lack of interconnected pores in this porogen leached sample would lead to the sample being less favorable for cell attachment and proliferation [3]. E on Figure 7 shows a controlled sample with lines present; these are solely for the beads of the 3D printed model. The analysis concluded that consistency was more favorable in the pore-casted samples. Additionally, the number of pores can be customized, due to the mold printing process, which in turn leads to a tunable and controlled porosity.

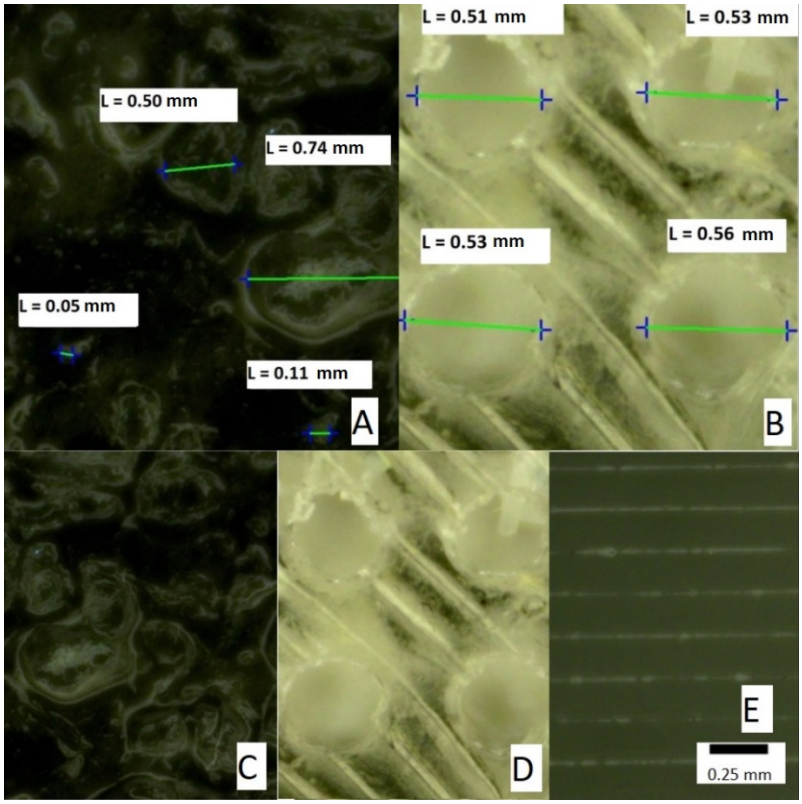


Figure 7: A) Dimensions of pores in porogen leached sample B) Dimension of pores in casted samples C, D, E) Micrograph of porogen leached, casted and control.

4. Conclusions and Future Work

The effects of various pore fabrication techniques on the swelling and degradation kinetics, mechanical properties and pore geometry of PVA hydrogel were investigated. The higher the number of interconnected pores in the hydrogel, the higher the initial swelling and degradation rate of the scaffold. The porogen leaching approach displayed difficulties to extract the inner porogens, leading to lower rates of swelling and degradation due to the properties of the PCL that remained embedded in the sample. This study has shown a method of pore fabrication that may enable tunable swelling and degradation behavior, which is useful when applied to different applications. Mechanical properties showed that with the presence of a larger proportion of pores, the weaker and less malleable the resultant scaffolds become. The ability to customize and acquire controlled porosity using this proposed method will help with the ability to tune the mechanical properties of scaffolds. Future studies will emphasize elucidating how different levels of porosity attained through the proposed method affect tunability. In addition, cell studies are suggested to further justify and validate the resultant biocompatibility that the use of this method of pore fabrication can provide. Lastly, further investigation into other physical cross-linking methods can be completed as comparison to the method used in this study.

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