A COMPARISON STUDY ON THE EFFECTS OF POLYCAPROLACTONE DEGRADATION USING DIFFERENT 3D BIO-PRINTING TECHNOLOGIES

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A COMPARISON STUDY ON THE EFFECTS OF POLYCAPROLACTONE DEGRADATION USING DIFFERENT 3D BIO-PRINTING TECHNOLOGIES

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ABSTRACT

Polycaprolactone (PCL) is a respected polymer for bio-implantation in the field of tissue engineering for scaffolding due to the its compatible and slow bio-degradation properties. While studies have been conducted on the mechanical properties of PCL before and after degradation, minor research has been done on PCL combined with hydroxyapatite (PCL/HA). HA occurs naturally in human bones and calcification, and when combined with PCL creates a rougher texture more suitable to cell adhesion. For this study, PCL and PCL/HA samples were constructed using two different printing methods (selective laser sintering and extrusion-based printing) before undergoing degradation testing. Sintered samples were composed of PCL/HA, while extruded samples were made of PCL-only or PCL/HA, and all three groups were made in a porous and solid version, to total six sample groups. Degradation testing in NaOH solution, to simulate the body's pH, was conducted with all six groups, for periods of 1, 2, and 4 weeks. While significant weight loss and smoothing of surface characteristics was shown across all sample groups, average Young's modulus from compression testing did not show a corresponding significant decrease from non-degraded samples. Further research could include be conducted to expand the range of mechanical testing to include bending tests, as well as longer degradation studies than the one month maximum used for this research to explore at what point degradation impacts structural integrity of scaffolding.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

3D bioprinting is a novel avenue for bio-implant creation, particularly in the field of tissue engineering. Typically, the design to be printed is created digitally, which can be as complex as a support scaffold for an organ or as simple as a rectangular prism for testing mechanical properties of a particular polymer. Polymers and their unique repeating structures offer physical properties that can be exploited for many different uses, including for implanted scaffolds.

In tissue engineering, scaffolds are placed in the body to simulate the extracellular matrix of the existing tissues, increasing desired cellular interactions, helping tissues to grow and stay healthy on the cellular level, as well as providing mechanical function. These scaffolds must be biocompatible in order for the body to accept them, while also degrading over a desired time period (Newman, 2013). One such polymer that meets both of those requirements is polycaprolactone (PCL), a biocompatible and biodegradable polyester with a very slow degradation rate in physiological conditions like the environment of the human body (Tokiwa, 2009). PCL has been FDA approved for use in several different applications to the human body, both internal and external (Woodruff and Hutmacher, 2010), and when combined with hydroxyapatite (PCL/HA), it creates a scaffold with slightly different properties that can be used for different applications (Park, 2011), (Du, 2017). HA is a naturally occurring mineral that is found in a modified state in human bones, enamel, and calcifications throughout the body, and is currently used in medical applications for bone grafting, dental work, or as a coating on implants (Habibah, 2020). Similar to PCL's reduced degradation rate, HA dissolves at a significantly lower rate than bone tissue (Zhu, 2018), but one of the important effects of combining the two

materials is a rougher scaffold surface that increases cell adhesion (Manoukian, 2019). Cell adhesion is a very desirable quality in tissue scaffolds, increasing the impact of the scaffold on regeneration in a specific area of the body. The trade-off is a decrease in stiffness, measured by Young's modulus, which can be a deciding factor when determining which combination of materials to select for a given application (Park, 2010). PCL and PCL/HA scaffolds are typically produced using one of two 3D printing methods, selective laser sintering or extrusion-based manufacturing (Yao, 2015)**,** (Kim, 2010), (Park, 2011), (Park, 2014). More information on the specifics of these manufacturing processes can be found in the methods and materials section of this paper.

Studies have been conducted on degradation of PCL *in vitro* and *in vivo*, showing minimal change in molecular weight *in vitro*, while molecular weight change was present in *in vivo* testing (Lam et. al, 2008). There has also been research on the impact of degradation on mechanical properties for PCL in combination with other materials (Mohamed, 2010), (Zaman, 2015), but not specifically about the degradation impacts on PCL/HA or comparisons to pure PCL. Building a library of expected mechanical properties of different combinations (printing processes, build directions, and polymers) would aid future researchers in picking the best combination of factors for their specific biomedical applications.

This study endeavored to add to the collective knowledge surrounding polymers and bio-implants, focusing specifically on the impact of degradation on the mechanical and surface properties. Samples were constructed through two different 3D printing, or additive manufacturing methods. Polymers that were compared against each other included a pure PCL polymer and a PCL/HA blend. A variety of tests were then conducted at each stage of

degradation (specified to simulate an *in vivo* environment) as well as on non-degraded samples,

to model potential degradation patterns in the human body.

CHAPTER 2
METHODS AND MATERIALS

Creation of Samples

As mentioned in the introduction, the samples required for degradation testing were created using different types of additive manufacturing and polymer blends to compare the impact of degradation on each combination. Two different types of additive manufacturing were employed in the creation of the samples tested. The first is Selective Laser Sintering (SLS), executed by the P110 3D printer. Sintering is the process of forming a cohesive, solid mass from particulate matter by heat, without melting (Deckard, 1986) and for this specific printer, layers of powder are sintered together by a precise laser. The P110 only prints with a specific PCL/HA mixture (4%). The second is extrusion-based printing, employed by the 3D BioPlotter. Extrusion based printing in this case heats up particulates to create a smooth stream of material that is then laid down, layer by layer, in the desired shape. The 3D BioPlotter can print with just PCL, or the PCL/HA mixture. This study aimed to compare (PCL) and PCL/HA (4%) and the two manufacturing techniques, SLS and extrusion-based printing.

Compression Testing

Compression testing of the polymer samples was conducted on the Instron 5944, a universal testing machine with a variety of attachments for mechanically testing samples of all shapes and sizes. For each test, the sample was centered on the immobile base of the Instron, and then the mobile top of the machine moved downward and applied force until the 2kN limit was reached. This is the upper limit of the force the Instron can provide (Illinois, n.d.), which is why that

specific metric was used as the stopping point for each test, rather than a time-based end point. While the sample was being compressed, displacement (in mm) and Force (in kN) were recorded.

Once the compression testing was complete, analysis was conducted using the following procedure to find Young's modulus, a measure of stiffness. First, actual displacement was calculated from the starting point of the data (where the force began increasing from zero), rather than from where the Instron began measuring prior to contact with the sample. Strain was computed using actual displacement divided by the height of the sample. Stress was computed using the kN force measured divided by the area of the sample. Sample dimensions were 10 mm in diameter and 4.8 mm in height. Strain and stress were then plotted in a cartesian plane, and the linear elastic portion of the graph was determined by observation. The slope of the linear portion is the Young's modulus. Higher values indicate stiffer materials.

Degradation Testing

An additional batch of samples with the same dimensions and groups as the initial, non-degraded compression tests, were used for the *in vitro* degradation testing for direct comparison across polymer blend, printing machine, and density (solid or porous).

As shown in Table 1, pictured below, six specific groups were created, based on a variety of factors. Which polymer was used in their creation, if the sample was solid or porous, which printing machine (and therefore method) was used, all contributed to creating the six groups. The table also includes the abbreviation for each group that will be used throughout the rest of this report.

Polymer	Solid or Porous	Printing Machine	Abbreviation
PCL/HA	Solid	P110	PHSP
PCL/HA	Porous	P110	PHPP
PCL/HA	Solid	3D BioPlotter	PHSD
PCL/HA	Porous	3D BioPlotter	PHPD
PCL only	Solid	3D BioPlotter	PSD
PCL only	Porous	3D BioPlotter	PPD

Table 1. Sample groups for degradation.
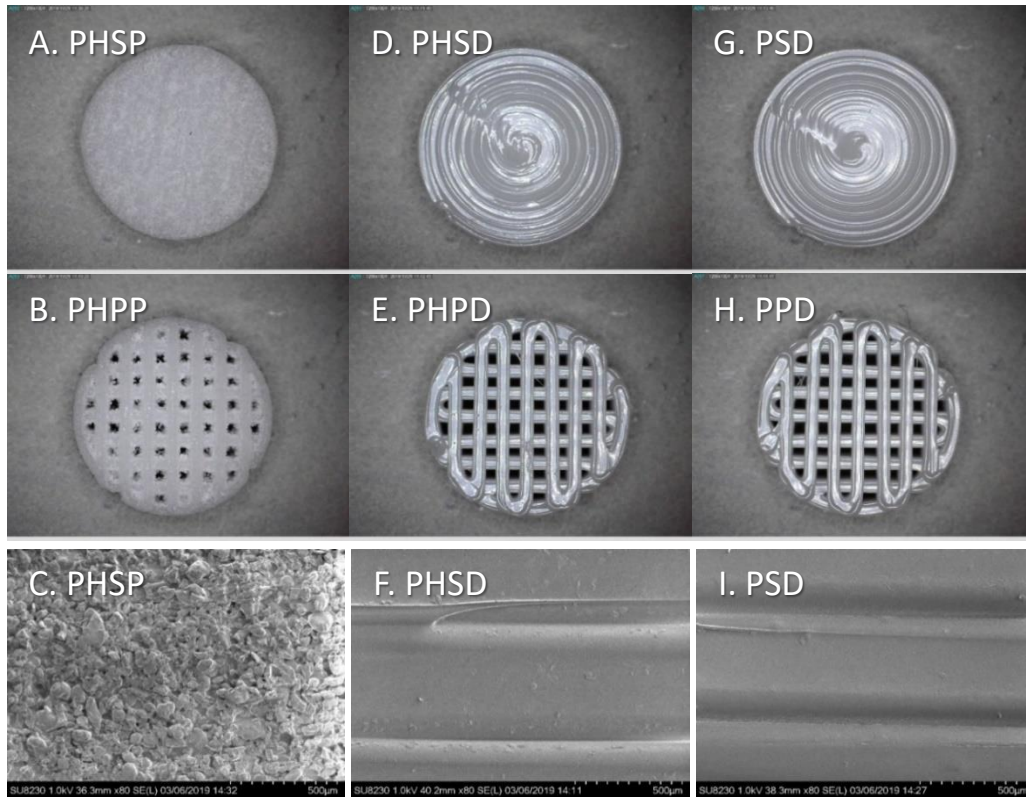
Figure 1. Examples of each sample group.

Figure 1 shows images of each sample group, including SEM close images of the surfaces.

Images C, F, and I are SEM close images of the surfaces of A, D, and G respectively. Each group

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was composed of five samples, making a total of thirty samples for a complete set. Three sets were assembled, for 1 week, 2 week, and 4 weeks of degradation. Thirty samples were additionally printed for a non-degraded control group and underwent compression testing without a waiting period. The environment for degradation was test tubes filled with 1 M NaOH solution. NaOH is a strong base that when added to water, raises the pH of the solution. This was done to simulate human body pH levels for the degradation period for a more accurate experiment.

Each tube was labelled with a number, and then filled with 10 mL of the NaOH solution and one sample. The three sets of thirty samples totaled 90 samples for the 1, 2, and 4 week degradation sets. Once filled, the tubes were then placed in an incubator at 37 degrees Celsius on a shaker, which continually moves the sample to allow fluid motion in and around the sample. When it was time for them to be removed with the rest of the set, they were transferred from the degradation tube to a new tube to be rinsed, in order to remove all traces of the NaOH solution. After a thorough rinse, the samples were dried and ready to be compressed. These compression tests were conducted in the same manner as the non-degraded samples, following the compression protocol above with the Instron 5944.

CHAPTER 3

RESULTS

Several different types of analysis were conducted for the degradation study. These

included mechanical testing to study Young's modulus, microCT analysis of surface and void

characteristics, weight change, and SEM images of surface changes.

**Initial microCT Surface and Void Volume Characteristics**
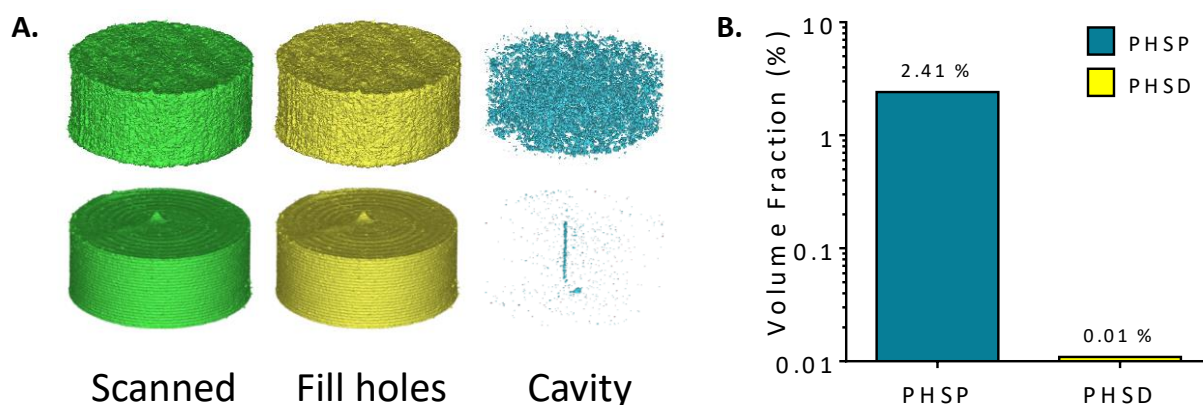
*Figure 2. A. microCT scans comparing PHSP (top) and PHSD (bottom).
B. Graph showing Volume Fraction (%) of cavity microCT scans.*

Figure 2, on the left side of the figure, shows microCT scans – comparing specifically

PHSP and PHSD, in an original scan, with holes filled by the software, and then cavity space.

PHSP is on the top line of the figure, manufactured by sintering with the P110 machine. PHSD,

on the bottom row of the figure, was extruded by the 3D BioPlotter. The microCT analysis was

performed with Mimics Research 21.0 software. For the scanned PHSP image (upper green

object in Figure 2), the threshold range was 1576-11620. For the filled PHSP image (upper

yellow object in Figure 2), the same threshold range from the scanned image was used. For the

cavity PHSP image (upper blue object in Figure 2), the scanned image was subtracted from the

filled hole image, and a Boolean operation was used to calculate the volume fraction shown on
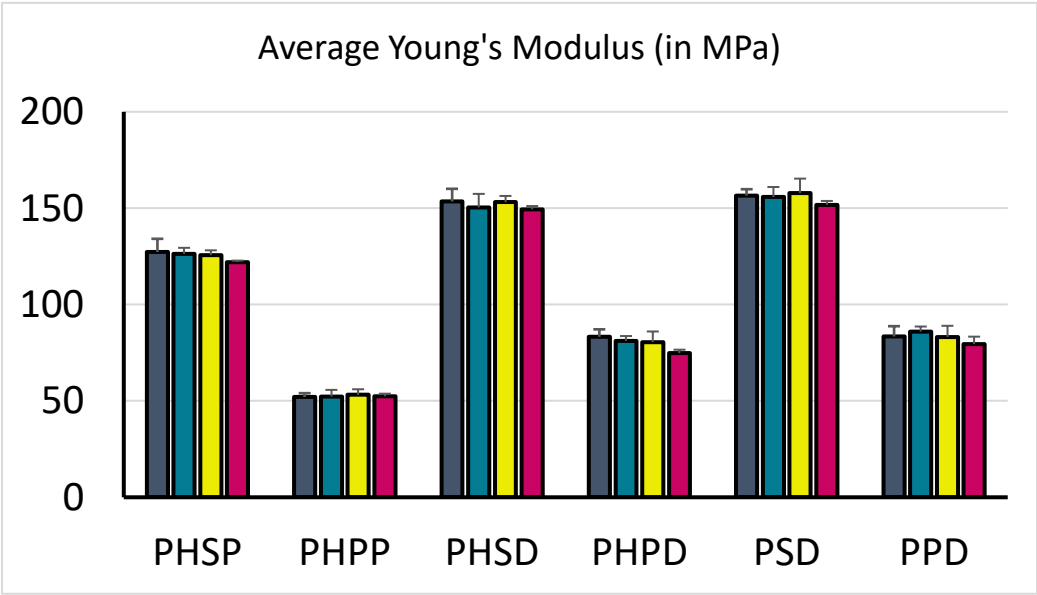
the right side of Figure 2. The same process was repeated for the PHSD in all steps, with new threshold values of 1782–18689.

Degradation Impact on Geometry and Porous Architecture
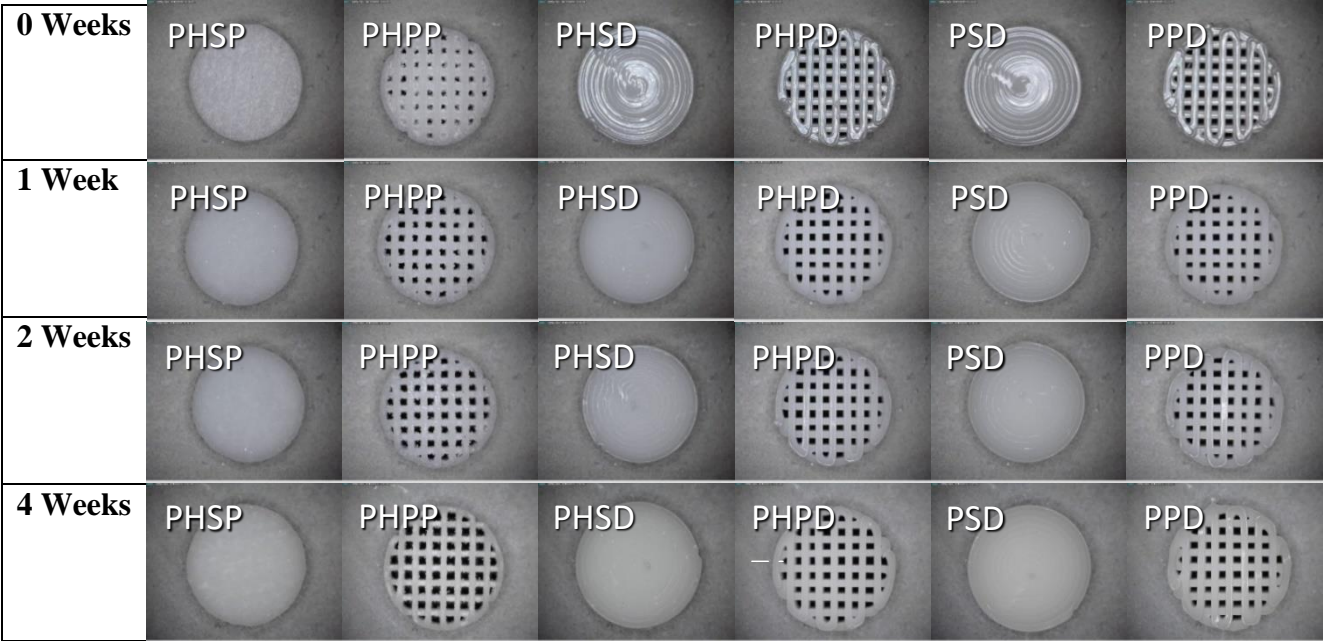
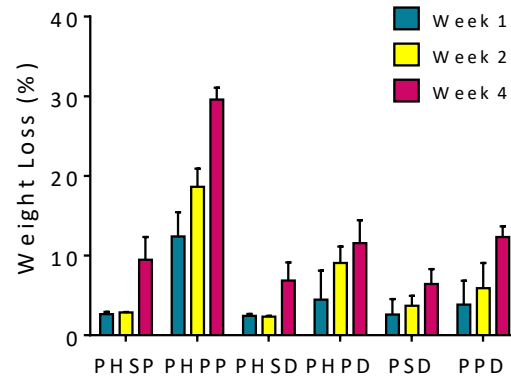*Figure 3. Gross images of the samples after 0, 1, 2, and 4 weeks of degradation.*

Degradation Impact on Young's Modulus

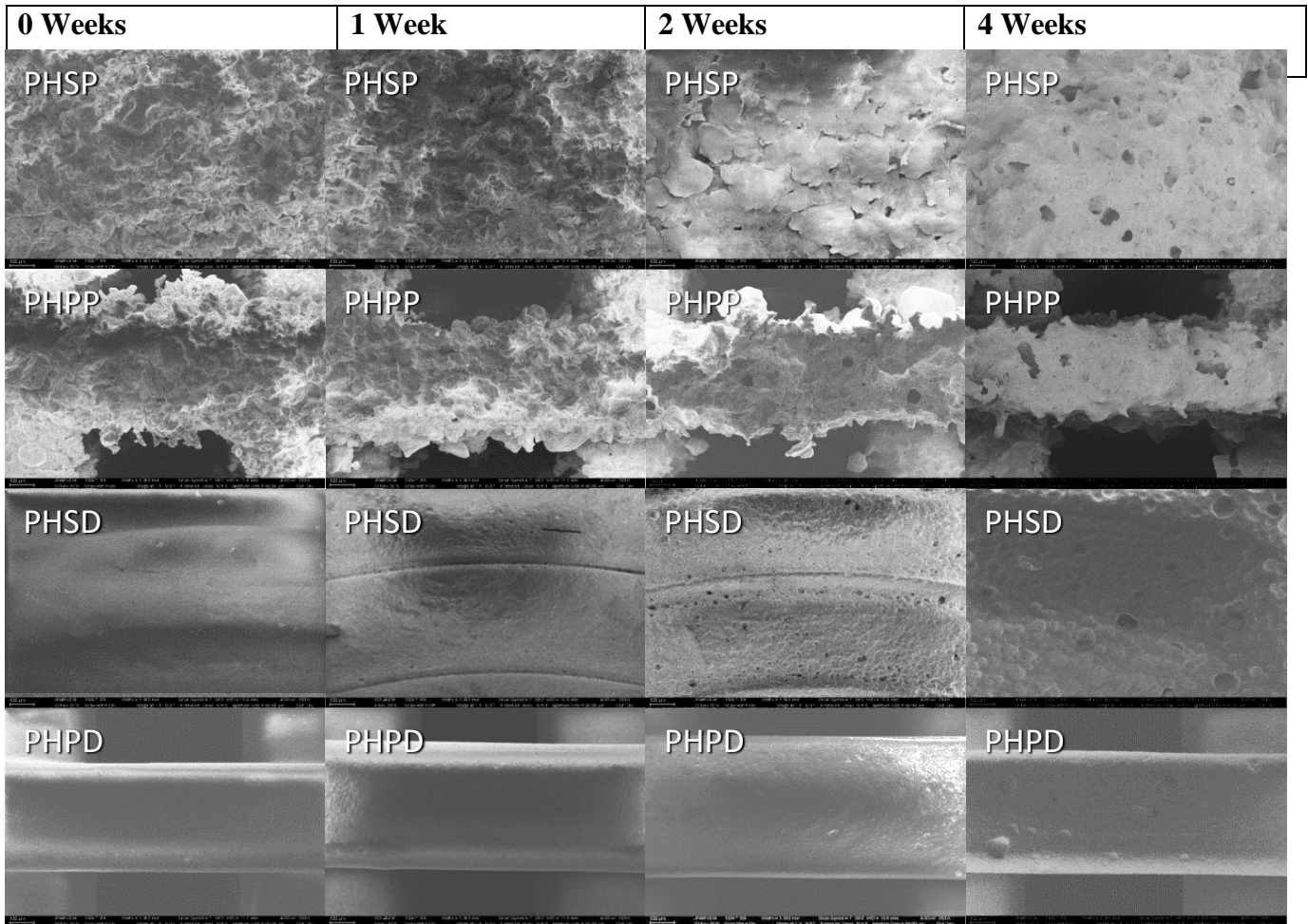*Figure 4: Average Young's Modulus for sample groups at 0, 1, 2, and 4 weeks of degradation.*

Degradation Impact on Weight

Figure 5. Graph of % weight loss compared to non-degraded (0 week) samples for 1, 2, and 4 weeks of degradation.

Degradation Impact on Surface

Figure 6. SEM images of surface changes of PCL/HA samples over the course of the degradation study.

CHAPTER 4

DISCUSSION

Discussion of Study Results

As shown in the images of samples after degradation (see Figure 3) at the standard timepoints (0 weeks, 1 week, 2 weeks, and 4 weeks), a smoothing effect takes place the longer a given sample is in degradation conditions, as the outer layers of the sample are degraded. This impact is visible across all combinations of printing, polymers, and porosity. It is also visible in the SEM images of the surfaces in Figure 6, again across all variations of samples present in the study. An example of the non-degraded solid samples is visualized in Figure 2, as the microCT scans of non-degraded samples show clearly the difference in laser sintered and extrusion based samples - PHSP had a void volume of 2.41%, while PHSD had a void volume of only 0.01%.

However, when viewed in combination with the data collected on average Young's modulus (see Figure 4), there was not significant decrease in the structural integrity of the samples, even when that smoothing had occurred for the maximum time of 4 weeks. As shown in Figure 5, all sample groups experienced weight loss due to degradation. Most notably, the porous groups lost between 10 and 30% of their initial weight by week 4, while solid groups never lost more than 10% of their initial weight. This is due to the increased surface area that came into contact with the degradation solution in the porous samples. As with the smoothing evident through the weeks of degradation, even though significant weight loss was found in these porous groups, there was not significant change in average Young's modulus.

Comparisons to Existing Literature

Comparisons will be drawn between several existing papers on other PCL-only and PCL hybrid studies on mechanical properties and surface changes. In particular, Poh 2016 and Arafat 2011 both have conducted similar studies and provide a baseline for the results gathered in this experiment. Poh's paper used extrusion based printing methods, and also cut down the scaffolds from larger prints, rather than individual prints as described in the materials and methods section of this paper. Poh found the compressive Young's modulus of non-degraded porous PCL-only samples to have a mean of 42.4 MPa. In comparison, the non-degraded porous PCL-only samples tested herein had a much higher average Young's modulus of over 80 MPa. This is most likely due to printing differences, changes in testing method, and the different sizes and porosities of the samples between the two studies.

In regard to the surface and weight changes seen in this study, the literature does support our findings of decreased weight due to the degradation of the samples. Notably, Woodard and Grunlan in their 2019 paper on PCL and PCL-PLLA (another PCL polymer combination) saw degradation of both materials, alone and in combination, under degradation conditions (Woodard, 2018). Figure 6 shows the emergence of HA particles as the PCL around the embedded HA was degraded, especially by week 4. This also falls in line with the findings of other research papers investigating *in vitro* degradation, including Diaz's 2014 paper on *in vitro* degradation of PCL/nHA composites. Díaz and her team found similar accelerated rates of PCL degradation compared to HA (Díaz, 2014).

CHAPTER 5

CONCLUSION

Degradation is an important facet of bio-implants, and it is useful to future researchers to have a strong understanding of which different combinations of polymers and printing methods produce specific mechanical properties and degradation rates. Many polymers can be used for a given implant, depending on the requirements and intended location. PCL is a popular choice as it is biocompatible, biodegradable, and degrades slowly in the body. This study examined printing method, PCL vs. PCL/HA (the addition of which increases cell adhesion), and the effects of degradation on the mechanical properties of the different samples. Overall, solid samples were stiffer than porous samples, and extruded samples were stiffer than sintered samples. Additionally, up to a month of degradation did not induce serious changes in the Young's modulus for any group of samples, regardless of which printing method or polymer combination were used. There was significant weight loss, especially for porous sample groups, and SEM imaging showed significant changes to the surface layers of the samples, but mechanical properties did not change in a significant way for any sample group at any point in the degradation testing. PCL/HA samples showed PCL degrading faster than HA, but smoothing was still evident from visual inspection using SEM imaging.

Opportunities for future research include the introduction of gel permeation chromatography (GPC) to determine molecular weight of degraded vs. non-degraded samples, or differential scanning calorimetry (DSC) to determine specific heat capacity and mechanical behavior. Additionally, more mechanical testing, such as bending tests on rectangular samples could be beneficial for understanding how the samples react not just under compression, but other stresses as well. It could also be beneficial to record longer degradation studies, as seen in

the literature, to determine how long it takes for degradation to impact mechanical properties like

Young's modulus in these sample groups.

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