SYNTHESIS AND CHARACTERIZATION OF MICROPARTICLES FOR TEMPLATING POROUS SHAPE MEMORY POLYMER SCAFFOLDS

An Undergraduate Research Scholars Thesis

by

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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. Duncan J. Maitland

May 2017

Major: Biomedical Engineering

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ABSTRACT

Synthesis and Characterization of Microparticles for Templating Porous Shape Memory Polymer Scaffolds

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Shape memory polymers (SMPs) are proposed for use in a variety of medical devices, such as neural and peripheral embolism coils for aneurysm occlusion. These "smart" materials have unique advantages over shape memory alloys, such as light weight, large shape recovery of up to 400% plastic strain, nontoxicity, nonmutagenicity, ease of processing. and low cost. Processing SMPs into porous forms increases their potential for use in a number of applications due to unique properties, such as increased thermal and electrical insulation, large volume changes on recovery from compressive strain, and low density.

Current SMP foams utilize a gas blowing technique to create the pores. This method results in inhomogeneous pore sizes and may result in shearing of the foams. By templating the SMP foam matrix with microparticles of controlled diameters, we hypothesize that we will be able to finely tune pore sizes within a set range and ensure pore interconnectivity.

Here, we fabricated alginate microparticles using a co-flow emulsion technique. The microparticles were sieved to a size range of 75 – 125 μm before utilizing them to template poly(dimethyl siloxane) (PDMS) matrices. The resulting polymer matrices were characterized in

terms of pore size and morphology. We found that utilizing the microparticles to template the matrices resulted in an interconnected pore matrix with homogeneous pores, which we hypothesize will allow for controlled expansion of the matrix. These results found using PDMS matrix will lay the groundwork for future generation of SMP foams with controlled porosity, reduced risk of shearing, and enhanced material properties for a variety of medical applications.

DEDICATION

I would like to dedicate this work to my parents, Dr. Balakrishna Haridas and Rashmi Balakrishna, who have always pushed, and encouraged me to pursue what I am passionate about. They championed me through college and have been my biggest supporters, providing me with more opportunities than anyone could ask for. Without their support, I would not be where I am today.

ACKNOWLEDGEMENTS

First, I would like to thank Dr. Duncan Maitland who allowed me to pursue this project in the BDL. Working with all of the students in the BDL has given me so many valuable experiences that I will continue to utilize throughout the entirety of my career, whatever path that I may take.

I would like to thank Ms. Grace Fletcher, who took me under her wing and advised me with the research, and explaining the variety of processes and laboratory techniques that I would use in my research. She has been instrumental throughout the entire Undergraduate Thesis process and working with her has been a pleasure.

Finally, I would like to thank Dr. Brandis Keller and Dr. Mary Beth Monroe, who spent countless hours helping me edit my thesis, and providing excellent and very constructive feedback on my scientific writing abilities.

NOMENCLATURE

SMP……Shape Memory Polymer

BDL……..Biomedical Device Laboratory

PU……Polyurethane

GDC……Gugliemi Detachable Coil

PDMS……..Poly(dimethyl siloxane)

SEM……..Scanning Electron Microscopy

RO……..Reverse Osmosis

CHAPTER I

INTRODUCTION

Clinical motivation and relevance

Cerebral aneurysms are weak, thin spots in the blood vessels within the brain that balloon out and fill with blood. Aneurysms can result from congenital defects; trauma or injury to the head; infections (mycotic aneurysms); high blood pressure; cancer and tumors found in the head or neck region; and other vascular diseases. Tobacco use and other forms of drug abuse are also associated with the formation of cerebral aneurysms. Cerebral aneurysms may rupture and cause hemorrhaging, or localized bleeding in the tissues of the brain [2]. This hemorrhaging is associated with severe complications, including hemorrhagic stroke, permanent nerve damage, or even death.

The goal of current cerebral aneurysm treatment is to cut off blood flow to the aneurysm and prevent rupture. A variety of surgical procedures are employed to cut off blood flow, such as microvascular clipping, during which a neurosurgeon will go through the skull and place a metallic clip on the aneurysm to cut off the blood supply, and bypass procedures, during which the surgeon clamps off the entire artery that feeds the aneurysm [2] and implants a bypass vessel to reroute blood flow around the damaged artery. Due to the invasive nature of these surgical procedures, there is a risk of damaging other blood vessels during the procedure, aneurysm recurrence, and post-operative stroke. These risks prompted physicians to pursue minimally invasive cerebral aneurysm treatment methods.

The current preferred method of cerebral aneurysm treatment uses platinum embolization coils (e.g. Gugliemi detachable coils, GDCs) that are delivered via catheter to tightly pack the aneurysm and block it from circulation, eventually forming a clot. All of the current treatments can be seen in **Figure 1**

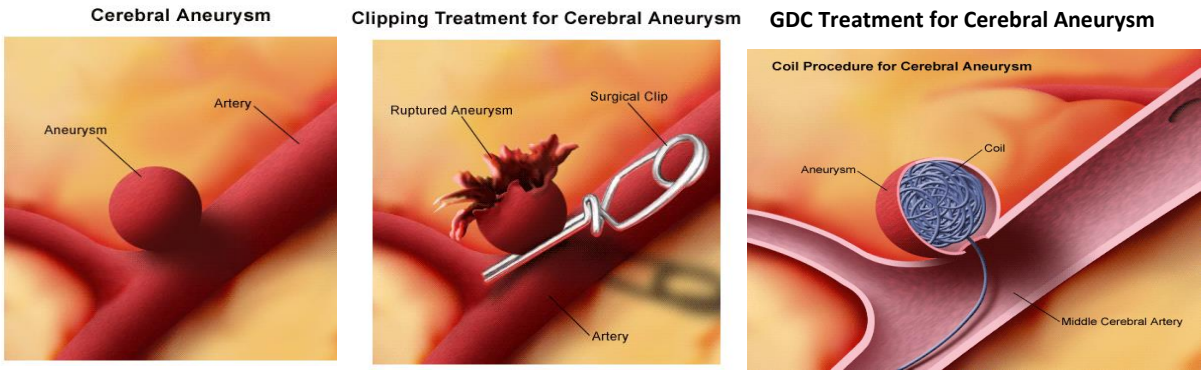

Figure 1: Current treatments for cerebral aneurysms: Cerebral aneurysm (left), Invasive surgical clipping of cerebral aneurysm (middle), Gugliemi detachable coil minimally invasive treatment of cerebral aneurysm (right). Images from Columbia Department of Neuroseurgery [12].

Current coil packing methods have limited efficacy due to complications, such as incomplete aneurysm occlusion, aneurysm rupture, or changes in coil position. As a result, patients who undergo aneurysm embolization treatment may need to receive multiple treatments.

Research objectives

Shape memory polymers (SMPs) are an emerging class of smart materials that can be deformed and stored in a temporary/secondary shape and thereafter actuated on demand via an external stimulus, such as heat, to return to their primary shape [9]. SMP foams are low density, interconnected porous materials that can be compressed into a secondary shape and actuated back to the expanded shape (**Figure 2)**, and have been proposed to replace platinum coils for aneurysm occlusion.

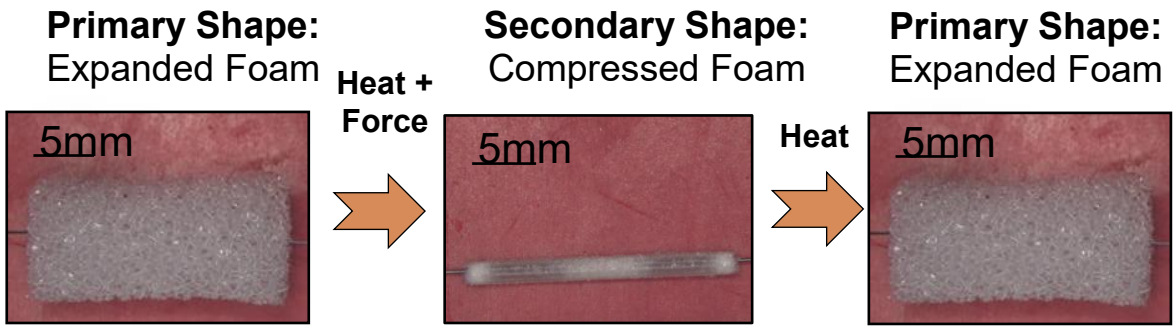

Figure 2: Shape memory polymer foams depicted undergoing shape memory effect

SMP foam-based devices can stay in their temporary compressed shape and delivered via catheter, allowing for efficient, non-invasive implantation, and they promote rapid blood clotting following expansion. These valuable properties suggest that SMP foams are ideal candidates to replace current embolization coils. Animal studies were conducted in a porcine sidewall aneurysm model using the proposed SMP foam-based aneurysm treatment compared to the standard Gugliemi detachable coil (GDC) treatment. Both the GDC coils and the SMP foams promoted clotting within the aneurysm [3]. Histological assessment verified that the SMP foams are biocompatible and effective at providing a biological scaffold with enhanced healing, as indicated by aneurysm shrinking and neointima formation across the aneurysm neck without compromising the parent vessel lumen [7].

Currently, gas blowing is utilized to fabricate SMP foams. However, this technique results in foams with inconsistent material properties due to highly heterogeneous pore sizes. Additionally, the SMP foams fabricated using this method are prone to tearing. We hypothesize that SMP foam pore sizes can be standardized using templating with polymeric microparticles with controlled diameters, which will improve consistency in properties and decrease the risk of tearing.

We propose to synthesize alginate microparticles using a microfluidic system. Optimization of this process includes manufacturing and sieving the microparticles to ensure homogeneous microparticle diameters. These sieved particles will be used to template poly(dimethyl siloxane) (PDMS) foams with finely controlled pore size homogeneity. PDMS will be crosslinked, or solidified, around tightly-packed alginate microparticles. The alginate particles will then be dissolved out of the foam, leaving interconnected pores. We hypothesize that this method will translate to production of polyurethane (PU) SMP foams with highly homogenous pores to enhance foaming reproducibility and reduce the risk of mechanical failure. The resulting foams will be characterized in future work in terms of pore size, density, and mechanical properties and compared to SMP foams currently in development for neural embolization devices.

CHAPTER II

METHODS

Alginate Microparticle

Fabrication

The small alginate microparticles were synthesized using an adapted co-flow emulsion technique [6], as see in **Figure 3**. A 3% alginate solution of alginate (Sigma-Aldrich) and reverse osmosis (RO) water was injected dropwise using a syringe pump (New Era Pump Systems, Inc.) into an external phase containing mineral oil with 1% Span-80 surfactant (Harvard Apparatus PHD 2000 Infuse/Withdraw Pump). The resulting microparticles were then deposited into a 7% calcium chloride solution to ionically crosslink. Needle gauge, continuous external phase flow rate, and alginate injection rate were varied to adjust microparticle size. Particles were fabricated using 1.6 mm ID tubing and a 23-gauge needle. The 3% alginate solution was injected at a rate of 15 μL/min.

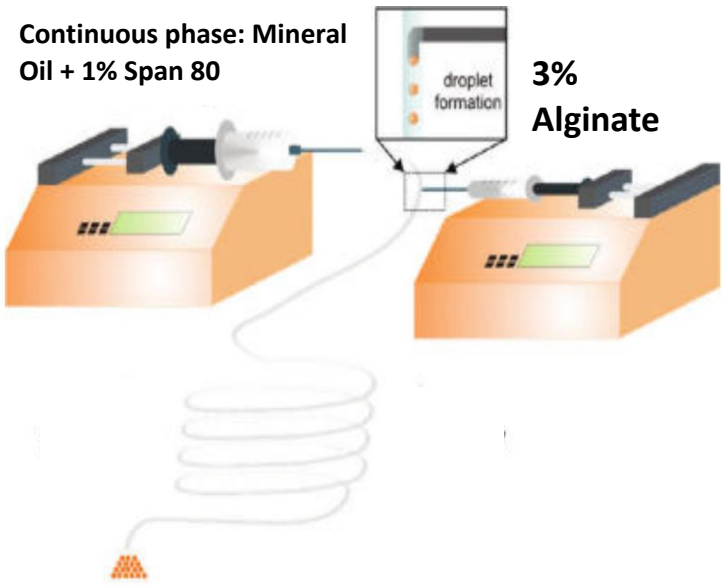

Figure 3: Co-flow emulsion microparticle setup for fabrication of small microparticles (50-127 μm). Adapted from Gokmen et al [5].

Larger particles were then fabricated as a means of comparison to the smaller particles as shown in **Figure 4**. The 3% alginate solution was injected dropwise directly into the 7% calcium chloride solution at a rate of 50 μm/min.

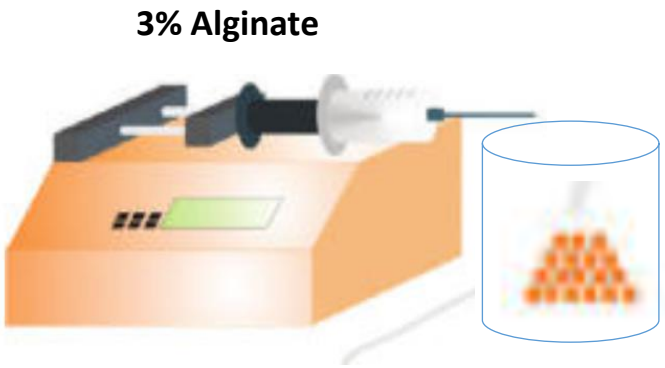

Figure 4: Standard microparticle setup for fabrication of large microparticles (2500-3000 μm). Adapted from Gokmen et al [5].

The mineral oil was removed via pipetting, and the microparticles were then filtered using a Geotech sand shaker with stainless steel mesh sieves. The sieve sizes that were used can be seen in **Table 1** below.

Table 1: Sieve designation and sizes used for filtering alginate Microparticles

Sieve Designation	Mesh Opening (mm)	US Standard Sieve No.
26 OPN	0.6604	25
15 OPN	0.3810	40
09 OPN	0.2286	60
055 OPN	0.1397	100
041 OPN	0.1041	140

Size Determination

A particle counter (PC5000, Chemtrac®) was used to quantify the mean microparticle size in solution. Microparticles were flown through the tube (pre-sieved and post-sieved) and the number of microparticles within a variety of size distributions was output. A histogram of the size distribution of particles was created from each run and used to determine the polydispersity index (PDI).

Poly-dimethyl Siloxane (PDMS) Polymer Matrix

Synthesis

PDMS matrices were synthesized by using 9.1 g of premade resin (Sylgard® 184 Silicone Elastomer Kit). The curing agent was then added to the cup and mixed for 30 seconds. The alginate microparticles were placed in rectangular containers and shaken so that they would be evenly dispersed and properly aligned within the mold. The contents of the FlakTek cup were poured over the microparticles in the rectangular mold, allowed to settle, and the mold was placed under vacuum inside a bell jar to remove any air bubbles. Once the bubbles were removed, the PDMS was cured for 1 hour at room temperature, followed by 90 °C for one hour. After curing, the polymer matrix was removed from the mold, and the two edges were cut to expose the templated pores. Blocks were dried for 48 hours. The remaining microspheres shrink and fall out of the matrix as a result of the drying process. A summary of the PDMS matrix synthesis can be seen in **Figure 5** below.

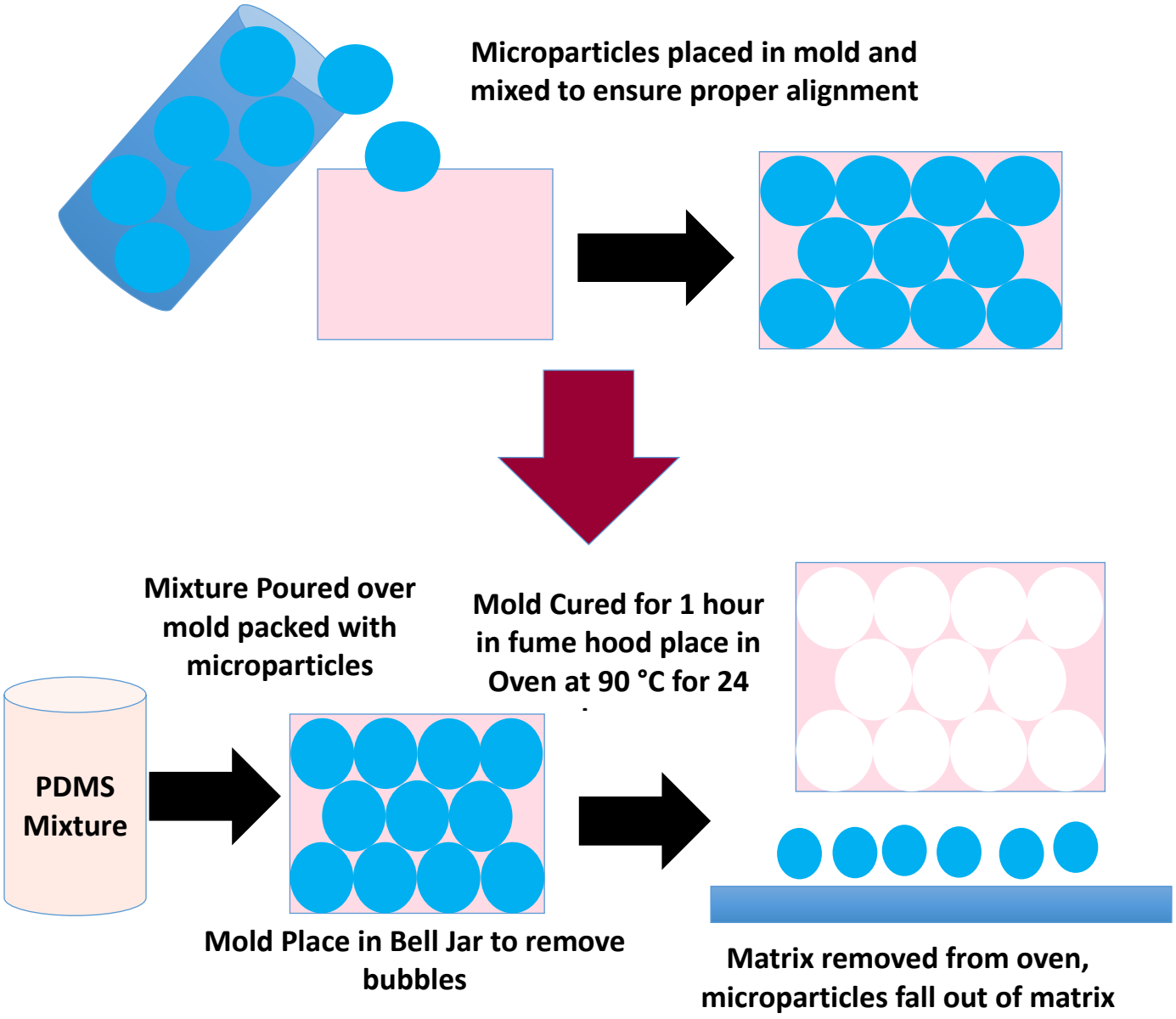

Figure 5: PDMS polymer matrix templating and synthesis

Matrix Characterization

Pore Size Measurements

Slices of the polymer blocks were cut in the axial and transverse directions and imaged using scanning electron microscopy (SEM). The diameter, d, of the pore was measured for the analysis by measuring the greatest distance between two walls of the pore.

Pore Morphology

To assess pore interconnectivity in the PDMS polymer matrix, SEM images were used. A piece of the polymer matrix was cut and attached to the SEM stub using electrically-conductive carbon tape. A sputter coater was used to coat the sample with gold, an electrically conductive material, in order to provide a high electron output for improved surface resolution.

CHAPTER III

RESULTS AND DISCUSSION

Alginate Microparticles

Fabrication and Optimization

Multiple run parameters were tested throughout the fabrication process for the smaller microparticles. The parameters were altered to homogenize the particle size and shape. These parameters include the continuous phase flow rate, alginate flow rate, concentration of alginate solution, temperature of the alginate solution, and position of the tubing in the receiving beaker. The final parameters that were selected for the fabrication of the microparticles were the 3% alginate solution being injected at a rate of 10 μL/min and the continuous mineral oil phase with 1% Span 80 being injected at a rate of 1 mL/min. These parameters would result in optimal small microparticle sizing with the distribution being improved post-sieving. The large microparticles were effectively fabricated when they were injected dropwise at a rate of 50 μL/min directly into the calcium chloride solution.

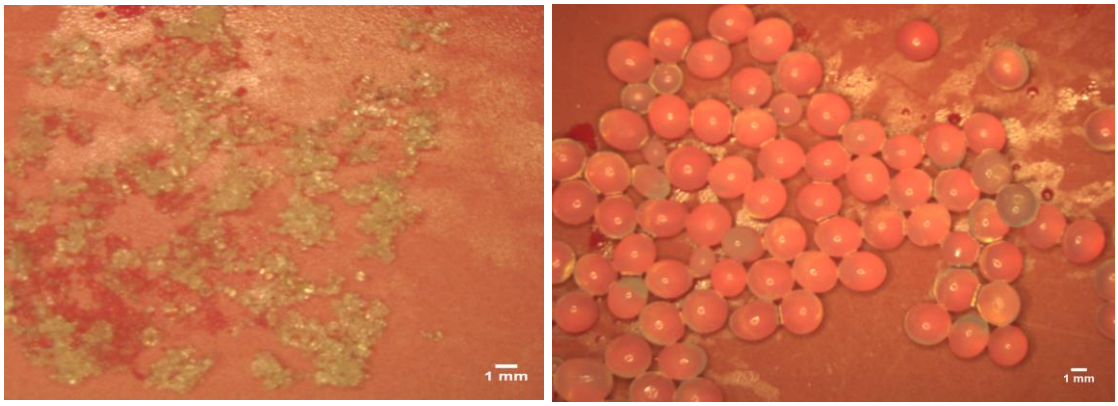

Figure 6: Small microparticles fabricated using the co-flow emulsion technique (right) and the large microparticles fabricated dropwise in solution (right)

Microparticle Size Distribution

The microparticle size distribution was tested before and after the particles were filtered using sieves ranging from 0.1041 mm to 0.6604 mm. Using the co-flow emulsion technique, the small microparticles were effectively fabricated and were constrained between 50 μm and 127 μm. The larger microparticles ranged from 2500 μm to 3000 μm, as seen in **Figure 7**. The average microparticle sizes and standard deviations for both the large and small alginate microparticles can be seen in **Table 2**.

Table 2: Average microparticle diameter and standard deviations

Type of Microparticle	Diameter of Microparticles (μm)	Standard Deviation
Large Microparticles	2822	255
Small Microparticles	67	15

PDMS Polymer Matrix

Pore Interconnectivity and Size Measurements

SEM cross-sectional images of the PDMS polymer matrices, shown in Figure 8, were used to quantitatively measure the interconnected pores as a result of the templating. The resulting matrix pore sizes were 59 ± 20 μm for the small alginate microparticles, and 1798 ± 185 μm for the large microparticles (**Table 3)**. The pore size of the of gas blown matrix was measured by measuring the long and short axis of the pore and averaging them together for the overall average diameter. The standard deviation for the gas blown pore size was much larger

compared to the pores created via the polymer templating, suggesting the pores formed during the gas blowing technique were inhomogeneous in size and shape.

Table 3: Average pore diameter and standard deviations

Type of Pore	Pore Size (µm)	Standard Deviation
Large Pore	1798	185
Small Pore	59	20
Gas Blown Pore	13437	4368

The pore morphology can be seen in the SEM images taken in **Figure 8**, depicting that the polymer matrices synthesized using the microparticle templating methods had a greater degree of interconnectivity than the polymer matrix developed using the current gas blowing technique. The images also show the homogeneity of the pores from the templated polymers compared to the non-homogeneous pore shapes and sizes that resulted from the gas blowing method, which indicates that foaming reproducibility could be improved using templating.

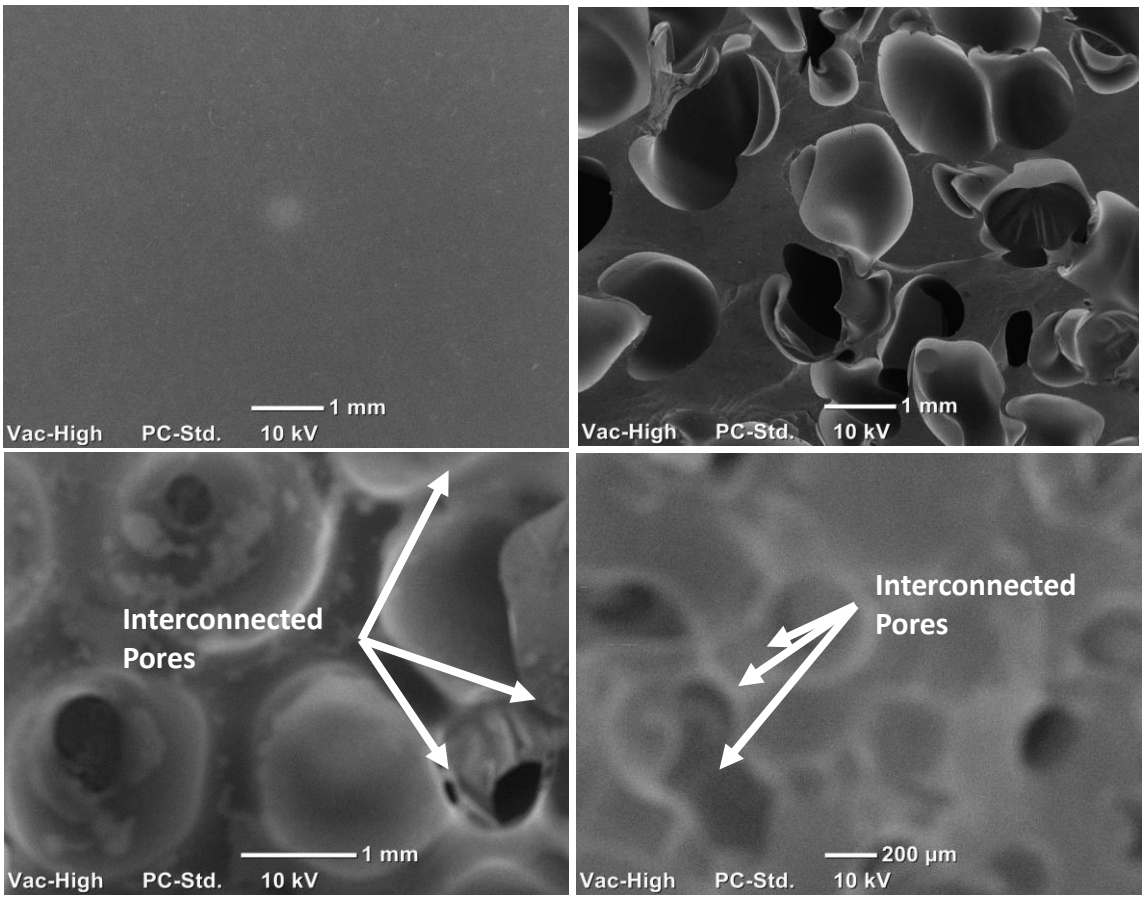

Figure 8: SEM images of PDMS matrices with no pores (top left), gas blown pores (top right), large templated pores (bottom left), and small templated pores (bottom right).

CHAPTER IV
CONCLUSIONS

Conclusions

This study characterized the use of alginate microparticles as a means for templating polymer matrices to create porous polymer matrices for applications in cerebral aneurysm occlusion. This was done via quantitative measurements, calculations, and comparisons of pore size, and microparticle diameter; as well as comparisons of the pore morphology in the polymer matrices. With the pore morphology showing an interconnected, and homogeneous pore network, it can be concluded that templating polymers with microparticles is an effective method for creating interconnected polymer matrices and foams. We were able to effectively fabricate alginate microparticles for use in templating polymers and developed parameters creating particles with a tunable size range. Additionally, we were able to further optimize the pore morphology in the PDMS polymer matrix by using the microparticles to template the PDMS and ensure pore homogeneity and interconnectivity. The resultant pore diameters closely match the diameter of the fabricated microparticles, which demonstrates the ability to precisely tune pore size via microparticle templating. In this work, we demonstrated a proof of concept that could be applied to polyurethane polymer matrices to develop SMP foams with homogeneous and interconnected pores, potentially reducing the risk of mechanical failure due to shear.

Future Work

In future studies, the alginate microparticles developed using the co-flow emulsion technique will be used to pattern and template the SMP matrix to demonstrate that the concept may be transferred. Pore morphology in the new polymer matrix must be characterized as well as the efficacy of the templated polymer to enhance mechanical properties and foaming reproducibility.

The use of microparticles to template SMP matrices shows promise, but requires further work and optimization for clinical translation.

REFERENCES

1. Spine, M.B. *Aneurysm Embolization: Coiling*. Endovascular Aneurysm Coiling 2016.

2. Medicine, U.S.N.L.o. *Cerebral Aneurysms Fact Sheet*. 2016.

3. Horn, J., et al., *Comparison of shape memory polymer foam versus bare metal coil treatments in an in vivo porcine sidewall aneurysm model.* J Biomed Mater Res B Appl Biomater, 2016.

4. Singhal, P., et al., *Controlling the Actuation Rate of Low-Density Shape-Memory Polymer Foams in Water.* Macromol Chem Phys, 2013. **214**(11): p. 1204-1214.

5. M. Talha Gokmen, ‡ Wim Van Camp,† Patrick J. Colver,‡ Stefan A. F. Bon,*,‡ and Filip E. Du Prez*,†, *Fabrication of Porous "Clickable" Polymer Beads and Rods through Generation of High Internal Phase Emulsion (HIPE) Droplets in a Simple Microfluidic Device.* Macromolecules, 2009. **42**: p. 9289-9294.

6. Rodriguez, J.N., et al., *In vivo response to an implanted shape memory polyurethane foam in a porcine aneurysm model.* J Biomed Mater Res A, 2014. **102**(5): p. 1231-42.

7. Calcagnile, P., et al., *Polymeric foams with functional nanocomposite cells.* RSC Advances, 2014. **4**(37): p. 19177.

8. *<Polyurethane- Foam- 17. Formulation, Preparation, and Charac of Polyurethane foams.pdf>*.

9. Wilson, T.S., et al., *Shape memory polymers based on uniform aliphatic urethane networks.* Journal of Applied Polymer Science, 2007. **106**(1): p. 540-551.

10. Moglia, R., et al., *Solvent-free fabrication of polyHIPE microspheres for controlled release of growth factors.* Macromol Rapid Commun, 2014. **35**(14): p. 1301-5.
11. Singhal, P., et al., *Ultra Low Density and Highly Crosslinked Biocompatible Shape Memory Polyurethane Foams.* J Polym Sci B Polym Phys, 2012. **50**(10): p. 724-737.

12. Neurosurgery, C.D.o. *Aneurysm*. 2017 [cited 2017 4/7/2017].