THE EFFECTS OF ETO STERILIZATION AND POLYDOPAMINE COATING ON PCL-DA/PLLA SEMI-IPN SCAFFOLDS

An Undergraduate Research Scholars Thesis

by

CHRISTOPHER HOUK

Submitted to the Undergraduate Research Scholars program at
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by Research Advisor: Dr. Melissa Grunlan

May 2019

Major: Biomedical Engineering

TABLE OF CONTENTS

ABSTRACT

The Effects of EtO Sterilization and Polydopamine Coating on PCL-DA/PLLA Semi-IPN Scaffolds

Christopher Houk
Department of Biomedical Engineering
Texas A&M University

Research Advisor: Dr. Melissa Grunlan
Department of Biomedical Engineering
Texas A&M University

The current gold standard for repair of cranial bone defects is an autograft procedure which, while having its advantages, lacks proper fitting and is limited by irregular wound geometries. A regenerative approach to cranial bone defects is self-fitting shape-memory polymers (SMPs) tissue scaffolds which are capable of complex geometries and conforming to fit of the defect. Poly(ε-caprolactone)-(diacrylate) (PCL-DA) is a widely studied SMP with few disadvantages such as slow degradation rates (~1-2 years) and lack of osteoconductivity. Thus, in order to improve upon these properties, poly(L-lactic acid) (PLLA) is included as a semi-interpenetrating (semi-IPN) component within the PCL-diacrylate (PCL-DA) network forming a network, wherein PCL-DA is chemically crosslinked while PLLA is independently embedded within the polymer network. Furthermore, in order to promote cell adhesion and aid in neotissue formation, scaffolds are coated with polydopamine to promote bioactivity. After fabrication, scaffolds undergo ethylene oxide (EtO) sterilization prior to implantation. In this study, shape-memory behavior, mechanical properties, and degradation of SMP scaffolds treated with and without EtO sterilization, coated and non-coated will be evaluated to ensure scaffold properties are not negatively affected and maintain desired functionality.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Grunlan, for providing me the opportunity to work in her biomaterials lab.

I extend my thanks to Felipe Beltran for mentoring and guiding me through my undergraduate research.

Lastly, thank you to my family and my girlfriend, for their love and support.

NOMENCLATURE

DMA	Dynamic Mechanical Analysis
EtO	Ethylene Oxide
IPN	Interpenetrating network
NS/C	Non-sterilized and coated
NS/NC	Non-sterilized and non-coated
PCL-(DA)	Poly(ε-caprolactone)-(diacrylate)
PLLA	Poly(L-lactic acid)
S/C	Sterilized and coated
S/NC	Sterilized and non-coated
SEM	Scanning electron microscopy
SMP	Shape-memory polymer

CHAPTER I

INTRODUCTION

The properties of PCL-DA tissue scaffolds prove favorable in cranial bone regeneration due to their ability to conform to the shape of the defect, acting as a lattice, allowing native bone tissue to grow in place of the scaffold, and has tunable degradation when PLLA is added as a semi-IPN component. [1] Thus, it is critical these properties remain consistent when prepared for *in vivo* studies.

PCL-DA shape-memory polymers are defined by two elements: 1) the crystalline lamellae of the PCL-DA "switching segments" and 2) chemical crosslinks "netpoints". [2] When heated above 55 ºC ($T > T_{trans}$), the crystalline lamellae become malleable and allows the scaffold to be temporarily press-fit into irregular defect geometries. Following implantation, the scaffold cools to body temperature (~37 ºC) causing reformation of crystalline lamellae resulting in a rigid and tight fit within the cranial defect.

By blending PCL-DA with PLLA, it is known that this lowers crystallinity by reducing chain mobility. [3] In lieu of a single crosslinked network, PCL-DA and PLLA are formed as a semi-IPN to yield desirable percent crystallinity and maintenance of shape-memory properties. [4] Additionally, by incorporating PLLA at various weight percent, the degradation rate of the semi-IPN can be tuned for suitable applications. [1]

By itself, semi-IPN PCL-DA/PLLA is hydrophobic which prevents cells from adhering and infiltrating the scaffold. Applying a coating of polydopamine to a semi-IPN SMP has shown to improve cell adhesion (by as much as 26-fold) and increase cell number and cell survival. [5] This indicates polydopamine is a viable candidate when considering methods to increase

biocompatibility. However, data regarding the effects on the degradation and physical properties on SMPs is scarce and should be investigated.

Recently, EtO sterilization has risen as one of the most prominent sterilizing methods because of its compatibility with a broad range of medical devices that are heat and/or moisture sensitive. [6] EtO sterilization is a favorable sterilization method due to the sterilizing agent's (ethylene oxide gas) proficiency in attacking cellular proteins/microorganisms and its low operating temperature ranges (25 – 55 ºC). The specific model of EtO sterilization to be used is the AN74*i*/AN75*ix* Anprolene® Sterilizer. Samples are placed in a vacuum chamber where ethylene oxide gas is evaporated within the chamber, killing microorganisms, sterilizing the samples. Other methods of sterilization such as steam or gamma irradiation have shown to degrade or change the physical properties of polymers, [7], [8] which leads to dysfunctionality of the material. Thus, EtO sterilization is the favored method to sterilize a wide array of biomedical devices due to reduced possible effects to device properties. As EtO sterilization effects are not fully understood, investigation is taking place to assess its effects on the properties and functionality of semi-IPN scaffolds.

CHAPTER II

METHODS

Fabrication of PCL-DA/PLLA Semi-IPN Scaffolds

Semi-IPN scaffolds were fabricated using the following PCL-DA/PLLA weight percent: 100:0 (PCL-DA control) and 75:25. Synthesis of PCL-DA and PLLA was preformed via ring-opening polymerization of ε-caprolactone or l-lactide, respectively, in the presence of ethylene glycol initiator and stannous 2-ethylhexanoate catalyst. Terminal hydroxyl groups of PCL-diol were converted to acrylate groups by reacting with acryloyl chloride. [1]

To maintain consistency with *in vivo* studies, fused salt templates were prepared in 20 mL glass scintillation vials. Polymer macromer solutions and 15wt% photoinitiator solution (10wt% DMP in NVP) were deposited into the template, centrifuged, then exposed to UV light for ~3 minutes. After drying overnight, the scaffolds were left in their glass vials and placed in a 50:50 DI water to ethanol solution with daily solution changes for one week. This process, known as particulate leeching, removes the salt thereby exposing pores and removes any unreacted polymer chains. On the second day, the scaffolds were removed from their vials and continued to soak in solution for five more days. The scaffolds were dried overnight at room temperature and polymer caps removed the next day. Casting and particulate leeching are illustrated in Figure 1.

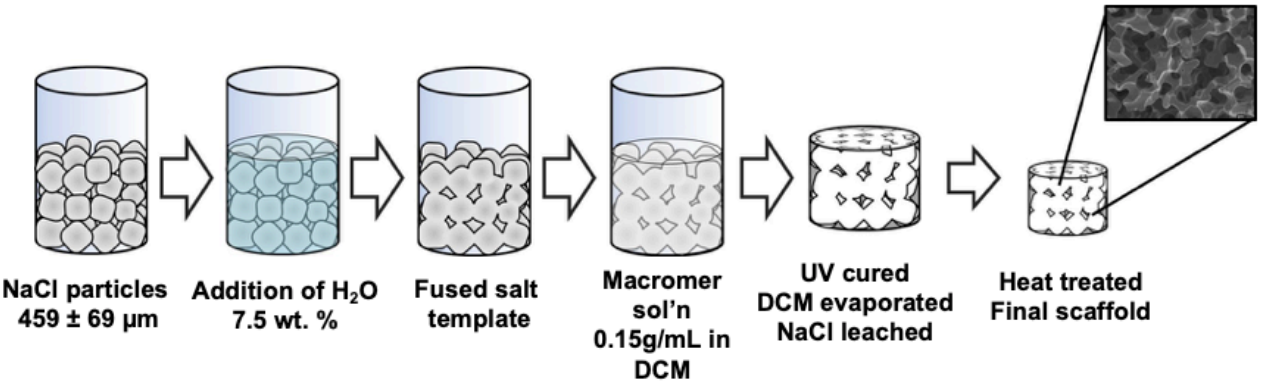

Figure 1: Fabrication of semi-IPN scaffolds. 0.75g of PCL-DA is used for the macromer solution for PCL-DA control (100:0).

Heat Treatment

Following the fabrication process, the scaffolds were placed into a vacuum oven to anneal. Fluctuations and variability of the internal oven temperature made the annealing process difficult without discoloring the PCL-DA/PLLA scaffolds. Discoloration of the scaffolds is indicative of the PLLA polymer degrading (T = 230°C). Various annealing times and temperatures were analyzed to determine what combination of time and heat was effective. Scaffolds were annealed for approximately 15 minutes at ~200°C then cooled to room temperature overnight. Scaffolds were secured to circular mounts using superglue and placed into a vibratome (Leica VT1000S) and cut horizontally into 2 mm slices.

Non-coated Scaffolds

The 2 mm scaffolds were categorized into four compositions for both the PCL-DA (100:0) and the PCL-DA/PLLA (75:25) series: non-coated/non-sterilized (NC/NS), coated/non-sterilized (C/NS), non-coated/sterilized (NC/S), and coated/sterilized (C/S) for a total of eight compositions (represented in Figure 2). Scaffolds organized into the non-coated category were punched into 6 mm samples.

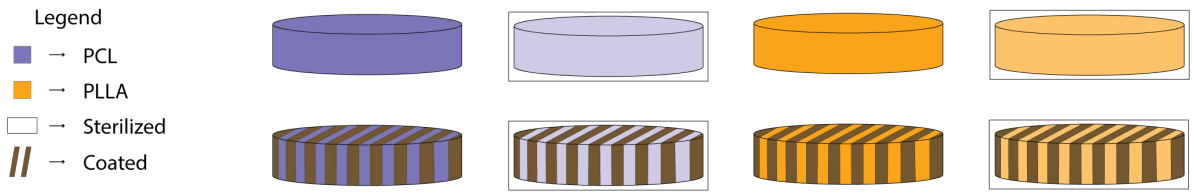

Figure 2: Illustration of the eight compositions to be tested.

Coated Scaffolds

Scaffolds determined to undergo a polydopamine coating were prepared accordingly. A 200 mL dopamine hydrochloride solution was prepared (2 mg/mL in 10mM Tris buffer) in a 1L container. The solution was stirred for ~30 minutes then transferred to a shallow Pyrex® dish. With a 20G needle and a wire wrapped around the hub, scaffolds were gently submerged into the polydopamine solution, and anchored to the rim of the dish. Scaffolds were degassed by attaching a syringe to the needle hub and pulling air out of the scaffold. The dish was placed on a stir plate for 16 hours followed by rinsing scaffolds with DI water. Scaffolds were dried overnight then punched into 6 mm samples. Figure 3 presents a visual for non-coated and coated samples.

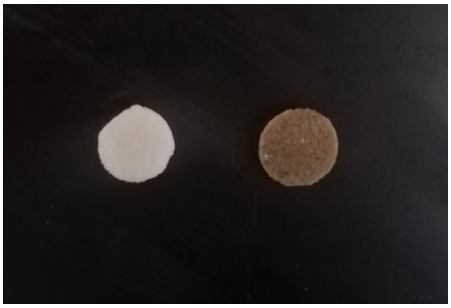

Figure 3: A non-coated sample (left, 6 x 2 mm) and coated sample (right, 6 x 2 mm).

Sterilization

Samples were wrapped and placed into a liner bag followed by a glass ampoule of liquid ethylene oxide encased in a gas release bag. The purge tube end was inserted into the liner bag with the bag secured to the neck of the purge tube via Velcro®. The bag was then purged inside

the sterilizer compartment. After completing the purge cycle, the glass ampoule was broken and the compartment door closed. The sterilization cycle was set for 24 hours. At the end of the cycle, the bags containing the samples were removed and sterilization strips indicated the samples were effectively sterilized.

Shape-Memory Behavior

To test shape-memory behavior, both qualitative and quantitative assays were conducted. A qualitative assay consisted of submerging samples in a warm saline solution (T = 60°C) for ~10 – 20 seconds. Upon removing from the solution, the scaffold was malleable and confined to an irregular shape then allowed to cool to room temperature. After some time, scaffolds were reintroduced into the saline solution for ~10 – 20 seconds and allowed to reform to permanent shape.

Dynamic mechanical analysis (DMA, TA Instruments® Q800) was used to quantitatively assess shape-memory properties. DMA was performed in triplicate for all eight compositions and the recovery and fixity of each cycle was calculated using equations 1 and 2 respectively.

$$R_r = \frac{final\ height}{initial\ height} * 100 \quad (1)$$

$$R_f = \frac{final\ compressed\ height}{initial\ compressed\ height} * 100 \quad (2)$$

Compression Testing

Compression properties were evaluated by placing samples (6 mm x 2 mm, $N = 6 – 10$) between compression clamps (Instron® 5944). The instrument compressed at a rate of 1.50 mm min^{-1} and the compressive Young's Modulus was calculated.

Accelerated Degradation

A model for accelerated degradation consisted of preparing a 0.2 M sodium hydroxide (NaOH) solution. Four timepoints were prepared in triplicate for each composition. Specimens

were weighed on an analytical balance then placed in 20 mL scintillation vials. 10 mL of NaOH solution was deposited into each vial and vials were placed in an incubation chamber and subjected to 60RPM at 37°C. Samples were removed at 24, 72, 120, and 168 hours, rinsed with DI water to remove excess solution, placed in a well plate to dry overnight in vacuo then weighed the following day.

Statistical Analysis

Results obtained are shown as the mean ± standard deviation. Values were compared using a Student's *t*-test to determine *p*-values.

CHAPTER III

RESULTS AND DISCUSSION

Shape-Memory Behavior

In Figure 4, qualitative results for each composition of PCL-DA/PLLA (75:25) samples are shown. The arrow indicated by 1 is when the sample is heated in a 60°C saline solution, deformed to an irregular shape then cooled to room temperature. Arrow 2 indicates when the sample is returned to the saline solution then allowed to cool without confinement.

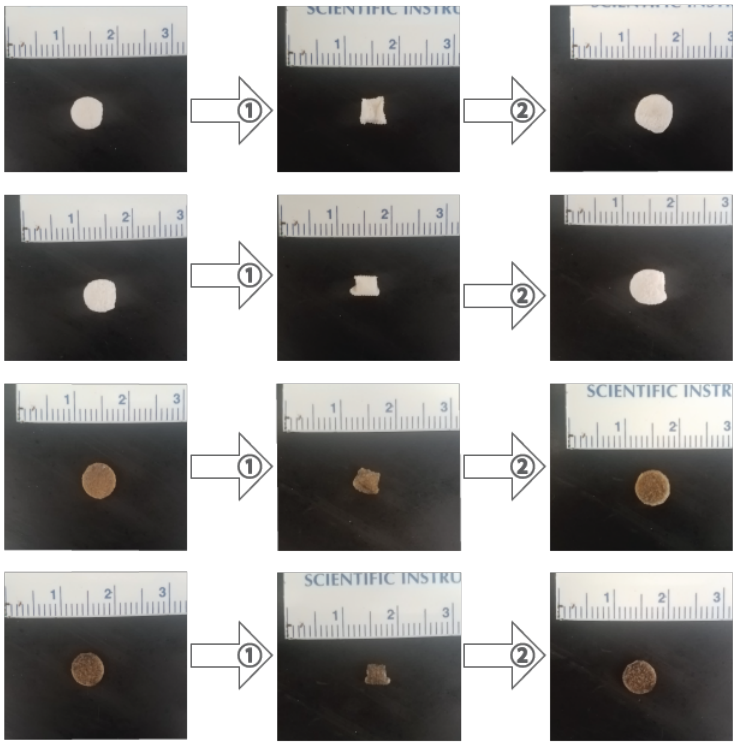

Figure 4: Qualitative shape-memory testing for PCL-DA/PLLA (75:25) for NC/NS, NC/S, C/NS, C/S respectively.

In the tables below (Table 1, Table 2), the results from DMA are quantitatively tabulated into shape fixity (R_f) and shape recovery (R_r) for cycle 1 and cycle 2.

Table 1: Shape fixity and shape recovery of PCL-DA (100:0) using DMA.

PCL-DA	$\mathbf{R_{f1}}$	$\mathbf{R_{r1}}$	$\mathbf{R_{f2}}$	$\mathbf{R_{r2}}$
NC/NS	99.3 ± 1.7	71.0 ± 4.0	100.4 ± 0.7	100.2 ± 0.4
NC/S	99.4 ± 0.5	88.4 ± 12.5	99.6 ± 0.3	99.6 ± 0.3
C/NS	100.8 ± 0.7	99.0 ± 3.5	100.3 ± 1.0	100.3 ± 1.0
C/S	99.9 ± 0.8	90.8 ± 5.8	100.1 ± 0.6	100.1 ± 0.6

Table 2: Shape fixity and shape recovery of PCL-DA/PLLA (75:25) using DMA.

PCL-DA/PLLA	$\mathbf{R_{f1}}$	$\mathbf{R_{r1}}$	$\mathbf{R_{f2}}$	$\mathbf{R_{r2}}$
NC/NS	100.7 ± 0.6	84.0 ± 15.6	99.7 ± 0.6	99.8 ± 0.4
NC/S	104.8 ± 5.3	81.4 ± 4.0	100.3 ± 0.5	99.2 ± 0.6
C/NS	100.3 ± 0.5	92.8 ± 3.3	100.3 ± 0.5	99.7 ± 0.3
C/S	100.6 ± 0.5	95.7 ± .0	100.3 ± 0.6	99.5 ± 0.0

Tabulated results indicate that regardless of coating or sterilization, shape-memory behavior was unaffected for scaffold samples ($p > 0.05$).

Mechanical Testing

The compressive modulus for both PCL-DA (100:0) and PCL-DA/PLLA (75:25) are shown in Figure 5.

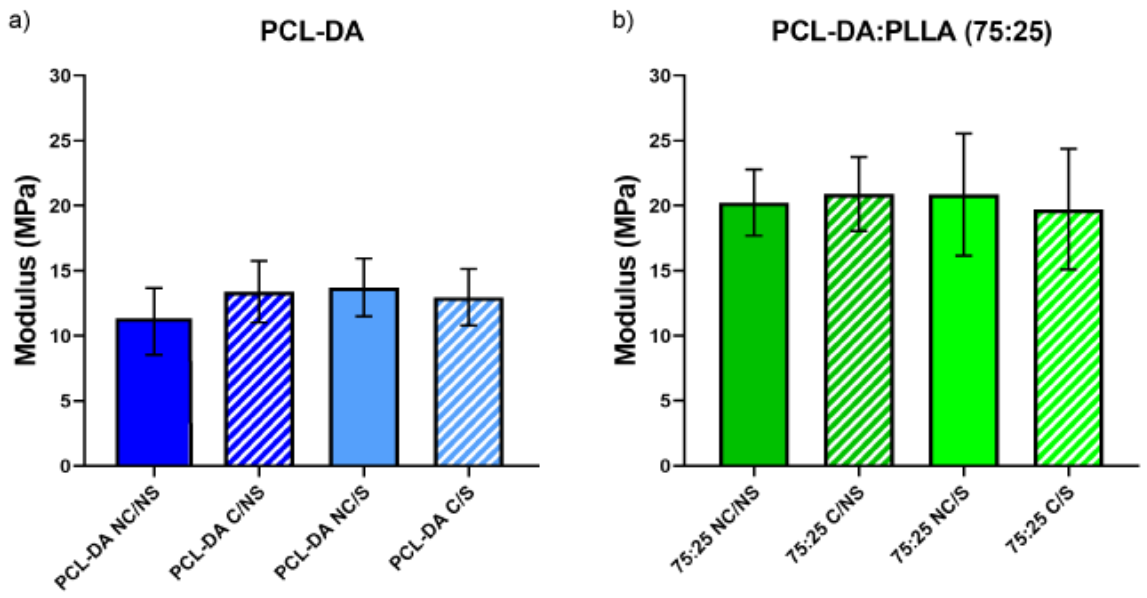

Figure 5: a) Compressive modulus for PCL-DA (100:0). b) Compressive modulus for PCL-DA/PLLA (75:25). For $p < 0.05$, no comparison met the criterion.

Compressive results show similar moduli for each series indicating that mechanical properties remain unaffected by sterilization or coating.

Accelerated Degradation

In Figure 6, the result from accelerated degradation is shown.

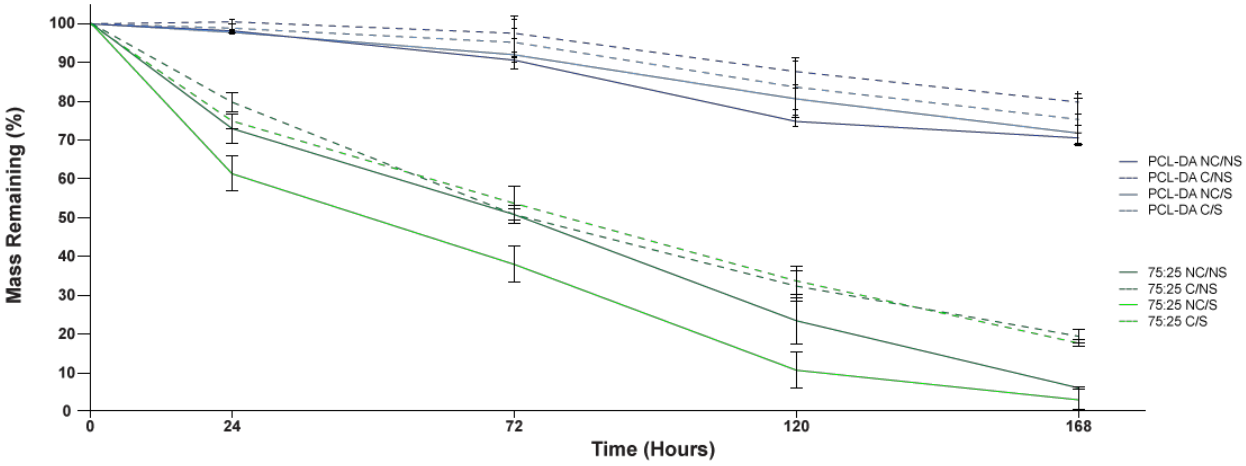

Figure 6: Accelerated degradation for both series over one week.

Mass remaining at 168 hours was converted to a bar graph (Figure 7) to determine difference among compositions.

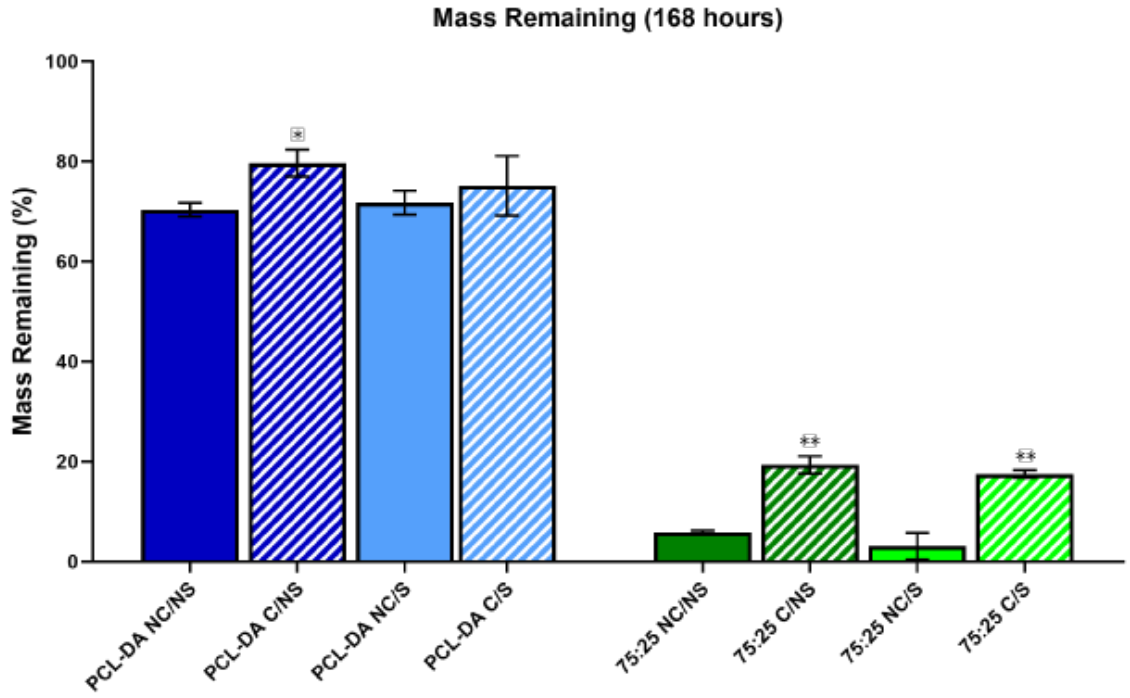

Figure 7: Mass remaining (%) after 168 hours for the eight compositions. *$p < 0.05$ vs corresponding PCL-DA control. **$p < 0.0001$ vs corresponding PCL-DA/PLLA (75:25).

At 168 hours, the mass remaining as a percentage of the initial mass for PCL-DA in sequential order was: 70.36 ± 1.35, 79.70 ± 2.67, 71.75 ± 2.40, 75.17 ± 5.98. Similarly, for PCL-DA/PLLA (75:25), the mass remaining was: 5.86 ± 0.37, 19.35 ± 1.72, 3.10 ± 2.68, 17.51 ± 0.76.

In Figure 8, the progression of degradation for PCL-DA/PLLA (75:25) is shown.

Figure 8: Left to right: 24, 72, 120, 168 hour timepoints. Top to bottom: NC/NS, NC/S, C/NS, C/S.

Accelerated degradation results show samples were not statistically different among sterilization groups. Coated scaffolds, however, show samples retained statistically more mass compared to non-coated scaffolds.

CHAPTER IV

CONCLUSION AND FUTURE WORK

In this paper, the effects of ethylene oxide sterilization and polydopamine coating were evaluated on PCL-DA and semi-IPN PCL-DA/PLLA (75:25) scaffolds. Shape-memory, compressive modulus, and accelerated degradation studies were conducted among the various compositions. Shape-memory results indicate that shape-memory behavior remained unaffected by these factors. Results from compression testing show moduli were also unaltered. With regards to accelerated degradation, sterilization does not affect rate of degradation. However, scaffolds coated with polydopamine show less mass loss after one week.

Examination into why coated scaffolds exhibit lower mass loss is currently being conducted. Comparative analysis among others in the Grunlan Lab coupled with individual studies will facilitate in drawing conclusions. SEM will be employed to analyze surface topography and porosity from the degradation study. Furthermore, C/NS and C/S samples will be fabricated and immersed in simulated body fluid to initiate mineralization of hydroxyapatite (HAp). Mineralization deposits formed on the samples will be analyzed via SEM-EDS (Energy-Dispersive Spectroscopy) and mechanically tested (following protocols mentioned in this paper) to verify HAp formation and effects on scaffold compressive moduli, respectively.

REFERENCES

[1] Woodard, Lindsay N., et al. "PCL-PLLA Semi-IPN Shape Memory Polymers (SMPs): Degradation and Mechanical Properties." *Macromolecular Rapid Communications* 37.23 (2016): 1972-77. Print.

[2] Lendlein, Andreas, et al. "Shape-Memory Polymers as a Technology Platform for Biomedical Applications." *Expert Review of Medical Devices* 7.3 (2010): 357-79. Print.

[3] Kweon, HaeYong, et al. "A Novel Degradable Polycaprolactone Networks for Tissue Engineering." *Biomaterials* 24.5 (2003): 801-08. Print.

[4] Lu, X. L., W. Cai, and Z. Y. Gao. "Shape-Memory Behaviors of Biodegradable Poly(L-Lactide-Co-E-Caprolactone) Copolymers." *Journal of Applied Polymer Science* 108.2 (2008): 1109-15. Print.

[5] Lynge, Martin, et al. "Polydopamine—a Nature-Inspired Polymer Coating for Biomedical Science." *Nanoscale* 3.12 (2011): 4916-28. Print.

[6] Angerer, J., M. Bader, and A. Krämer. "Ambient and Biochemical Effect Monitoring of Workers Exposed to Ethylene Oxide." *International Archives of Occupational and Environmental Health* 71.1 (1998): 14-18. Print.

[7] Alariqi SAS, Mutair AA, Singh RP. "Effect of Different Sterilization Methods on Biodegradation of Biomedical Polypropylene." *Journal of Environmental and Analytical Toxicology* 6:373 (2016). Print.

[8] Ahmed, Maqsood, et al. "Effects of Sterilization Treatments on Bulk and Surface Properties of Nanocomposite Biomaterials." *Journal of biomedical materials research. Part B, Applied biomaterials* 101.7 (2013): 1182-90. Print.