

***IN- VITRO* REAL-TIME DEGRADATION OF “SELF-FITTING”  
PCL-DA/PLA SEMI-IPN SCAFFOLDS FOR CRANIAL BONE DEFECTS**

An Undergraduate Research Scholars Thesis

by

RABIA ALI

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 A. Grunlan

May 2020

Major: Biomedical Engineering

# TABLE OF CONTENTS

	Page
ABSTRACT.....	1
ACKNOWLEDGMENTS .....	3
NOMENCLATURE .....	4
CHAPTER	
I. INTRODUCTION .....	5
II. MATERIALS AND METHODS.....	9
Materials .....	9
Polymer Syntheses .....	9
Scaffold Fabrication.....	11
Characterization .....	13
Accelerated Degradation.....	14
Neutral Degradation.....	15
Statistical Analysis.....	15
III. RESULTS .....	16
Scaffold Fabrication Verification .....	16
Base-catalyzed Degradation.....	19
Real-time Degradation .....	21
Post-degradation Scaffold Characterization .....	25
IV. CONCLUSION.....	32
REFERENCES .....	33

## ABSTRACT

*In-vitro* Real-time Degradation of “Self-fitting”  
PCL-DA/PLA Semi-IPN Scaffolds for Cranial Bone Defects

Rabia Ali  
Department of Biomedical Engineering  
Texas A&M University

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

Autografts remain the current “gold standard” for treating craniomaxillofacial (CMF) bone defects despite the difficulty in fitting rigid grafts into complex shaped defects. To improve CMF bone treatment, the Grunlan Lab has proposed “self-fitting” shape memory polymer (SMP) scaffolds based on a poly( $\epsilon$ -caprolactone)-diacrylate (PCL-DA) network. Upon heating ( $T_{\text{trans}} \sim 55^\circ\text{C}$ ), the scaffolds become malleable and can be easily press-fitted into defects for improved osseointegration. Recently, to accelerate the degradation rate of the scaffolds to more closely match bone regeneration, various poly(lactic acid) (PLA) thermoplastics were incorporated into the thermoset PCL-DA network. The resulting PCL-DA/PLA semi-interpenetrating networks (semi-IPNs) demonstrated tunable degradation rates while maintaining SMP behavior. However, the study was limited to nonporous solid films and degradation studies were limited to base-catalyzed conditions (1.0 M NaOH, pH $\sim$ 14, 37 °C). Herein, PCL-DA/PLA semi-IPN porous scaffolds were examined for their degradation behavior under both base-catalyzed and neutral, more physiologically relevant, conditions (1X PBS, pH $\sim$ 7.4, 37 °C). The

base-catalyzed conditions altered the hydrolysis kinetics of several compositions and thus did not accurately predict which compositions degraded quickly under neutral conditions. Under neutral conditions, low  $M_n$ , amorphous, and hydrophilic PLA thermoplastics promoted the fastest scaffold degradation. Additionally, post-degradation thermal analyses showed that even in the fastest degrading PLC-DA/PLA semi-IPNs, both PCL and PLLA were degrading, proving that the incorporation of PLAs can promote accelerated degradation of PCL.

## **ACKNOWLEDGMENTS**

I would like to thank Michaela Pfau and Dr. Melissa A. Grunlan for their constant mentorship and guidance throughout this project as well as during my two years in Grunlan Lab. Research has had a profound effect on my time at Texas A&M and it is thanks to them. I would like to thank my parents for their unending support of my goals and for motivating me to excel not only in research but in all areas of life. Thanks to LAUNCH offices for giving me the opportunity to partake in this amazing program and gain a new perspective on research.

## NOMENCLATURE

CMF	Craniomaxillofacial
DSC	Differential Scanning Calorimetry
PBS	Phosphate Buffered Saline
PCL-DA	Poly( $\epsilon$ -caprolactone)-diacrylate
PGA	Poly (glycolic acid)
PLA	Poly (lactic acid)
PLGA	Poly (lactic-co-glycolic acid)
PLLA	Poly (L-lactic acid)
SCPL	Solvent-casting Particulate-leaching
SEM	Scanning Electron Microscopy
Semi-IPN	Semi-Interpenetrating Network
SMP	Shape Memory Polymer
TGA	Thermal Gravimetric Analysis

# CHAPTER I

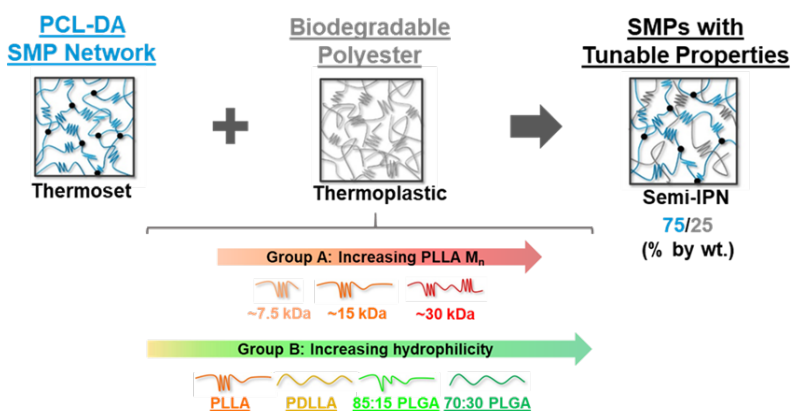
## INTRODUCTION

Despite their shortcomings, autografts remain the most prevalent method for treating craniomaxillofacial (CMF) bone defects including congenital defects like cleft palate and confined cranial bone defects such as those commonly caused by traffic accidents.<sup>2,3</sup> Along with the primary obstacle of shaping the autograft to fit into complex defects, there is also risk of infection, limited supply of donor tissue, the need for second surgeries, and other risks such as hematoma or sensory loss.<sup>4</sup> To overcome these barriers in treating CMF bone defects, the Grunlan Lab proposed shape memory polymers (SMPs) as biodegradable “self-fitting” scaffolds as a regenerative tissue engineering treatment. The SMP scaffolds are based on a poly( $\epsilon$ -caprolactone)-diacrylate (PCL-DA,  $M_n \sim 10$  kDa) network where PCL crystalline lamellae act as switching segments responsible for the temporary shape and chemical cross-links act as netpoints responsible for the permanent shape. In a clinical setting, scaffolds can be warmed in saline ( $T > T_{trans}$ ;  $T_{trans} = \sim 55$  °C) allowing them to become malleable. Then, the softened scaffolds can be easily “press-fitted” into the precise shape of the bone defect to achieve a tight fit between the scaffold and the defect surface, essential for improving osseointegration.<sup>5</sup> Once the scaffold cools to body temperature ( $\sim 37$  °C), it return to its mechanically robust, rigid state and stays locked into the conformational shape of the defect. PCL-DA SMP scaffolds were fabricated via solvent-casting particulate-leaching (SCPL) resulting in highly interconnected pores ( $d \sim 200$   $\mu\text{m}$ ,  $\sim 70\%$  porous), necessary for osteoconductivity.<sup>6</sup> The porous PCL-DA scaffolds were also shown to feature robust, non-brittle mechanical properties with a compressive strength (CS) of  $\sim 20$  MPa.<sup>7</sup>

Although PCL-DA SMP scaffolds showed promising properties for treating CMF bone defects, slow degradation of PCL *in vivo* (2+ years)<sup>8</sup> may limit effective bone regeneration (~3-12 weeks).<sup>1</sup> Therefore, thermoplastic poly(L-lactic acid) (PLLA,  $M_n \sim 15$  kDa) was incorporated into the thermoset PCL-DA network as a semi-IPN at varying weight ratios [100% PCL-DA, 90/10, 75/25, and 60/40 PCL-DA/PLLA]. PCL-DA/PLLA semi-IPNs proved advantageous as the PLLA did not disturb PCLs crystallinity, necessary for the SMP “self-fitting” behavior. In addition, PCL-DA/PLLA semi-IPNs demonstrated dramatically accelerated degradation with increasing PLLA content compared to the 100% PCL-DA scaffolds (1.0 M NaOH, pH~14, 37 °C).<sup>1, 9</sup> Notably, the 75/25 PCL-DA/PLLA semi-IPN scaffolds demonstrated the most improved compressive modulus (more “bone-like”) coupled with accelerated degradation (towards bone neotissue formation). Phase separation between PCL and PLLA detected and was shown to be linked to the accelerated degradation of PCL-DA/PLLA semi-IPNs. The phase separation may have led to greater diffusion of water into the scaffolds which ultimately resulted in faster hydrolysis. However, in follow-up studies where degradation was performed under neutral conditions (1X PBS, pH~7.4, 37 °C) the PCL-DA/PLLA semi-IPNs did not degrade as rapidly, but still degraded significantly faster than 100% PCL-DA controls.<sup>10, 11</sup> The degradation behavior of PCL-DA based semi-IPNs was further probed by varying structural properties of the thermoplastic PLLA component including  $M_n$ , hydrophilicity, and crystallinity while maintaining wt. % at 75/25 (PCL-DA/PLA). As shown in Figure 1, *Group A* compositions included semi-crystalline poly(L-lactic acid) (PLA) with varying  $M_n$ s [ $\sim 7.5$ ,  $\sim 15$ , and  $\sim 30$  kDa]. For *Group B*,  $M_n$  was held constant at  $\sim 15$  kDa and hydrophilicity was varied via copolymerization of PLLA and poly(glycolic acid) (PGA) at two varying mol ratios (85:15 and 70:30 LA:GA); amorphous poly(D,-L-Lactide) (PDLLA) was also included to assess differences

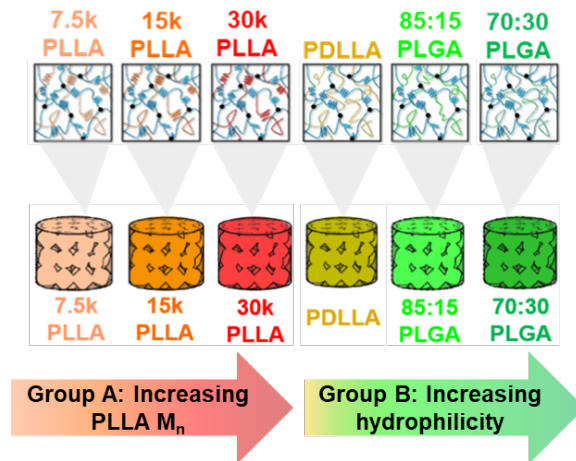


in crystallinity. The study was limited to solid films and base-catalyzed degradation (1.0M NaOH, pH~14, 37 °C)<sup>9</sup> but the PCL-DA/PLA semi-IPNs demonstrated tunable degradation rates with well-maintained SMP and mechanical properties. Again, the accelerated degradation rates were linked to relative PCL/PLA miscibility or phase separation. Selected compositions were annealed at a higher temperature (160 °C compared to 85 °C previously) and the resulting semi-IPNs showed reduced phase separation and slowed degradation.



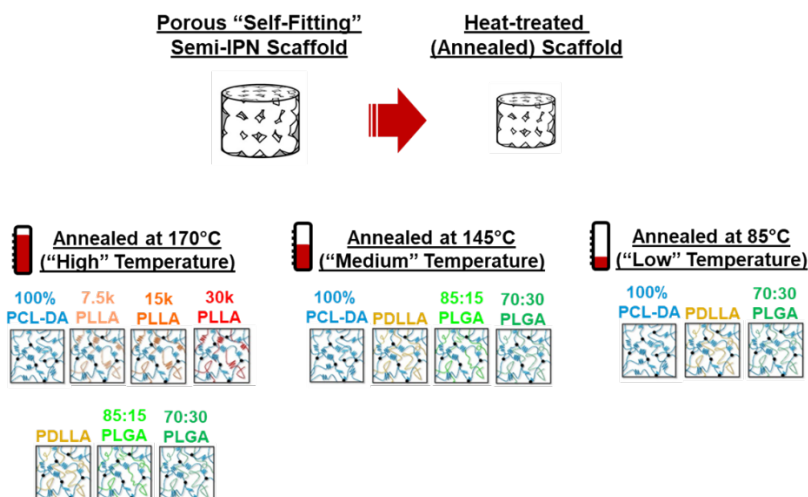
**Figure 1.** PCL-DA based semi-IPNs were prepared using PLAs with varying  $M_n$  and with varying hydrophilicity.

Herein, analogous porous scaffolds (Figure 2) will be examined for their degradation behavior under both base-catalyzed and neutral conditions. Previous work in our group and literature indicate that base-catalyzed conditions may not accurately predict hydrolysis kinetics of polyesters under neutral conditions. High pH conditions tend to produce surface erosion as the rate of hydrolysis is greater than the rate of diffusion, while, neutral conditions tend to exhibit bulk degradation as the rate of diffusion is greater than the rate of hydrolysis.<sup>12</sup> This study aims to determine the differences in PCL-DA/PLA semi-IPN scaffold hydrolysis kinetics under base-catalyzed and neutral conditions in order to more accurately predict their degradation rates and associated erosion behavior *in vivo*.



**Figure 2.** PLC-DA semi-IPN scaffolds designated by their thermoplastic components.

In addition to the varying semi-IPN compositions, three different temperatures were used to anneal the scaffolds: “High Temperature” at 170 °C, “Medium Temperature” at 145 °C, and “Low Temperature” at 85 °C (Figure 3). Annealing temperature has been shown to affect pore size, with scaffolds containing semi-crystalline PLLA shrinking to the size of 100% PCL-DA controls only when heated above the PLLA melting temperature ( $T_m$ ).<sup>1</sup> This study was designed to examine the impact of annealing temperature on phase separation and subsequent degradation without varying pore size. As such, annealing temperatures were tuned and assigned to selected scaffold compositions as dictated in Figure 3 in order to achieve consistent pore size.



**Figure 3.** Annealing temperatures were also varied (170 °C, 145 °C, and 85 °C), and compositions selected for each temperature to achieve consistent pore size are shown.

## CHAPTER II

### MATERIALS AND METHODS

#### Materials

Poly( $\epsilon$ -caprolactone)-diol (PCL,  $M_n \sim 10$  kg/mol), 3,6-dimethyl-1,4-dioxane-2,5-dione (D,L-lactide), (3S)-cis-3,6-dimethyl-1,4-dioxane-2,5-dione (L-Lactide), glycolide, stannous (II) 2-ethylhexanoate  $\text{Sn}(\text{Oct})_2$ , triethylamine (Et<sub>3</sub>N), acryloyl chloride, 4-dimethylaminopyridine (DMAP), 2,2-dimethoxy-2-phenylacetophenone (DMP), 1-vinyl-2-pyrrolidinone (NVP), potassium carbonate ( $\text{K}_2\text{CO}_3$ ), magnesium sulfate ( $\text{MgSO}_4$ ), sodium chloride (NaCl), sodium hydroxide (NaOH), phosphate-buffered saline (PBS, 1X), ethylene glycol, and solvents such as dichloromethane (DCM) and methanol were obtained from Sigma-Aldrich. Solvents and ethylene glycol were dried over 4 Å molecular sieves before use and all monomers were vacuum dried prior to use. All glassware was dried for at least 12 hours at 120°C prior to use.

#### Polymer Syntheses

All reactions were run under a nitrogen ( $\text{N}_2$ ) atmosphere with a Teflon-covered stir bar. Chemical structures and  $M_n$  of the purified polymers were confirmed with  $^1\text{H}$  NMR spectroscopy (Inova 500 MHz spectrometer in the FT-mode with  $\text{CDCl}_3$  as the standard). Polymers had been previously well characterized via  $^1\text{H}$  NMR end group analysis to verify that target  $M_n$  and percent acrylation had been achieved through synthesis.<sup>9</sup>

#### *PCL-DA Synthesis*

PCL-diacrylate (PCL-DA) (95% acrylation) was prepared via reaction of PCL-diol ( $M_n = 10$  kDa;  $T_g = -65^\circ\text{C}$ ,  $T_m = 53^\circ\text{C}$ , 46.5% crystallinity) with acryloyl chloride, as previously reported.<sup>6</sup>

### *PLLA and PDLLA Synthesis*

Thermoplastic PLLA of varying  $M_n$  (~7.5, ~15, and ~30 kDa) was synthesized via ring opening polymerization (ROP) of L-lactide.<sup>13</sup> L-lactide was combined with ethylene glycol as the initiator and Sn(Oct)<sub>2</sub> as the catalyst and was allowed to react at 120°C overnight. Control over  $M_n$  was achieved via controlling the ratio of the L-lactide monomer to the ethylene glycol initiator as shown in Table 1. Crude product was dissolved in minimal chloroform and was precipitated into methanol, followed by vacuum filtration to recover purified PLLA.

**Table 1.** Monomer to initiator ([M]/[I]) ratios for each of the PLLA syntheses.

PLLA Target $M_n$ (kDa)	[M]/[I] Ratio
7.5	52
15	104
30	208

PDLLA ( $M_n$  ~15 kDa) was synthesized via ROP as well, using D, L-lactide instead of L-lactide as the monomer ([M]/[I] = 104). Final product was obtained through the same method of dissolving in chloroform, precipitating in methanol, and finally retrieving purified product through vacuum filtration.

### *PLGA Synthesis*

85:15 PLGA and 70:30 PLGA (LA:GA by mol) were synthesized and purified according to an analogous procedure.<sup>13</sup> The mole ratio of L-lactide and glycolide monomer feedstock was varied according to Table 2, but  $M_n$  was maintained at ~15 kDa ([M]/[I] = 107).

**Table 2.** Lactic Acid: Glycolic Acid ratios for each of the PLGA syntheses.

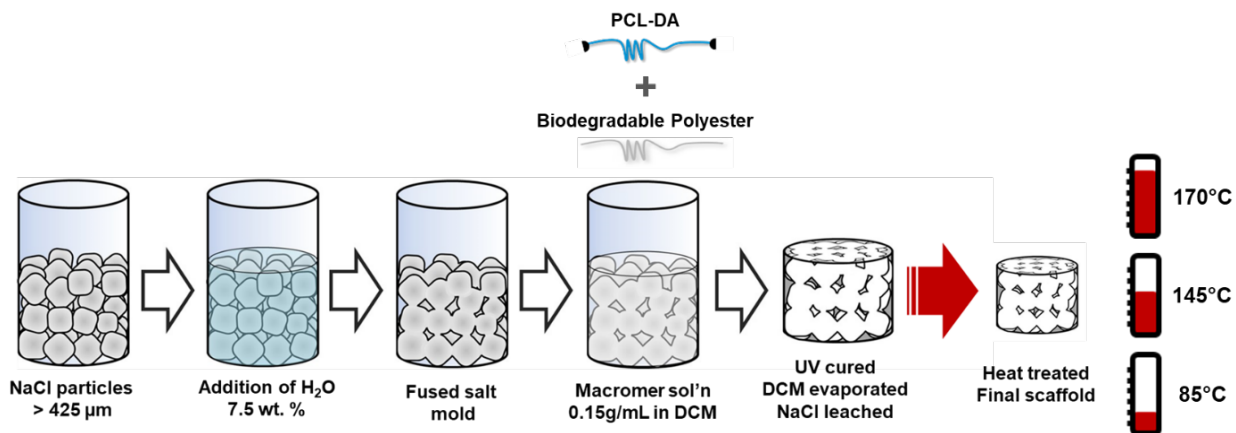
Composition	L-Lactide: Glycolide Ratio (g)
85:15 PLGA	5.25: 0.75
70:30 PLGA	4.46: 1.54

## Scaffold Fabrication

Scaffolds were prepared using a previously reported solvent-casting particulate-leaching (SCPL) method. To obtain scaffolds with the desired pore sizes, NaCl particles were separated using a 425  $\mu\text{m}$  sieve, resulting in particles  $459 \pm 69 \mu\text{m}$  in diameter, which was confirmed via ImageJ software from SEM images.<sup>1,6</sup> To fabricate scaffolds, 10.0 g of the sieved salt were measured and placed in 20 mL glass vials. To create interconnected pores, NaCl particles were fused with 7.5 wt% deionized water added in four additions and were mixed well between increments to allow for even distribution. The vials were then centrifuged (3,220 x g, 15 minutes), dried in a fume hood (~60 minutes), and finally dried *in vacuo* (30 in. Hg, RT, ON).

Once fused salt molds were dried, they were injected with macromer solutions. These solutions were prepared by dissolving 75/25 PCL-DA/PLA wt% ratios in DCM (0.15 g polymer/mL) in addition to photoinitiator (PI) solution (15% by vol., DMP in NVP 10% by wt.). *Group A* included PLLAs varying in  $M_n$  (7.5 kDa, 15 kDa, and 30 kDa) as the incorporated thermoplastic. *Group B*, consisting of PDLLA and PLGAs [85:15 and 70:30 molar ratios of L-lactide to glycolide], included PLAs of varying hydrophilicity as the incorporated thermoplastics ( $M_n \sim 15$  kDa). Herein, semi-IPN scaffolds are referred to their thermoplastic component (e.g. *7.5k PLLA* for the PCL-DA/7.5 kDa PLLA semi-IPN, PDLLA for the PCL-DA/PDLLA semi-IPN and *85:15 PLGA* for the PCL-DA/85:15 PLGA semi-IPN). A control scaffold was prepared, which consisted of only 100% PCL-DA (*100% PCL-DA*; i.e. no thermoplastic). The final solution was mixed thoroughly using a vortexer until all particles had visibly dissolved. The monomer solutions were then added to the vials until the entirety of the salt mold was submerged, and centrifuged (1,260 x g, 10 minutes) before curing via exposure to UV light (6 mW  $\text{cm}^{-2}$ , 365 nm) for 5 minutes.

Once solvent-casting was completed, vials were air dried overnight to evaporate all DCM. Once dried, scaffolds were soaked in a water and ethanol solution (1:1 C<sub>2</sub>H<sub>5</sub>OH volume: H<sub>2</sub>O volume) for ~5 days to leach out all salt particulates. Scaffolds were then air-dried and were subsequently annealed. In this study, three varying anneal temperatures were utilized. The three temperatures will be referred to as “high” (~170 °C), “medium” (~145 °C) and “low” (~85 °C) throughout this study. Since PCL had a T<sub>m</sub> lower than all three annealing temperatures, the control 100% PCL-DA scaffold was annealed at all three temperatures. Amorphous PLA containing semi-IPN scaffolds (*PDLLA, 70:30 PLGA*) were also included at all annealing temperatures due to their lack of PLLA crystallinity and T<sub>m</sub>. The *85:15 PLGA* semi-IPN scaffolds (T<sub>m</sub> = 143.4 ± 0.16) had a decreased T<sub>m</sub> due to decreased PLLA crystallinity and therefore was annealed at both “medium” and “high” temperatures. Since the *7.5 kDa PLLA, 15 kDa PLLA*, and *30 kDa PLLA* contained PLLA crystalline regions (T<sub>m</sub> ~155), *Group A* semi-IPN scaffolds were only annealed at the “high” temperature. Scaffolds were annealed *in vacuo* for 7 minutes, 10 minutes, and 30 minutes for “high”, “medium”, and “low” annealing temperatures, respectively. These varying times were selected via examining porous scaffolds for sufficient pore shrinkage. Once annealed, scaffolds were sliced via vibratome (Leica VT 1000 S) to achieve specimens of 2 mm thickness. Sliced scaffolds were biopsy punched (Integra Miltex Disposable Biopsy Punch) to 6 mm in diameter. The fabrication process is illustrated below in Figure 4.



**Figure 4.** Overview of SCPL scaffold fabrication. Salt particles were fused with the addition of 7.5 wt% water and once dried, were casted with macromer solution of 75/25 [PCL-DA/PLA] followed by UV curing. Salt was then leached in a solution consisting of equal parts water and ethanol. Next, scaffolds were annealed at the three different temperatures to achieve sufficient pore shrinkage.

## Characterization

### Scanning Electron Microscopy

Pore size and morphology were visualized via SEM prior to degradation as well as at each timepoint post-degradation. Specimens were secured onto SEM stages with carbon tape and coated with Au-Pt (~7 nm) via sputter deposition. Images were taken using an SEM (Tescan Vega 3) with an accelerating voltage of 10 kV. ImageJ software was utilized to obtain average pore size from SEM images (N = 2). Porosity (%) was calculated using Equation 1 shown below where P (%) is percent porosity within the scaffold,  $\rho_{\text{solid SMP}}$  is the density of corresponding non-porous films, and  $\rho_{\text{porous SMP}}$  is the density of the porous scaffolds.

$$(1) \quad P (\%) = \frac{\rho_{\text{solid SMP}} - \rho_{\text{porous SMP}}}{\rho_{\text{solid SMP}}} \times 100$$

Densities of the analogous non-porous films ( $\rho_{\text{solid SMP}}$ ) were gravimetrically determined to be 1.108 (PCL-DA), 1.140 (PDLLA), 1.099 (7.5k PLLA), 1.140 (15k PLLA), 1.129 (30k PLLA), 1.157 (85:15 PLGA), and 1.204 g cm<sup>-3</sup> (70:30 PLGA). Films were annealed at 85°C for 60 minutes *in vacuo* (30 in. Hg, RT).

## *DSC and TGA*

Thermal properties of PCL and PLA in scaffolds were obtained using Differential Scanning Calorimeter (DSC, TA Instruments Q100) and Thermal Gravimetric Analysis (TGA, TA Instruments Q50) to determine PCL-DA/PLA wt% ratios. Thermal transitions ( $T_m$  and  $T_g$ ) as well as enthalpy change ( $\Delta H_m$ ) were collected from DSC thermograms. Specimens ( $\sim 5\text{-}8$  mg,  $N = 3$ ) were sealed in hermetic pans and heated from room temperature to  $190^\circ\text{C}$  at  $5^\circ\text{C}/\text{min}$ . Percent crystallinity of PCL-DA and PLA within the scaffolds were obtained using Equation 2 which includes a correction factor accounting for the weight percent (wt%) of each polymer in the scaffold. Here,  $\% \chi_c$  is the percent crystallinity within the scaffold,  $\Delta H_m$  is the enthalpy of fusion determined from the integral of the endothermic melt peak,  $\Delta H_m^\circ$  is the theoretical value for 100% crystalline PCL ( $139.5$  J/g)<sup>14</sup> or PLLA ( $93.0$  J/g)<sup>15</sup>, and  $W$  is the corresponding mass fraction of the polymer.

$$(2) \quad \% \chi_c = \frac{\Delta H_m}{\Delta H_m^\circ \times W} \times 100$$

The same specimens utilized in DSC were used in TGA analysis as well ( $\sim 5\text{-}8$  mg,  $N = 3$ ) and were run under  $N_2$  in a platinum pan from room temperature to  $500^\circ\text{C}$  at  $10^\circ\text{C}/\text{min}$ . Since PLLA is known to thermally degrade at lower temperatures than PCL,<sup>16</sup> weight percent of each polymer in the semi-IPN scaffold was quantified and used to correct DSC data.

## **Accelerated Degradation**

An accelerated degradation study was conducted under base-catalyzed conditions ( $0.2$  M NaOH,  $37^\circ\text{C}$ ) with 5 total time points, each 24 hours apart. Scaffold specimens ( $N=3$ , per timepoint) were each submerged in 10 mL of  $0.2$  M NaOH ( $\text{pH}\sim 14$ ) in sealed 20 mL vials. All vials were collectively placed in an incubator (VWR Benchtop Shaking Incubator Model 1570) held at 60 rpm and  $37^\circ\text{C}$  to mimic physiological conditions. At each timepoint, specimens were



removed from vials and air dried, followed by drying *in vacuo* (30 in Hg RT, ON) to retrieve their final mass and thickness of the scaffolds at those time points. Equation 3 was utilized to determine mass loss where  $m_{\text{initial}}$  is the recorded mass before degradation and  $m_{\text{final}}$  is the post-degradation weight.

$$(3) \quad \text{Mass Loss (\%)} = \frac{m_{\text{initial}} - m_{\text{final}}}{m_{\text{initial}}} \times 100$$

### Neutral Degradation

A real-time degradation study was conducted under neutral conditions (PBS, 37°C, pH~7.4) with 5 total time points, each 3 months apart. Scaffold specimens (N=3, per timepoint) were each submerged in 10 mL of PBS (pH~7.4, changed monthly to maintain neutral pH) in sealed 20 mL vials. All vials were collectively placed in an incubator (VWR Benchtop Shaking Incubator Model 1570) held at 60 rpm and 37°C to mimic physiologic conditions. At the designated timepoints, scaffolds were removed from solution, measured at their wet weight, and dried *in vacuo* (30 in Hg RT, ON) to retrieve their final mass. Water uptake was calculated using Equation 4 where  $m_{\text{wet}}$  is the wet weight of the scaffolds and  $m_{\text{dry}}$  is the mass of the scaffolds after drying *in vacuo*.

$$(4) \quad \text{Water Uptake (\%)} = \frac{m_{\text{wet}} - m_{\text{dry}}}{m_{\text{dry}}} \times 100$$

### Statistical Analysis

Data was reported as the mean  $\pm$  standard deviation. Semi-IPN scaffolds were compared to the control 100% PCL-DA scaffolds annealed at the same temperature using t-tests and One-way ANOVA to determine p-values. All tests were analyzed at a 99% confidence interval.

## CHAPTER III

### RESULTS

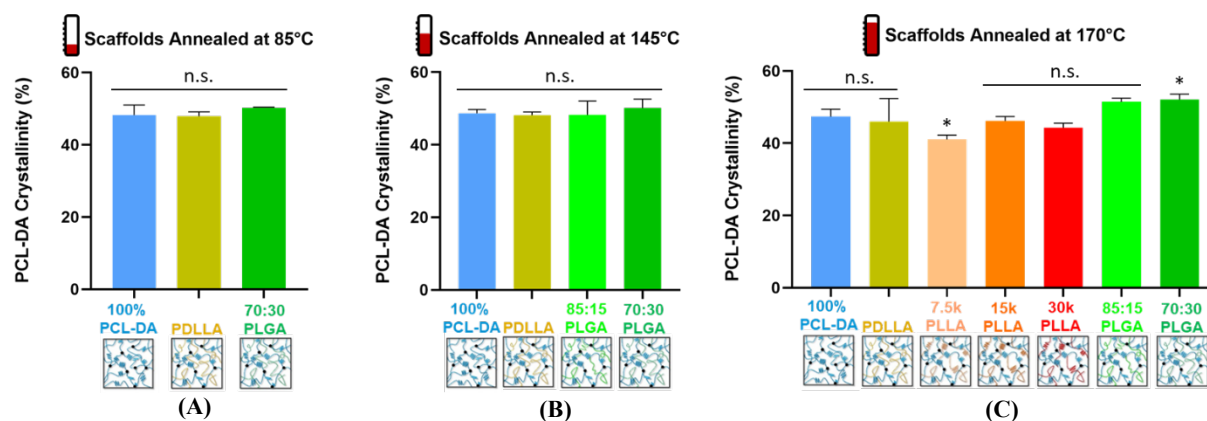
#### Scaffold Fabrication Verification

<sup>1</sup>H NMR analysis verified that targeted  $M_n$  for all semi-IPN compositions was successfully achieved via end group analyses. This method also confirmed that targeted molar ratios of PLGA-containing compositions were successfully achieved, as shown below in Figure 5. Additionally, DSC data confirmed that PLA crystallinity (%) was held constant across all PLLA-containing semi-IPN compositions.

Polymer	$M_n$ (kDa) (NMR)	Ratio L:G (NMR)	$T_g$ (°C) (DSC)	$T_m$ (°C) (DSC)	% Cryst (DSC)
7.5k PLLA	8.3	-	$44.9 \pm 0.93$	$153.1 \pm 0.35$	$48.1 \pm 1.9$
15k PLLA	15.1	-	$45.1 \pm 0.72$	$155.4 \pm 0.35$	$52.7 \pm 0.14$
30k PLLA	27.5	-	$46.3 \pm 2.78$	$158.8 \pm 0.85$	$56.2 \pm 1.4$
PDLLA	11.3	-	$28.4 \pm 0.13$	-	-
85:15 PLGA	14.2	84:16	$41.2 \pm 1.49$	$137.4 \pm 6.2$	$3.17 \pm 0.74$
70:30 PLGA	12.7	67:33	$37.3 \pm 2.22$	-	-

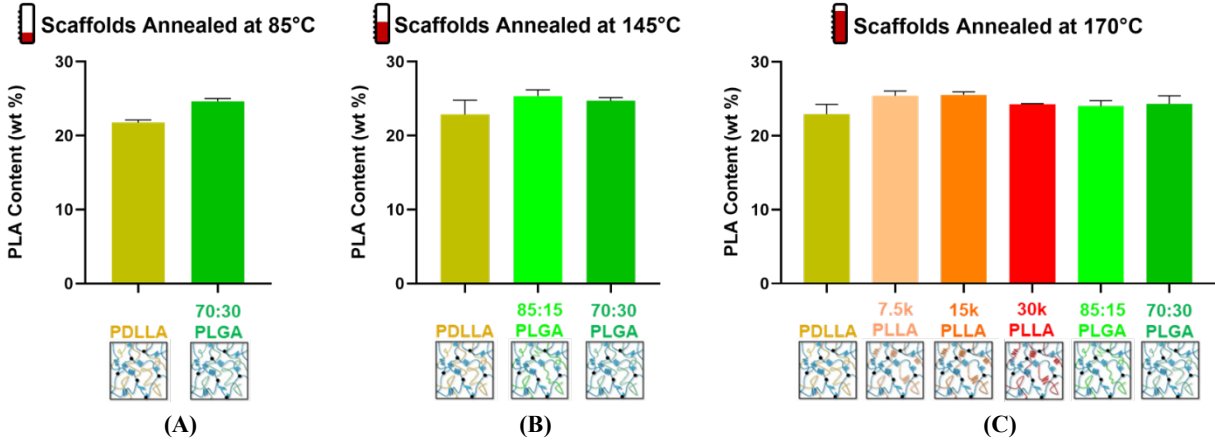
**Figure 5.** All semi-IPN compositions are shown with their corresponding  $M_n$ , molar LA:GA ratios (for 70:30 PLGA and 85:15 PLGA), thermal properties ( $T_m$  and  $T_g$ ), and PLA crystallinity (%).

Upon DSC analysis to verify scaffold synthesis, it was shown that initial PCL-DA crystallinity was not significantly varied between annealing temperatures and compositions, as shown in Figure 6.



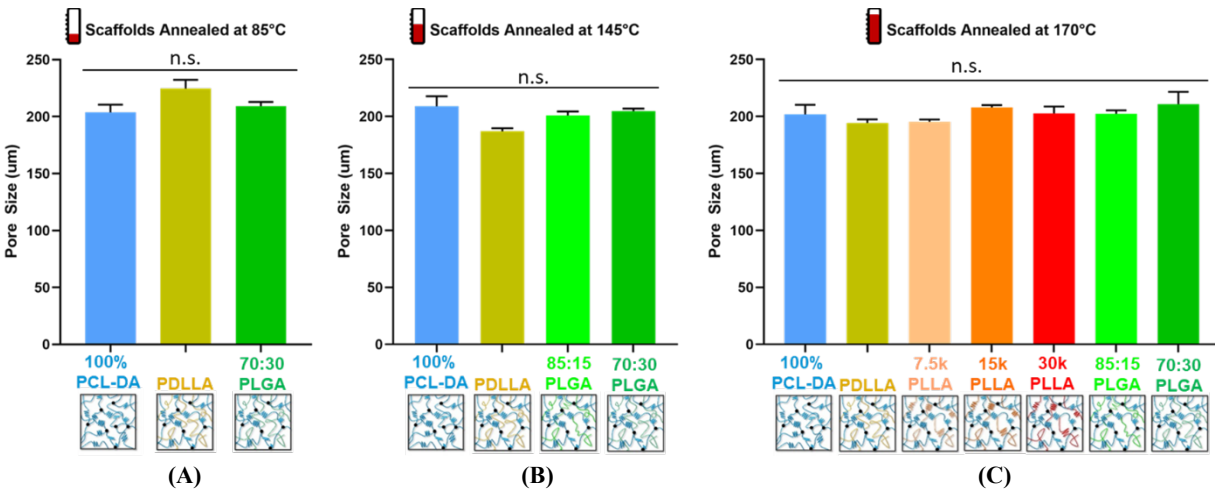
**Figure 6.** Initial PCL-DA crystallinity (%) of scaffolds, shown above at their given annealing temperatures: 85 °C (A), 145 °C (B), and 170 °C (C). Compositions annealed at the “low” (85 °C) and “medium” (145 °C) temperatures did not significantly vary from the 100% PCL-DA control. 7.5k PLLA and 70:30 PLGA annealed at the “high” (170 °C) temperature (C) varied slightly according to t-test analysis, however, did not vary enough to affect degradation. Semi-IPN scaffolds were compared to the control 100% PCL-DA (\* $p < 0.05$ ).

As shown in Figure 6A and 6B, all semi-IPN compositions annealed at the “low” (85 °C) and “medium” (145 °C) temperatures did not vary significantly from the 100% PCL-DA control. The same was true for most semi-IPN compositions annealed at the “high” (170 °C) temperature with the exception of 7.5k PLLA and 70:30 PLGA, as shown in Figure 6C. Although t-test analysis showed variance from the 100% PCL-DA control, 7.5k PLLA (initial PCL-DA crystallinity ~41.0%) and 70:30 PLGA (initial PCL-DA crystallinity ~52.1%) did not vary from the 100% PCL-DA control (initial PCL-DA crystallinity ~47.4) enough to notably affect degradation results. Figure 7 shows initial PLA content of polymers obtained via TGA analysis, confirming that the target ~25 wt. % PLA had successfully been achieved during synthesis.



**Figure 7.** Initial PLA content (wt. %) of scaffolds, shown above at their given annealing temperatures: 85 °C (A), 145 °C (B), and 170 °C (C). Semi-IPN compositions at all annealing temperatures achieved the targeted ~25 wt % PLA.

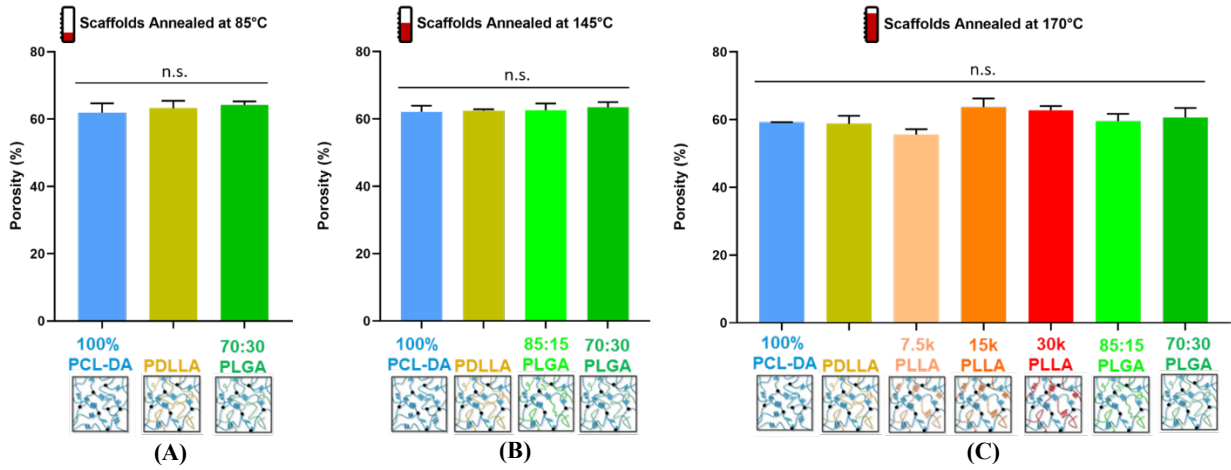
Prior to degradation, scaffolds were analyzed for pore size across all compositions and annealing temperatures. ImageJ software was utilized to confirm that average pore size between all compositions and annealing temperatures had remained consistent. Successfully, pore sizes amongst all annealing temperatures were maintained around the desired ~200  $\mu\text{m}$  ( $d = 203.87 \mu\text{m} \pm 8.84$ ) with insignificant variations between compositions, as shown in Figure 8.



**Figure 8.** ImageJ and SEM were utilized to quantify average pore size for each semi-IPN composition, shown above at their given annealing temperatures: 85 °C (A), 145 °C (B), and 170 °C (C). Semi-IPN scaffolds were compared to the control 100% PCL-DA (\* $p < 0.05$ ).

Percent porosity was also calculated using Equation 1. Porosity of semi-IPN compositions annealed at 85 °C, 145 °C, and 170 °C are shown in Figures 9A, 9B, and 9C,

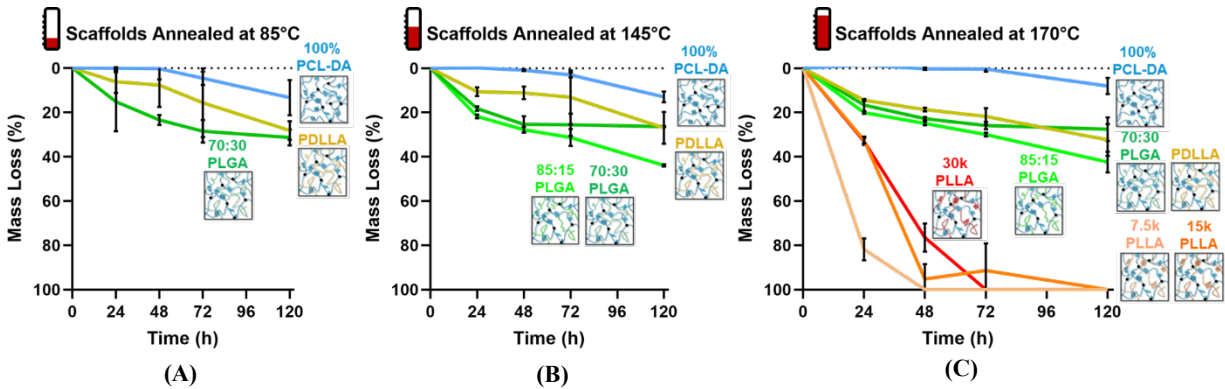
respectively. Consistent porosity was achieved throughout varying compositions and annealing temperatures, as shown.



**Figure 9.** Percent porosity was shown to be maintained at ~60% for all compositions at their given annealing temperatures: 85 °C (A), 145 °C, and 170 °C. PCL-DA/PLA semi-IPN compositions were compared to the 100% PCL-DA control (\* $p < 0.05$ ).

### Base-catalyzed Degradation

Gravimetric mass loss was monitored at 24-hour intervals under base catalyzed degradation conditions (0.2 M NaOH, 37 °C, 60 rpm) for all compositions shown in Figure 10.



**Figure 10.** Gravimetric mass loss under base-catalyzed conditions (0.2 M NaOH, 37°C, 60 rpm) for PCL-DA/PLA semi-IPN scaffolds annealed at 85 °C (A), 145 °C (B), and 160 °C (C). All semi-IPN compositions degraded significantly faster than the 100% PCL-DA controls. The annealing temperature did not significantly impact scaffold degradation rate.

Under basic conditions, all semi-IPN scaffolds degraded significantly faster than the 100% PCL-DA control. Figure 10A compares the degradation rates of scaffolds annealed at the “low” 85 °C temperature. At this annealing temperature, the 70:30 PLGA semi-IPN composition

demonstrated the fastest degradation likely due to the 70:30 PLGAs hydrophilic and amorphous nature, followed by the *PDLLA* semi-IPN composition likely due to *PDLLA*'s more hydrophobic nature. The 85:15 *PLGA* semi-IPN scaffold demonstrated the fastest rate of degradation when annealed at the “medium” 145 °C temperature as shown in Figure 10B. This is somewhat counterintuitive given that the 85:15 *PLGA* copolymer has partial *PLLA* crystallinity due to a high L-lactide content, however, this is consistent with trends previously observed in our group under base-catalyzed conditions. At the “medium” 145 °C annealing temperature, the 70:30 *PLGA* and *PDLLA* scaffolds degraded at an intermediate rate, slower than the 85:15 *PLGA* but still significantly faster than the 100% *PCL-DA* control. For scaffolds annealed at the “high” 160 °C temperature, all semi-crystalline *PLLA* containing semi-IPN scaffolds (7.5k *PLLA*, 15k *PLLA* and 30k *PLLA*) displayed rapid degradation with 100% mass loss at 120 h as shown in Figure 10C. Of those compositions, the 7.5k *PLLA* degraded the fastest with 100% mass loss at just 48 hours; this may be due to the 7.5k *PLLA*'s lower  $M_n$ . The *PDLLA*, 85:15 *PLGA*, and 70:30 *PLGA* scaffolds degraded at a slower rate than the *PCL-DA/PLLA* semi-IPN compositions but were all still significantly faster than the 100% *PCL-DA* control. These results demonstrate the potential to both accelerate and fine-tune scaffold degradation rate using a *PCL-DA/PLA* semi-IPN design.

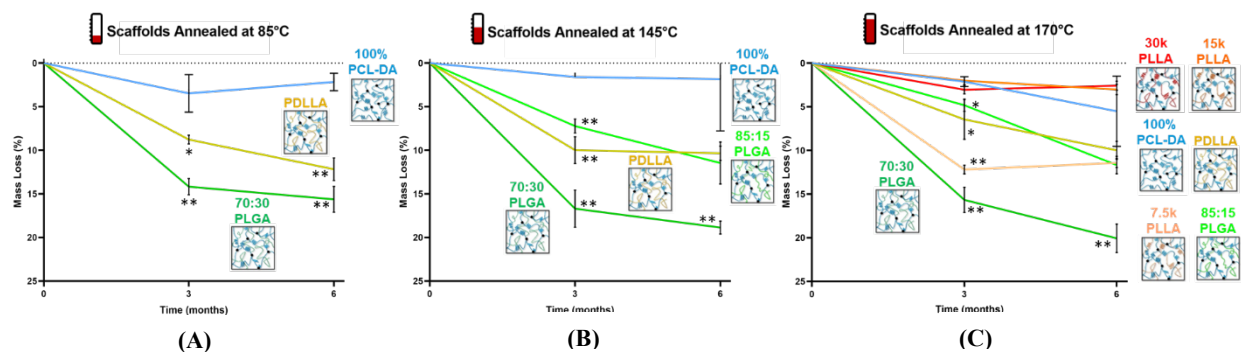
Varying the annealing temperature of scaffolds did not significantly alter scaffold degradation trends of a given composition. The 70:30 *PLGA* scaffolds showed a slight decrease in mass loss with increased annealing temperature, however t-test analyses (\* $p < 0.05$ ) showed that the difference was insignificant. This was unexpected based on previous base-catalyzed degradation studies performed on analogous solid films where a higher annealing temperature was shown to slow degradation.<sup>9</sup> For porous *PCL-DA/PLA* semi-IPN scaffolds, chemical

composition of the PLA component had a profound impact on degradation rate, but scaffold degradation was not dependent on scaffold annealing temperature.

## Real-time Degradation

### Mass Loss

Gravimetric mass loss was monitored at 3-month intervals under neutral, physiologically relevant conditions (1X PBS, pH~7.4, 37 °C, 60 rpm) as shown in Figure 11.



**Figure 11.** Gravimetric mass loss under neutral conditions (1X PBS, pH ~7.4, 37 °C, 60 rpm) for PCL-DA/PLA semi-IPN scaffolds annealed at 85 °C (A), 145 °C (B), and 160 °C (C). Notably across all annealing temperatures, the 70:30 PLGA scaffolds and the 7.5k PLLA scaffolds degraded the fastest followed by the PDLLA and 85:15 PLGA scaffolds. The 15k PLLA and 30k PLLA semi-IPNs degraded slow, at a rate similar to 100% PCL-DA controls. PCL-DA/PLA semi-IPN compositions were compared to the 100% PCL-DA control (\*\*p<0.01 and \*p<0.05).

The study was planned for a total of 15 months and thus far the 3-month and 6-month timepoints have been removed, but the study is still in progress. As shown in Figure 11A and Figure 11B, all PCL-DA/PLA semi-IPN scaffolds annealed at the “low” 85 °C and “medium” 145 °C temperatures showed significantly enhanced degradation rates compared to the 100% PCL-DA control. Amongst scaffolds annealed at the “low” 85°C temperature, the 70:30 PLGA semi-IPNs showed the most dramatic mass loss compared to the control at both 3 and 6 mo. The PDLLA scaffolds degraded slower than the 70:30 PLGA scaffolds but were still significantly faster than the 100% PCL-DA controls. As shown in Figure 11B, all PCL-DA/PLA semi-IPN scaffolds annealed at the “medium” 145 °C temperature also showed substantial mass loss at 3 and 6 mo., compared to the control. The 70:30 PLGA and PDLLA compositions followed similar

trends as what was reported for scaffolds annealed at the “low” 85 °C temperature. The *85:15 PLGA* scaffolds degraded at a similar rate as the *PDLLA* scaffolds annealed at the “medium” 145 °C temperature; they both degraded at an intermediate rate, slower than the *70:30 PLGA* but faster than the *100% PCL-DA* scaffolds.

PCL-DA/PLA semi-IPN scaffolds annealed at the “high” 160 °C temperature showed a wider range of fast and slow degradation rates as illustrated in Figure 11C. Following the trend from the “low” 85 °C and “medium” 145 °C annealing temperature groups, the *70:30 PLGA* semi-IPN scaffolds annealed at the “high” 160 °C temperature demonstrated the fastest degradation rate at both 3 and 6 mo. with >20% mass loss. The *7.5k PLLA* semi-IPNs showed similar mass loss to the *70:30 PLGA* semi-IPNs at 3 mo., however this high rate of degradation was not maintained at the 6 mo. timepoint. Next, the *PDLLA* and *85:15 PLGA* displayed an intermediate degradation rate that was slower than the *70:30 PLGA* and *7.5k PLLA* semi-IPNs but was still significantly faster than the *100% PCL-DA* controls. Finally, the *15k PLLA* and *30k PLLA* semi-IPNs degraded at a slow rate similar to the *100% PCL-DA* controls. In short, a variety of fast, intermediate and slow scaffold degradation rates were achieved using PCL-DA/PLA semi-IPNs.

The tunable semi-IPN degradation rates can be explained based on the structural properties of the thermoplastic PLA components. At all annealing temperatures, the *70:30 PLGA* composition degraded the most rapidly because *70:30 PLGA* thermoplastic is hydrophilic and amorphous; both properties promote solution diffusion making the polymer chains more susceptible to hydrolysis. The *PDLLA* composition degraded slightly slower because *PDLLA* is amorphous but not hydrophilic. The *85:15 PLGA* composition degraded at a similar rate to the *PDLLA* composition at both “medium” 145 °C and “high” 160 °C annealing temperatures,



thereby demonstrating the competing impacts from crystallinity and hydrophilicity.

Thermoplastic 85:15 PLGA is somewhat hydrophilic leading to enhanced rates of hydrolysis but is also partially crystalline, which inhibits hydrolysis. At 3 mo. the 7.5k PLLA composition degraded at a rapid rate similar to the 70:30 PLGA composition due to 7.5k PLLA thermoplastics lower  $M_n$ . The 15k PLLA and 30k PLLA compositions degraded the slowest, which can be explained by 15k PLLA and 30k PLLA thermoplastics semi-crystallinity, hydrophobicity, and relatively high  $M_n$ - all properties that inhibit diffusion and hydrolysis. In conclusion, hydrophilic, amorphous and low  $M_n$  PLA thermoplastics promoted the fastest scaffold degradation.

Notably, some scaffolds degraded following similar hydrolysis kinetics to the base-catalyzed study while other scaffolds degraded following different hydrolysis kinetics. The fast and intermediate PCL-DA/PLA semi-IPN compositions [7.5k PLLA, 70:30 PLA, 85:15 PLGA and PDLLA at all annealing temperatures] degraded under similar linear hydrolysis kinetics under both degradation conditions. It is promising that these scaffold compositions degraded following linear kinetics under neutral real-time degradation conditions because this implies that the scaffolds are undergoing surface degradation, which is preferable in biomedical applications due to high maintenance of properties (i.e. mechanical) with degradation.<sup>12</sup> The slow degrading semi-IPNs [15k PLLA, 30k PLLA] degraded rapidly with linear kinetics under base-catalyzed conditions (Figure 10), however under neutral degradation conditions they have yet to experience significant mass loss. We hypothesize that under neutral conditions, these scaffolds are following bulk degradation kinetics whereby minimal mass loss is followed by a sudden and dramatic decrease in mass. We expect to see this sudden mass loss at a later timepoint in the study. This change in hydrolysis kinetics from surface to bulk degradation under basic and neutral conditions respectively has been previously reported for other poly( $\alpha$ -hydroxy acids)

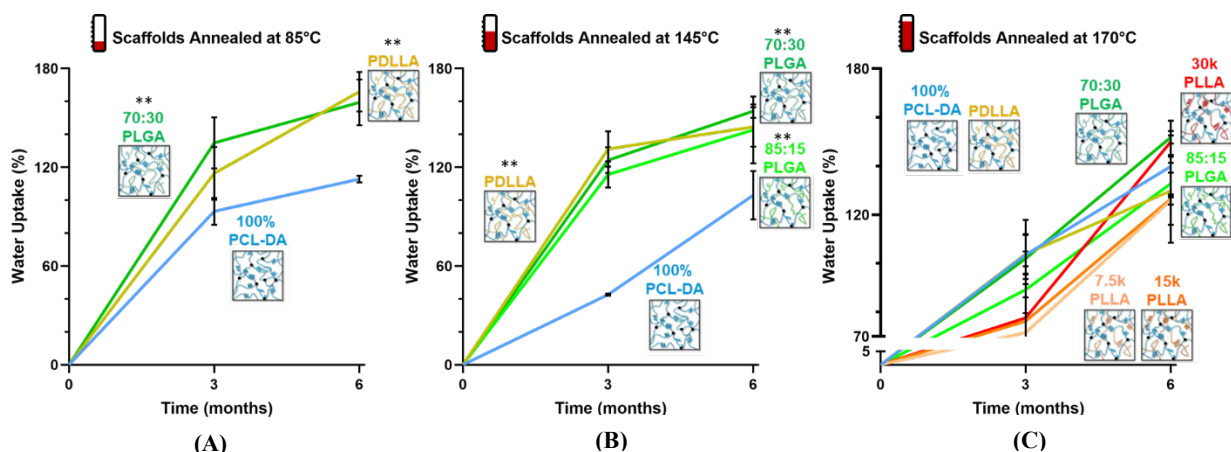
(PAHAs).<sup>12</sup> However, we have yet to understand why some PAHAs degradation kinetics can be predicted using base-catalyzed studies while other cannot. Based on this study, degradation kinetics were drastically altered for compositions using semi-crystalline PLLAs with relatively high  $M_n$  in PCL-DA based semi-IPNs.

Under basic conditions there were no significant differences in scaffold degradation rate across different annealing temperatures, but under neutral conditions there were some subtle differences. The 85:15 PLGA composition did not appear to show any differences in degradation when annealed at “medium” 145 °C or “high” 160 °C temperatures. However, the PDLLA composition showed decreasing mass loss with increased annealing temperature. This can be explained based on previous studies on analogous solid films where a higher annealing temperature reduced phase separation and thus slowed degradation.<sup>9</sup> Interestingly, the 70:30 PLGA composition followed the opposite trend where mass loss was shown to increase with increased annealing temperature. Post-degradation analyses may help to further explain this unexpected trend.

#### *Water Uptake*

Water uptake of degraded scaffolds was monitored to quantify solution diffusion (Equation 4) as that may be related to the previously described degradation trends. As shown in Figure 12, a lower annealing temperature typically resulted in significantly higher water uptake. Figure 12A and Figure 12B both show that all semi-IPN scaffolds annealed at the “low” 85 °C and “medium” 145 °C temperatures demonstrated significantly higher water uptake than the 100% PCL-DA control. The 70:30 PLGA semi-IPN scaffolds annealed at the “high” 160 °C temperature (Figure 12C) also showed higher water uptake than controls but only at the 6 mo. timepoint, not at the 3 mo. timepoint. This trend was also observed in the PDLLA and 85:15

*PLGA* compositions which showed decreased water uptake with increased annealing temperature. This can be attributed to decreased phase separation of PCL and PLA when annealed at higher temperatures, which likely reduced solution diffusion.<sup>11</sup> The increased water uptake at “low” 85 °C and “medium” 145 °C annealing temperatures can be attributed to immiscibility and phase separation of PCL and PLA in the semi-IPN scaffolds resulting in increased water permeability and uptake. This suggests that scaffold annealing temperature may impact the degradation mechanism in some way even though it does not significantly affect gravimetric mass loss or apparent degradation rate.



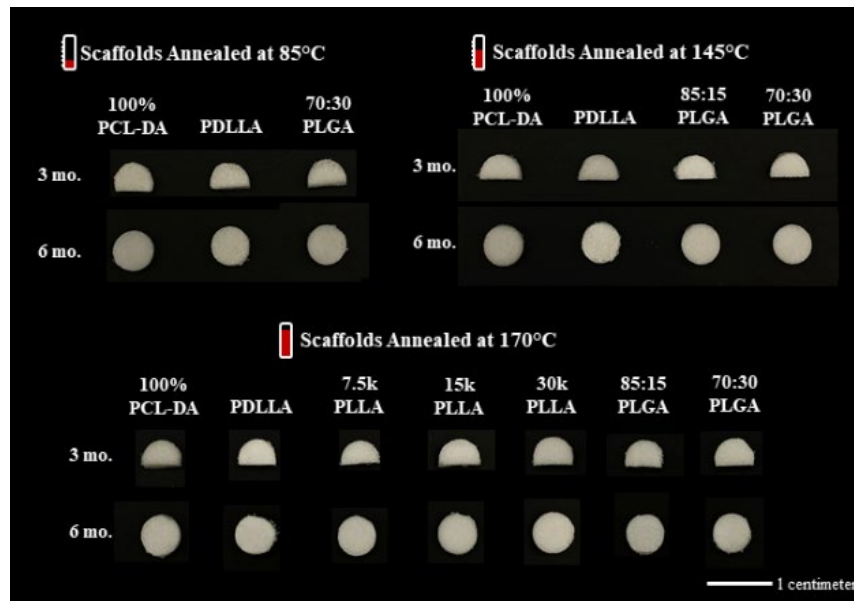
**Figure 12.** Water uptake percentage following exposure to neutral degradation conditions (1X PBS, pH ~7.4, 37 °C, 60 rpm) for semi-IPN scaffolds annealed at 85 °C (A), 145 °C (B), and 160 °C (C). Greater water uptake was observed for scaffolds annealed at the “low” and “medium” temperatures compared to those scaffolds annealed at the “high” temperature. PCL-DA/PLA semi-IPN compositions were compared to the 100% PCL-DA control (\*\*p<0.01 and \*p<0.05).

## Post-degradation Scaffold Characterization

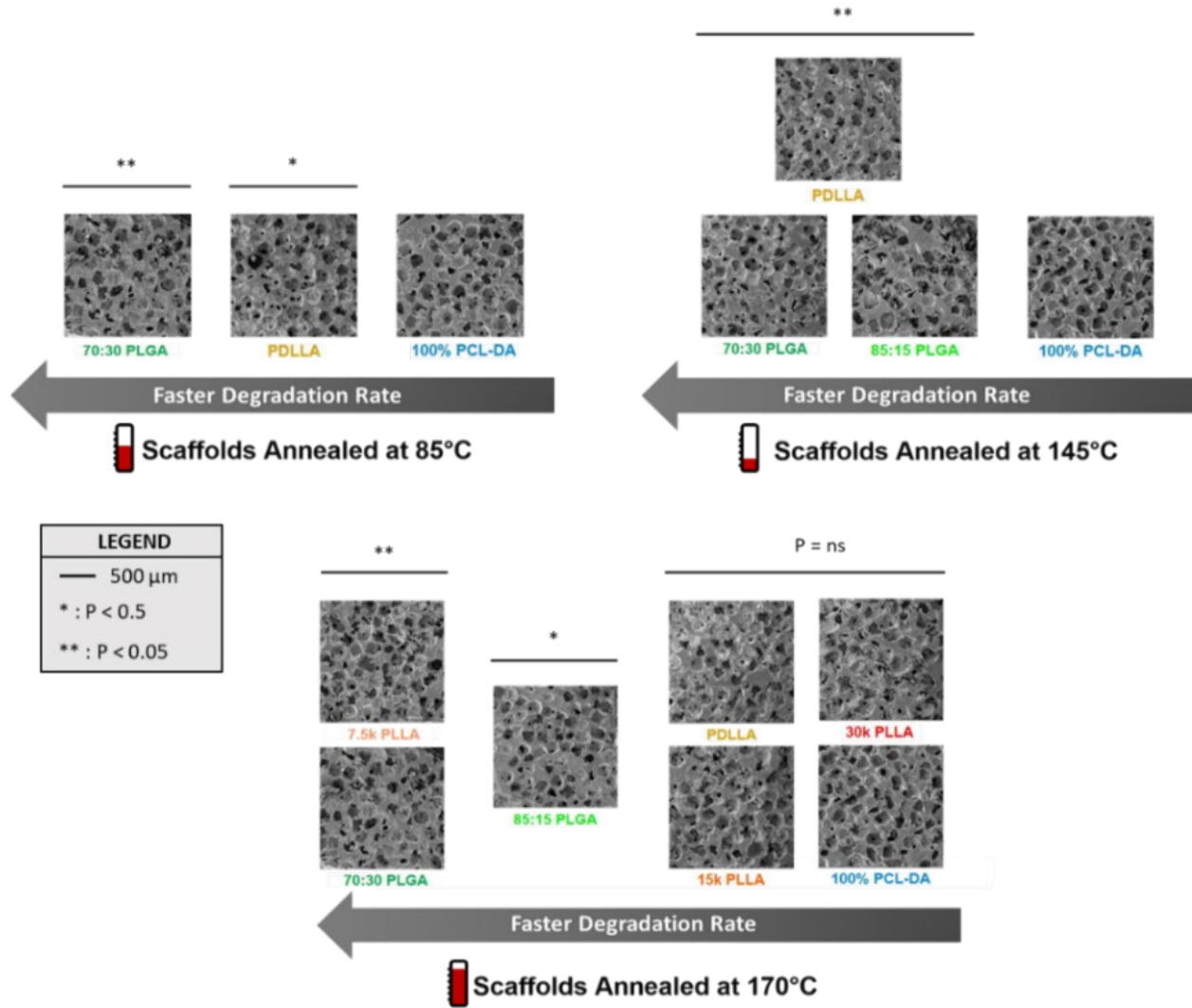
### Visualization

Following exposure to neutral degradation conditions (1X PBS, pH ~7.4, 37 °C, 60 rpm), scaffolds were visualized macroscopically using photos and microscopically using SEM images. Photos of the scaffolds were compiled and are shown in Figure 13. Note that the 3-month timepoint samples were used for other testing prior to taking the photos, so scaffold halves were photographed. Macroscopic visualization did not show any significant signs of erosion, even for

scaffold compositions that had demonstrated significant mass loss. So, scaffolds were further visualized microscopically via SEM to qualitatively compare pore morphology after 3 months of degradation as shown in Figure 13. Substantial differences in pore morphology across scaffold compositions can be seen where interconnected pores start to morph together for fast degrading compositions, as denoted by their degradation statistics shown in Figure 14. This was expected for fast degrading compositions and further demonstrates the potential of those scaffolds to more effectively accommodate growing bone tissue.



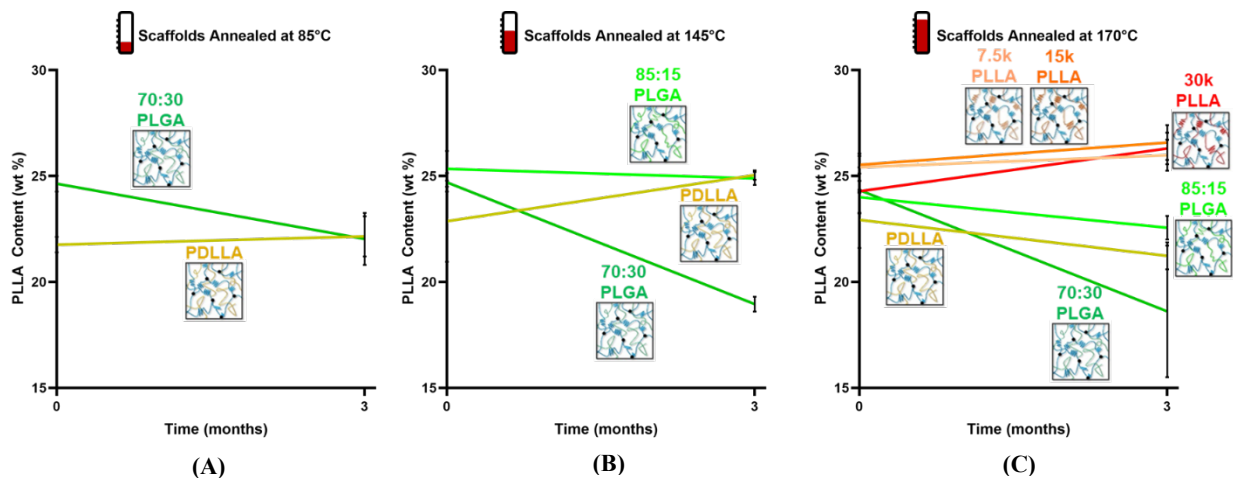
**Figure 13.** Post-degradation, scaffolds were compared macroscopically and did not show significant signs of degradation or erosion. Varying shapes of scaffolds at 3 mo. And 6 mo. in this figure were due to other testing conducted for this study.



**Figure 14.** SEM images were taken after 3 months of neutral degradation to analyze qualitative effects of degradation on pore morphology. Statistics for the mass loss data reported in Figure 11 were included here; PCL-DA/PLA semi-IPN compositions were compared to the 100% PCL-DA control (\*\*p<0.01 and \*p<0.05).

### Composition

The change in weight percent (wt %) of PLA in PCL-DA/PLA semi-IPNs was analyzed from 0 to 3 mo. to determine which polymer in the semi-IPN scaffold was degrading first, as shown in Figure 15.



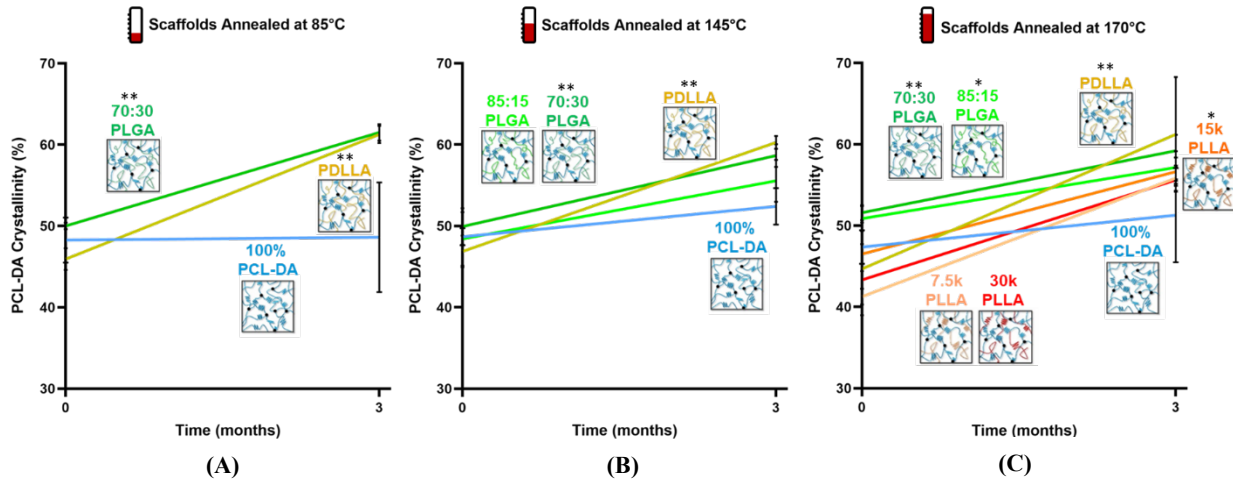
**Figure 15.** PLA content (wt %) in PCL-DA/PLA semi-IPN scaffolds over 3 mo. degradation. As shown for scaffolds annealed at the “low” (A) and “medium” (B) temperatures, *PDLLA* displayed an increase of PLA content indicative of greater PCL than PLA degradation. This was also true for *7.5k*, *15k*, and *30k PLLA* annealed at the “high” temperature (C). *85:15 PLGA* annealed at both the “medium” and “high” temperatures displayed much higher PLA degradation than PCL degradation. This trend was also, most notably, repeated by *70:30 PLGA* at all annealing temperatures.

The *70:30 PLGA* showed a slight decrease in PLA content that followed degradation mass loss trends at the respective annealing temperatures. However, the decrease in relative weight percent of PLA did not account for all mass loss (i.e. ~5% PLA lost based on TGA versus ~10% degradation mass loss) meaning that for the *70:30 PLGA* scaffolds both PCL and PLA were likely degrading, but PLA degradation may have been greater. This was also true for the *PDLLA* and *85:15 PLGA* annealed at the “high” 160 °C temperature. However, for the *PDLLA* and *85:15 PLGA* annealed at the “medium” 145 °C and at the “low” 85 °C temperature, PLA content was constant or even increased slightly following degradation. This may indicate that in these semi-IPN scaffolds, degradation was promoted more in the PCL component. All of the semi-crystalline PLLA containing semi-IPNs showed a slight increase in PLA content following degradation. This was particularly notable for the *7.5k PLLA* which showed >10% mass loss after 3 mo. of degradation. Again, this implies that for these scaffold compositions, degradation was promoted in the PCL component. Moreover, compositional results following degradation

showed that PLA thermoplastics were not simply degrading out of scaffolds first, but rather they were also promoting the degradation of PCL within the semi-IPN scaffolds.

### *Crystallinity*

The percent crystallinity of PCL and PLLA in degraded scaffold samples was quantified using DSC and TGA using Equation 2. Interestingly, PCL crystallinity was observed to increase significantly for all PCL-DA/PLA semi-IPN scaffolds as shown in Figure 16. An increase in PCL crystallinity is indicative of PCL degradation; typically, the amorphous regions hydrolyze first leading to an initial increase in polymer crystallinity following degradation.<sup>17</sup> This implies that the PCL portion in the PCL-DA/PLA semi-IPNs was undergoing hydrolysis for all semi-IPN compositions, even for those that did not show significant mass loss (*15k PLLA* and *30k PLLA*). These results supports our previously mentioned prediction that as this study progresses, the *15k PLLA* and *30k PLLA* compositions will undergo a dramatic mass loss and will ultimately still degrade faster than the controls. The *100% PCL-DA* controls did not exhibit a similar increase in PCL crystallinity further supporting the notion that the PCL in control scaffolds did not undergo significant hydrolysis. In general, annealing temperature did not appear to significantly impact PCL crystallinity following degradation.

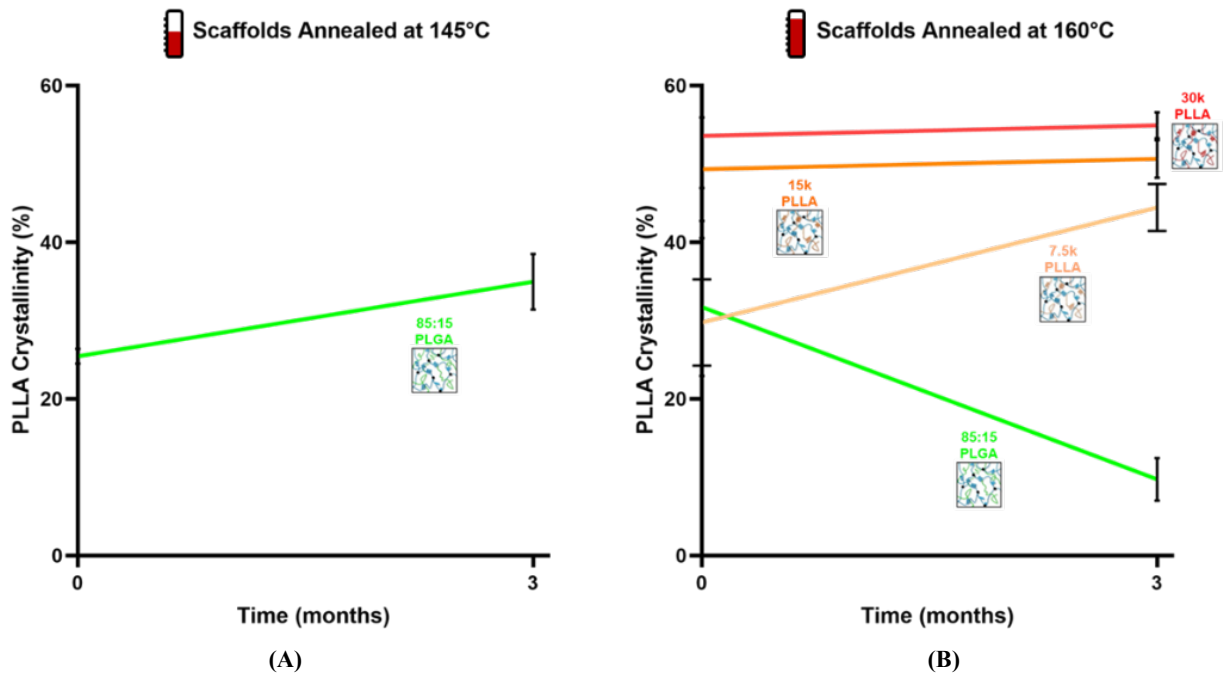


**Figure 16.** TGA results were used to determine PLA content or wt % in scaffolds annealed at 85 °C (A), 145 °C (B), and 160 °C (C) following degradation. PLA content was reduced in some fast degrading scaffold compositions but was well-maintained and even slightly increased for other compositions, indicating that the PLA thermoplastics were not degrading out of scaffolds first followed by PCL.

Figure 17 shows the change in PLLA crystallinity post-degradation for scaffolds; semi-IPNs containing an amorphous PLA thermoplastic and controls (no PLA thermoplastic) were omitted due to lack of PLLA crystallinity. PLLA crystallinity was observed to increase for the 85:15 PLGA annealed at the “medium” 145 °C temperature and for the 7.5k PLLA composition annealed at the “high” 160 °C temperature. Like the previous explanation regarding increased PCL crystallinity, this was indicative of PLLA hydrolysis in those scaffolds due to degradation in amorphous PLLA regions. The 85:15 PLGA annealed at the “high” 160 °C temperature unexpectedly showed a decrease in PLLA crystallinity after 3 months of degradation. The compositional data obtained via TGA analysis also displayed a decrease in PLA content, which indicated that more PLA (including crystalline regions) had already undergone hydrolysis in these scaffolds. This result also highlights that while degradation rate in terms of mass loss was not significantly impacted by annealing temperature, the degradation mechanism of scaffold compositions may be affected by annealing parameters. The 15k PLLA and 30k PLLA annealed at the “high” 160 °C temperature showed no change in PLLA crystallinity following



degradation. This was reasonable considering that neither of these compositions showed significant mass loss at 3 months. However, in light of the PCL crystallinity results this was somewhat interesting because these compositions showed evidence of PCL hydrolyzing but no evidence of PLLA hydrolyzing. As the study continues, these results will continue to provide more detailed information about how the various PCL-DA/PLA semi-IPN scaffolds degrade compared to 100% PCL-DA controls.



**Figure 17.** The change in PCL crystallinity following 3 months of degradation for scaffolds annealed at 145 °C (A), and 160 °C (B); all compositions lacking a semi-crystalline PLLA component were omitted. PLLA crystallinity was increased for the 85:15 PLGA at 145 °C and for the 7.5k PLLA, was maintained for the 15k PLLA and 30k PLLA and was decreased for the 85:15 PLGA at 160 °C. Crystallinity values for a given composition at 3 mo. were compared to initial PCL crystallinity values (\*\*p<0.01 and \*p<0.05).

## CHAPTER IV

### CONCLUSION

Thus far, various PCL-DA/PLA semi-IPN compositions such as the *7.5k PLLA, 85:15 PLGA, 70:30 PLGA* and *PDLLA* showed tunable and accelerated degradation under neutral conditions compared to *100% PCL-DA* controls. These scaffold compositions achieved significant mass loss at 3 mo., a timescale that is relevant to bone tissue regeneration, thus demonstrating their potential to improve regenerative treatment of CMF bone defects. The rate of degradation (in terms of mass loss) of PCL-DA/PLA semi-IPNs containing semi-crystalline PLLA thermoplastics of relatively high  $M_n$  were significantly altered under neutral and basic conditions but were similar for all other semi-IPN compositions under both conditions. Results such as these can help to provide a better understanding of the link between base-catalyzed and non-catalyzed *in vitro* degradation studies, which can ultimately be useful in predicting scaffold degradation for future *in vivo* studies. Scaffold annealing temperature did not appear to impact scaffold degradation based on mass loss under base-catalyzed and neutral conditions. However, differences were detected in water uptake results indicating that scaffolds annealed at the “high” 170 °C temperature showed reduced solution uptake, likely due to reduced phase separation of PCL and PLA. Post-degradation analyses showed that even in fast degrading semi-IPN scaffold compositions, both PCL and PLA components were degrading. This further illustrates how this study was designed to not only monitor degradation rate of PCL-DA/PLA semi-IPN scaffolds but also to analyze the degradation mechanism of such scaffolds in order to better predict their degradation and erosion behavior for future use *in vivo*.

## REFERENCES

1. Woodard, L. N.; Kmetz, K. T.; Roth, A. A.; Page, V. M.; Grunlan, M. A., Porous Poly( $\epsilon$ -caprolactone)-Poly(l-lactic acid) Semi-Interpenetrating Networks as Superior, Defect-Specific Scaffolds with Potential for Cranial Bone Defect Repair. *Biomacromolecules* **2017**, *18* (12), 4075-4083.
2. Hallur, N.; Goudar, G.; Sikkerimath, B.; Gudi, S.; Patil, R., Reconstruction of Large Cranial Defect with Alloplastic Material (Bone Cement-Cold Cure Polymethyl-Methacrylate Resin). *Journal of maxillofacial and oral surgery* **2010**, *9*, 191-4.
3. Rawashdeh, M. A., Morbidity of iliac crest donor site following open bone harvesting in cleft lip and palate patients. *International Journal of Oral and Maxillofacial Surgery* **2008**, *37* (3), 223-227.
4. Kalk, W. W. I.; Raghoobar, G. M.; Jansma, J.; Boering, G., Morbidity from iliac crest bone harvesting. *Journal of Oral and Maxillofacial Surgery* **1996**, *54* (12), 1424-1429.
5. Zhang, D.; George, O. J.; Petersen, K. M.; Jimenez-Vergara, A. C.; Hahn, M. S.; Grunlan, M. A., A bioactive “self-fitting” shape memory polymer scaffold with potential to treat cranio-maxillo facial bone defects. *Acta Biomaterialia* **2014**, *10* (11), 4597-4605.
6. Nail, L. N.; Zhang, D.; Reinhard, J. L.; Grunlan, M. A., Fabrication of a Bioactive, PCL-based "Self-fitting" Shape Memory Polymer Scaffold. *J Vis Exp* **2015**, (105), e52981.
7. Woodard, L. N.; Page, V. M.; Kmetz, K. T.; Grunlan, M. A., PCL-PLLA Semi-IPN Shape Memory Polymers (SMPs): Degradation and Mechanical Properties. *Macromolecular Rapid Communications* **2016**, *37* (23), 1972-1977.
8. Gilding, D. K.; Reed, A. M., Biodegradable polymers for use in surgery— polyglycolic/poly(lactic acid) homo- and copolymers: 1. *Polymer* **1979**, *20* (12), 1459-1464.
9. Pfau, M. R.; McKinzey, K. G.; Roth, A. A.; Grunlan, M. A., PCL-based shape memory polymer (SMP) semi-IPNs: The role of miscibility in tuning degradation rate. *Biomacromolecules* [under review].

10. Roth, A. A. Assessment of Degradation for Porous PCL-PLLA Semi-IPN Shape Memory Polymer (SMP) Implants for Cranial Defect Repair. 2019.
11. Woodard, L. N.; Grunlan, M. A., Hydrolytic degradation of PCL-PLLA semi-IPNs exhibiting rapid, tunable degradation. *ACS Biomater Sci Eng* **2019**, *5* (2), 498-508.
12. von Burkersroda, F.; Schedl, L.; Gopferich, A., Why degradable polymers undergo surface erosion or bulk erosion. *Biomaterials* **2002**, *23* (21), 4221-31.
13. Kaihara, S.; Matsumura, S.; Mikos, A. G.; Fisher, J. P., Synthesis of poly(L-lactide) and polyglycolide by ring-opening polymerization. *Nature Protocols* **2007**, *2* (11), 2767-2771.
14. Pitt, C. G.; Chasalow, F. I.; Hibionada, Y. M.; Klimas, D. M.; Schindler, A., Aliphatic polyesters. I. The degradation of poly( $\epsilon$ -caprolactone) in vivo. *Journal of Applied Polymer Science* **1981**, *26* (11), 3779-3787.
15. Siparsky, G.; Voorhees, K.; Dorgan, J.; Schilling, K., Water transport in polylactic acid (PLA), PLA/ polycaprolactone copolymers, and PLA/polyethylene glycol blends. *Journal of Environmental Polymer Degradation* **1997**, *5*, 125-136.
16. Navarro-Baena, I.; Kenny, J. M.; Peponi, L., Crystallization and thermal characterization of biodegradable tri-block copolymers and poly(ester-urethane)s based on PCL and PLLA. *Polymer Degradation and Stability* **2014**, *108*, 140-150.
17. Tsuji, H.; Miyauchi, S., Poly(l-lactide): 7. Enzymatic hydrolysis of free and restricted amorphous regions in poly(l-lactide) films with different crystallinities and a fixed crystalline thickness. *Polymer* **2001**, *42* (9), 4463-4467.