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UNIVERSITY OF CALIFORNIA
Los Angeles

Materials Approach to Novel Endovascular Coils with Enhanced Wound Healing for Intracranial Aneurysms

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Biomedical Engineering

by

Arnold Tirapat Suwarnasarn

2015
ABSTRACT OF THE DISSERTATION

Materials Approach to Novel Endovascular Coils with Enhanced Wound Healing for Intracranial Aneurysms

by

Arnold Tirapat Suwarnasarn

Doctor of Philosophy in Biomedical Engineering

University of California, Los Angeles, 2015

Professor Benjamin Wu, Chair

An intracranial aneurysm (ICA) is a local distension within the arterial wall of the brain. Within the United States it is estimated that 15 million people have some form of an ICA, with an additional incidence rate of 75,000 patients per year. When a patient experiences an aneurysm rupture, 15% of patients will die before reaching the hospital. However shockingly, 50% of patients that receive treatment with existing technology will die within the first 30 days. Therefore, current technology must be improved to for fast and effective treatment. The goal of this research was to utilize inherent biomaterial properties for the purposes of accelerating the ICA wound healing process.

We have developed a novel endovascular device around clinical practice with high potential for commercial development. Requirements for technological development were to
limit friction during deployment, validate coating quality for commercial fabrication, and achieve accelerated intracranial aneurysm wound healing above bare platinum coils (BPC).

Research has shown that materials can influence the wound healing cascade. It was hypothesized that inducing a stronger inflammatory reaction will lead to accelerated wound healing mechanisms downstream. We have shown that fast degrading acid modified poly(lactic-co-glycolic) polymers (aPLGA) can further accelerate this process by early induction of the inflammatory phase. To further understand the mechanism behind accelerated ICA wound healing, we performed in vitro analysis of low molecular weight aPLGA. Cellular response to aPLGA showed increased levels of inflammatory cytokines as well as collagen deposition. In addition, in vivo tests were performed in swine and showed significant healing above BPCs and another commercially available coil type.

Degradable metals were investigated for the potential to induce accelerated ICA healing in vitro. Candidate degradable metals were chosen based on biocompatibility and commercial feasibility for production. Electroplated iron on BPCs showed an increase of inflammatory cytokines in vitro as well as collagen deposition against controls. Proof of concept for commercial development was shown by atomic sputter deposition.

The goal of this research was to develop novel solutions to achieve accelerated ICA wound healing through inherent biomaterial properties. This goal was achieved by elucidating candidate materials which induced enhanced inflammatory reactions, and then constructed processes for commercial fabrication.
The dissertation of Arnold Tirapat Suwarnasarn is approved.

James Dunn

Min Lee

Maie St. John

Benjamin Wu, Committee Chair

University of California, Los Angeles

2015
DEDICATION PAGE

This thesis is dedicated to my loving family. Their unconditional love gives me strength to overcome my weaknesses.

I would also like to thank my loving fiancé. Without her pushing me forward, I would have never completed this thesis. (80% me, 20% you… it’s in writing…)
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<tr>
<td>3D</td>
<td>Three dimensional</td>
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<td>3DP</td>
<td>Three dimensional printing</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>aPLGA</td>
<td>Acid modified Poly(lactic-co-glycolic) acid</td>
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<td>CaCl₂</td>
<td>Calcium Chloride</td>
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<td>BPC</td>
<td>Bare platinum coil</td>
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<tr>
<td>DI-H₂O</td>
<td>Distilled water</td>
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<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle Medium</td>
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<td>DPBS</td>
<td>Dulbecco’s Phosphate-Buffered Saline</td>
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<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
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<td>FBS</td>
<td>Fetal bovine serum</td>
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<td>Food and Drug Administration</td>
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<td>Human foreskin fibroblast</td>
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<td>Intracranial Aneurysm</td>
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<td>MCP-1</td>
<td>Monocyte Chemotactic Protein 1</td>
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<tr>
<td>PGLA</td>
<td>Poly (glycolic-co-lactic acid</td>
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<tr>
<td>PLGA</td>
<td>Poly (lactic-co-glycolic) acid</td>
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<tr>
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<td>Scanning electron microscopy</td>
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<tr>
<td>SCC</td>
<td>Spiral coated coil</td>
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<tr>
<td>TGF-b</td>
<td>Transforming growth factor beta</td>
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<td>Tumor necrosis factor alpha</td>
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A Bioactive Spiral Coil Coating. UCLA Case No. 2011-135-11
CHAPTER ONE - BACKGROUND

1.1 INTRODUCTION

Disease State and Progression of Intracranial Aneurysms

Intracranial aneurysms (ICA) are a vascular disease where a local distension of the arterial wall within the brain is enlarged. When an ICA ruptures into the brain, permanent damage and death most often results. Despite modern treatment the mortality rate for ruptured aneurysms is between 30-40% (1). It is estimated that between 3.6-6% of the general population exhibit an unruptured aneurysm (2). In the United States alone this amounts to almost 18 million individuals. However, 99% of these patients will experience significant quality of life ailments which include: seizures, loss of balance, speech complications, mental processing problems, and decreased concentration.

The anatomical structure of a saccular aneurysm consists of a dome region and a neck region (1, 3). Turbulent high-pressure and pulsatile blood flow exists in the dome region which can increase the size of the aneurysm. The neck region connects the dome to parental blood flow. These ICAs can manifest at any point in the brain, however they are most often presented within the Circle of Willis at bifurcation points as well as slightly downstream of bifurcations.

Normal healthy arterial vessels are composed of three discrete cellular layers, separated by two elastic lamina. The tunica intima lines the lumen of the vessel, wherein a monolayer of endothelial cells provide a non-clotting surface with the blood. Adjacent to the intima lies the internal elastic lamina which reinforces and aids in arterial compliance. The middle layer is the tunica media which is composed of concentrically oriented vascular smooth muscle cells, elastin, and collagen. The function of this layer is principally to modulate blood pressure. An external elastic lamina then separates the tunica media from the tunica adventitia; the outer most layer.
This layer is composed of dense longitudinally oriented collagen fibers which provide additional strength to high blood pressures seen in arteries.

The pathology of arteries with saccular intracranial aneurysms possess key differences. These differences include: significantly reduced elastin within the tunica media, absence of an external elastic lamina, as well as the nonappearance of a tunica adventitia (4). The presence of these abnormalities, and in combination with high-pressure pulsatile blood flow, contribute to the growth and eventual rupture of these aneurysm (1). In addition, due to tissue damage and rupture, a higher concentration of inflammatory cells are seen within the vessel wall that comprises the saccular aneurysm.

1.2 REVIEW OF LITERATURE

Current Technology for Intracranial Aneurysm Treatment

Due to the nature of this disease, ICAs present significant problems not only to the patient, but also for physicians and engineers to develop minimally invasive tools for treatment. The pathway of cerebral vasculature can be tortuous, present small caliber vessels, and bifurcation points which require dexterous tools for endovascular surgery. In addition, the presence of high arterial blood pressure and pulsatile turbulent flow within the aneurysm itself demands an even higher degree of precision for device implantation.

Bare platinum coils (BPC), developed at UCLA, have been the gold standard for treating saccular ICAs. These first generation helical shape-memory BPCs revolutionized the treatment of ICAs since patient recovery times were drastically reduced when compared to open brain surgery (5). The minimally invasive procedure requires a small incision in the femoral vein, wherein a small microcatheter is inserted, navigated past the aorta, and to the site of the ICA.
Once the tip of the microcatheter is placed within the aneurysm, BPCs larger and stronger in size are deposited one at a time to “frame” the aneurysm sack. Subsequently, smaller coils are deposited into the interior to increase packing density and “fill” the aneurysm (5).

The primary function of BPCs is to occlude the ICA from parental blood flow. This requires a mechanically strong medical device to withstand hemodynamics, but still remain flexible and dexterous for precise placement at over 1 meter away without direct manipulation of the BPCs. If the aneurysm is insufficiently packed or the BPCs used are not mechanically strong, blood flow may re-enter the aneurysm resulting in recanalization and the genesis of a secondary aneurysm. These secondary aneurysms are significantly more difficult to treat. When treated with BPCs, aneurysm eventually heal after 6 months.

Second generation bioactive coils have been developed to accelerate ICA healing. While multiple commercially available designs exist, many achieve accelerated healing through the use of biodegradable polymers. The typical healing time of smaller aneurysms with well-defined neck regions is between 4-6 months. Current commercially available bioactive coils – coils which utilize polymers to enhance aneurysm healing – still possess significant limitations. These limitations include: increased friction during delivery, exhibit inadequate strength due to industrial fabrication methods, contain sub-optimal healing speed do to polymer selection, and can only be used for the inner most region of the aneurysm sac. In addition, since they are mechanically weaker due to their construction – 70% polymer, 30% platinum by volume - they are used in conjunction with first generation BPCs, and limited to the interior of the aneurysm to increase packing density.

Metallic stents and flow diverters are also used to treat ICAs as well. However, these products have limitations. Metallic stents and flow diverters present the possibility of thrombus
formation on struts and within the lumen of the device. These require anti-thrombotic drugs for prolonged periods, if not for the remainder of the patient’s life. In addition, while flow diverters have been attempted to treat aneurysms at bifurcation points, they have drastically reduced patient outcomes, making them unsuitable for these types of ICAs.

Considerations for Medical Device Design

In the field of bioengineering, the goal is to develop translational medicine. A myriad of material classes can be used. In addition, the form-factor and design of a medical device can vary widely even for the same application. Furthermore, the presence of numerous drugs and proteins, compounds the complexity for medical device design. Therefore to simplify the situation and produce useable and innovative devices, three factors should be considered prior to inception: usability, manufacturability, and regulation. First, usability considers the ease at which a device can be used by medical providers. If a tool is too difficult to use or execute properly, adoption of the device to the field will be extremely slow. In fact, patient outcomes may even be reduced due to improper use. However, if patient outcomes are significantly increased, a device may still be adopted at the expense of years of training. Second, consistent and precise manufacturability must be achieved. While laboratory scale tests are useful for uncovering possible innovations translation to production may be halted due to significant costs for intricate medical device designs. Lastly, the resulting device must be able to pass regulation to be used. This entails years of experimentation and validation to prove efficacy and safety. In addition, combination devices which serve two functions – a drug eluting stent, for example – will require even longer for regulatory review. These are the barriers-to-entry for a successful medical device.
To reduce the barriers-to-entry, the goal of this project was to develop an innovative medical device for endovascular delivery for the treatment of saccular ICAs. Our core construct utilizes the BPC platform, which increase the potential for adoption by leverage of a gold-standard device already used in the field, as well as reduces regulatory review timeframe based on predicate devices. In addition, this work has shown the potential for accelerated vascular healing through the use of inherent biomaterial properties. By selecting materials that have been used in other medical devices approved by the FDA, this drastically reduces regulatory review requirements when compared to material/drug combinations. Lastly, we have developed two fabrication systems for translation of laboratory prototypes to large scale production.

The benefits of utilizing inherent biomaterial properties in medical device design is realized by comparison of drugs and proteins. Growth factors and chemokines such as Transforming Growth Factor Beta (TGF-b) and Monocyte Chemotactic Protein-1 (MCP-1) have been shown to accelerated wound healing through enhancing cellular proliferation and recruitment of inflammatory cells. However, utilization of these molecules for clinical application – i.e. the development of a medical device – possess significant limitations. First, proteins most often require a carrier material for local administration; typically a polymer. As consequence, development costs can be significant since product validation must be performed to ensure that: 1) proteins are pure and free of other contaminating molecules, 2) proteins are not denatured due to the carrier, 3) the correct dose is administered to prevent deleterious growth, 4) have limited shelf life since proteins inevitably degrade over time, 5) increase production and storage costs due to refrigeration, and 6) longer time for FDA review for a combination device. While a medical device utilizing inherent biomaterial properties also require product validation for safety and efficacy, the requirements are significantly lower and faster for biomaterials alone.
Normal wound healing proceeds by initiation of hemostasis, followed by localized inflammation, a proliferative phase, and finally remodeling of the site. Hemostasis is the formation of a stable fibrin clot through aggregation of platelets, fibrinogen, and other proteins within the blood. Inflammation follows with infiltration by neutrophils and monocytes cells which are present throughout the wound healing cascade to orchestrate productive healing. Proliferation of fibroblasts respond to inflammatory signals and begin to deposit collagen type III, in addition, angiogenesis will also ensue to provide nutrients to highly active cells within the wound. Finally resolution of the wound proceeds by conversion of collagen type III to collagen type I, as well as recession of vasculature. While each one of these phases are important to normal healing, it is important to understand that the wound healing cascade is a continuum of events where cells are responding to preceding cells, deposited extracellular matrices, proteins, and biomaterials.

The role of the inflammatory phase cannot be understated since downstream events of the wound healing cascade will be defined by the magnitude of inflammatory cytokines expressed. In addition, the introduction of biomaterials to the site can have dramatic effects on the inflammatory phase which will determine the biocompatibility of implanted materials. Factors which are known to cause an inflammatory response include: surface chemistry, surface roughness, degradation particles, and wear particles.

For decades, inflammation has been perceived as a problematic hurdle for medical devices, and thus, biomaterials have been engineered such that the inflammatory phase is quickly resolved within the host. However, growing evidence indicates that inherent biomaterial properties can produce beneficial accelerated healing through an enhanced, yet transient,
inflammatory response. Specifically, poly(lactic-co-glycolic) acids (PLGAs) have been shown to accelerate the healing of intracranial aneurysms \textit{in vivo} when compared to aneurysms treated without PLGA. In addition, the production of inflammatory cytokines is enhanced by acidic PLGAs when cocultured with Human Foreskin Fibroblasts (HFF) and Human derived monocytes (U937) in vitro. While the precise mechanism of this phenomena is poorly understood, it is believed that the speed of degradation and composition of degradation products are key factors which produce accelerated healing.

\textit{Cell Response to Biomaterials}

Biocompatibility is typically defined as the benign host response to implanted materials, whereby the presence of inflammatory cells subsides, long after implantation. However, when biomaterials are first implanted, these materials will undergo host immunity. Subsequently, contact with blood proteins will allow inflammatory cells to determine “self” from “non-self” materials. If materials are perceived as non-self, blood proteins as well as inflammatory cells will respond by initiating either the innate or adaptive immune systems (6). Macrophages are the first to respond to implanted materials.

Macrophages are responsible to determine the outcome of the biomaterial – i.e. degradation, phagocytosis, or encapsulation – through secreted cytokines, chemokines and growth factors (7). Macrophages sense and bind to materials via integrin-mediated adhesion (8). Upon binding, these interactions result in changes in the actin cytoskeleton, activation of cellular responses, gene transcription and cell movement (9). It is believed that blood proteins adsorbed to the material trigger specific cellular responses (10, 11). The role of macrophages in wound healing is further highlighted in cell plasticity and polarization of these inflammatory cells.
Classically activated or M1 pro-inflammatory phenotype macrophages produce inflammatory cytokines such as TNF-α and IL-6. These cytokines are known to influence cell proliferation in monocytes and fibroblasts, collagen deposition, and differentiation of monocytes. The alternatively activated M2 phenotype, is believed to take part in tissue repair through high levels of IL-10 (12). The activation state of macrophages can be induced \textit{in vitro} by administration of either LPS or INF-γ to induce the M1 inflammatory phenotype, or IL-4 to induce the M2 repair phenotype (13).

\textit{Mechanism for Accelerated Vascular Healing through Acidic Polymers}

Polymers are extremely appealing to biomedical device design due to their ease of processing and the ability to chemically modulate their material and physiological properties. Of interest, the physio-chemical properties of PLGAs will be explored to suggest a potential mechanism for accelerated ICA healing.

Under differential physiological conditions such as inflammation, extracellular pH shows a distinct decrease surrounding inflamed tissues. Extracellular acidosis is thought to be the result from the release from active inflammatory cells surrounding these tissues (15). In addition, it has been shown that extracellular modulation of pH produces an increased secretion of IL-1b in human monocytes (16), and increased secretion of TNF-α in fibroblasts (14). The mechanism of action in these two cell types were attributed to lower intracellular pH; induced caspase-1 activity and the conversion of pro-IL-1b to IL-1b stimulating inflammation in monocytes, and a MAP kinase dependent stress-induced pathway in fibroblasts (14, 16).

Previous studies \textit{in vivo} have shown that it is possible to induce extracellular acidosis via PLGAs. In one study, different ratios of lactic-to-glycolic acid – 50/50, 65/35, 75/25, and 85/15 -
were implanted as a polymer ribbons in swine, and observed for the degree of healing through collagen deposition. It was found that the highest degree of collagen deposition was attributed to the faster 50/50 PLGA, whereas slower degrading 85/15 PLGA produced less deposition (17). To utilize these mechanisms and produce a higher degree of extracellular acidosis, 50/50 PLGA with acid modified end group and low molecular weight (aPLGA-2A) was used in this work.

Mechanism for Accelerated Vascular Healing through Selected Degradable Metals

The role of metals in medical devices is pervasive in almost every field. However, most devices which utilize metals are for structural purposes only. This is highlighted in the use of metallic stents for vascular diseases. However, with the advent of degradable metal stents, the focus of the literature is still segregated to long term outcomes which are biocompatibility in the form of thrombosis, strength in relation to the rate of healing, and final endothelialization of the implant. While some metals have been investigated for their influence on biological phenomena, little focus has been given towards utilizing metals as a mode for accelerated bioactive healing itself.

The role of zinc in vivo can be appreciated by its importance to immune functions (18), wound healing (19), protein synthesis (20), and DNA synthesis (20, 21). It has been shown that zinc ions can induce chemotaxis and enhance phagocytosis of polymorphonuclear cells and leukocytes in vitro (22, 23). Zinc has also been shown to influence adaptive immunity. In addition, in response to zinc deficiency, T-cells decreased production of IFN-γ, TNF-α, and IL-2, (24). These findings suggest that zinc is inherently vital for inflammatory reactions through modulation of protein expression and cellular activity. In addition the dose of zinc is of key importance to achieve desired outcomes.
Systemic iron is also vital to life. Adult humans require a daily intake of iron of 1-2 mg/day, however, hemoglobin synthesis require much higher amounts of 20 mg/day (25, 26). To compensate for these requirements, macrophages are responsible for iron recycling through phagocytosis of expired erythrocytes (25). The direct role of iron in inflammation can be appreciated by macrophage polarization. When macrophages are classically activated or M1 proinflammatory phenotype via lipopolysaccharide (LPS), iron retention within macrophages and low iron export is seen, which is thought to restrict the availability of iron to opportunistic bacterium (29). Polarization if macrophages to alternatively activated or M2 anti-inflammatory phenotype through IL-4 or IL-13, results in high levels of iron export which aids in tissue remodeling (30). The role of iron in inflammation is further realized by experiments which inhibit iron release from macrophages. Mice which lack the plasma membrane protein ferroportin (Fpn1), result in iron laden macrophages with pronounced increase in TNF and IL-6 production (27). Other studies in which iron is depleted, macrophages produce less amounts of TNF and IL-6 (28). These studies suggest that iron is not only vital to normal immune function, but also has potential to modulate wound healing as well.

*In Vitro Models for Inflammatory Response and Accelerated Vascular Healing*

In an effort to elucidate the underlying mechanisms for accelerated vascular wound healing, *in vitro* models provide an environment which can be well controlled. However, since *in vitro* models are significantly different from the physiological environment – multiple cell types, complex ECM materials, hemodynamic blood flow, etc. – the conditions of the *in vitro* model used must be scrutinized to accurately represent *in vivo* phenomena.
Saccular aneurysm which have been treated with BPCs provide further insight towards determining appropriate conditions for in vitro investigation. Upon implantation, a preliminary platelet plug forms around the platinum surface, followed by stabilization of the plug by catalyzing coagulation reactions leading to a 3D fibrin network. Subsequently, leukocytes are the first cells to respond to implanted materials and infiltrate the fibrin network. At which point, material-dependent properties – surface chemistry, degradation products, wear products, hydrophobicity, etc. – influence the degree of inflammation experienced, and in turn inflammatory biomolecules released from inflammatory cells. The smooth non-degradable platinum surface which comprises a BPC is considered to be biologically inert. However, monocytes will still respond to the foreign material and direct the formation of a fibrous capsule around the implant. Histological samples of resected aneurysms treated with BPCs indicate the presence of granulation tissue comprised of macrophages and foreign body giant cells directly around the platinum surface, with the presence of fibroblasts, myofibroblasts, and organized collagen dispersed between BPC segments. Typical healing in vivo can take as long as 3-6 months through BPCs alone. However, when compared with bioactive coils comprised of a 90/10 PGLA braided suture wrapped around a BPC core, healing can be seen between 2-4 months. Similarly, histological analysis of these treated aneurysms shows an increase in inflammatory cells proximal to biomaterials, with more organized collagen deposited within the aneurysm.

A suitable in vitro model for investigating physiological processes should be conceived to accurately portray the in vivo system. Three in vitro models used in this work and were designed to capture early events of inflammation, as well as indicators of accelerated healing through collagen deposition. Therefore we have constructed a 3D fibrin model to which cells were
exposed in monoculture or coculture using HFF fibroblasts, and U937 monocytes. In addition, since our prototype medical device has the potential to replace the need for first generation BPCs – i.e. coils that are still used to frame the aneurysm, and in direct contact with the aneurysm wall – we were interested if direct contact of pro-inflammatory materials to cells has an effect. Therefore, cells in fibrin were either directly exposed to proinflammatory materials and encapsulated in 3D fibrin, or diffusely dispersed within the 3D fibrin which require remote signaling from proinflammatory materials via degradation products. Lastly, to assess the influence of the surrounding 3D network on inflammation, 3D collagen type I was used. The utility of these model is appreciated by the potential to test multiple candidate biomaterials in parallel, at reduced cost and time.
1.3 RESEARCH OBJECTIVES

a) Demonstrate feasibility and manufacturability of discrete and thin pro-inflammatory coatings on complex 3D helical geometries
   - Robot assisted atomized-polymer deposition
   - Electroplating from binary electrolyte solutions

b) Assess relevant material properties for prototype medical devices fabricated
   - Friction due to thin coatings on aneurysm coils
   - Coating wear due to clinically relevant deployment
   - Degradation assessment \textit{in vitro}

c) Assess bioactivity of thin pro-inflammatory coatings
   - Inflammatory cytokine expression \textit{in vitro}
   - Collagen deposition \textit{in vitro}
   - Assessment of accelerated healing \textit{in vivo} in swine model
1.4 CHAPTER 1 REFERENCES


CHAPTER TWO - DEGRADABLE POLYMERS FOR ACCELERATED WOUND HEALING

2.1 INTRODUCTION

A growing need exists towards the development of minimally invasive tools for intracranial aneurysm treatment. The current gold standard for small saccular intracranial aneurysm treatment is minimally invasive endovascular coiling (1). These bare platinum coils (BPC) are composed of a platinum/tungsten alloy with which are extremely flexible, yet strong enough to withstand hemodynamic forces which include high blood pressure, under pulsatile movement, and complex due to bifurcation of the vasculature (2). While this procedure significantly reduces patient complication from open brain surgery, patient outcomes can still be improved. Patients with small saccular aneurysm which receive treatment through BPCs will have a success rate of 79-89% with complete occlusion, however secondary complications through recanalization—bloodflow into the aneurysm due to incomplete healing or insufficient packing density – or incomplete occlusion are as high as 20% (1,3, 8). However, treatment of wide-neck or large saccular aneurysms have lower success rates of 30-40% with complete occlusion, and 10-40% with secondary complications (1, 8).

Second generation bioactive coils have been developed to increase the speed at which saccular aneurysm heal. These bioactive coils are composed of 70% 90/10 Poly(glycoic-co-lactic) acid (PGLA), and 30% platinum by volume (4). It is believed that the speed and degradation products induce an inflammatory response to accelerate vascular wound healing (5, 6, 7). Bioactive coils are used in conjunction with BPCs, however, since they possess less mechanical strength they are only used after mechanically strong BPCs (4). Patient outcomes using these bioactive coils have an 87% of complete occlusion, with 24% secondary complications (8). However, it should be noted that assessment of these patient outcomes are
influenced by friction perceived during delivery of these bioactive coils, which often result in lower packing densities of the aneurysms (9).

Due to the nature and location of this disease, a safe and efficacious device must be developed such that it provides significant benefit to patient outcomes. To achieve this, the device itself must be conceived with equal weight given to biological efficacy and ease of use for clinical delivery. Therefore we have developed a novel bioactive aneurysm coil with significantly accelerated vascular wound healing and mechanical properties above current commercially available products, addressed limitations of previous technologies, and designed fabrication methods for manufacturing. In addition to these improvements, this novel technology utilizes inherent biomaterial properties alone.

As opposed to products which incorporate drug delivery systems – i.e. a polymer combined with a drug or protein - this technology has the potential to expedite FDA review by limiting requirements for approval which are mechanical in nature. In addition, this technology has the potential for high user adoption since the core device is based on gold-standard intracranial aneurysm coils already used in the field.

Due to the tortuous nature, small size of aneurysms, and dexterity required for clinical placement, it is imperative to intracranial aneurysm coil device design to limit friction during deployment. During treatment, multiple coils are used to sufficiently pack the aneurysm to occlude it from parental arterial blood flow and obliterate the aneurysm. After the first coil is placed within the aneurysm sac, the second coil will experience coil-on-coil friction due to advancement and retraction of the coil for precise placement. If the surface of the coil is not sufficiently smooth, high levels of traction and even ratcheting - non-linear stop-and-go motion -
is perceived. The consequence of this is reduced coil-packing, reduced efficacy for long term healing, and the potential for a secondary aneurysm through recanalization.

BPCs are composed of platinum/tungsten wire, wound into a helical geometry with shape memory. These helical BPCs were designed such that flexibility and precise placement within saccular aneurysm could be achieved through bending of the coil between helical turns. Thus to maintain this flexibility we have devised a coating system which allows polymer to be deposited only on the surface of the platinum, leaving the grooves devoid of material, resulting in a spray coated coil (SCC). To achieve a micron-level coating we have designed and fabricated an automated polymer-atomizing computer-controlled robot.

Theoretical parameters which govern atomized polymer deposition are through mechanisms which influence droplet breakup in air, and solvent evaporation with respect to time. The maximum droplet size \( D_{\text{max}} \) is governed by (10):

\[
D_{\text{max}} = \frac{8\sigma}{C_D \cdot \rho \cdot U_R^2}
\]

Where \( \sigma \) is the surface tension of the liquid, \( C_D \) is the drag coefficient of the droplet, \( \rho \) is the density of the liquid, and \( U_R \) is the relative velocity of the droplet with respect to the gas phase.

In addition, droplets also experience evaporation defined by:

\[
d^2 = d_0^2 - (\lambda_{\text{evap}} \cdot t)
\]

Where \( d_0 \) is the initial droplet diameter prior to evaporation, \( \lambda_{\text{evap}} \) is the evaporation constant of the solvent, and \( d \) is the resulting droplet diameter at time \( t \). While these two equations represent theoretical limits for droplet formation and evaporation, in practice engineering parameters which may be modulated include: solvent selection, solution concentration, solution flowrate, and the number of layers deposited,
To determine if our prototype SCC was robust under clinically-relevant settings, we subject our construct to materials analysis including: 3-point flexation, friction within dry storage sheaths, friction within a hydrate microcatheter, and polymer wear due to storage sheath and microcatheter. In addition, to understand potential mechanisms behind accelerated aneurysm healing as well as construct durability, material analysis was also performed for: polymer swelling, polymer degradation through change in solution pH, and material hardness after processing.

To validate biological efficacy, two in vitro models were used. The first model consisted of a 3D fibrin gel over monocultured U937 monocytes, monocultured Human Foreskin Fibroblasts (HFFs), or Co-Cultured HFF/U937s. The purpose of this model was to determine if pro-inflammatory materials can induce collagen deposition in vitro. The second model consisted of the same cellular conditions, however 3D collagen was used as the cellular matrix. The purpose of the second model was adapted from previous work to collect inflammatory cytokine expression. Lastly, a swine model was used to compare uncoated BPCs, SCCs, and a commercially available 90/10 PGLA bioactive coil in vivo.

2.2 Materials & Methods

Sample Preparation

Polymer solutions were made from 2.5% (w/w) acid modified PLGA-2A (molecular weight 18 kDa) (aPLGA-2A) (Evonik Industries, Parsippany, NJ) in acetone (Sigma, St. Louis, MO); polymers were allowed to fully solvate overnight. 18mm round glass slides (Fisher Scientific, Pittsburgh, PA) were solvent cast with polymer to achieve a dry weight of 0.75, 1.5, and 4.5mg. Samples were allowed to air dry for 20 minutes, and transferred to an oven at 37°C.
overnight to remove residual solvent. For cell culture samples were sterilized in 70% ethanol for 10 minutes and then rinsed in Dulbecco’s Phosphate-Buffered Saline (DPBS) (Life Technologies, Grand Island, NY) for 10 minutes each.

*Coating Parameters by Atomized Polymer Deposition*

Parameters for atomized polymer deposition on BPCs were performed using a manual air-assisted airbrush (TCP Global, San Diego, CA) connected to an air compressor. Solution flow rate, distance away from coil targets, number of layers deposited, air pressure, solution concentration, and solvent selection were modulated to determine optimal coating parameters. To determine suitable processing parameters for atomized polymer deposition, three solvents with disparate physical properties were used to solvate aPLGA-2A and were used for proof-of-concept (table 1). aPLGA-2A was dissolved as a 2% (w/w) solution in either acetone, chloroform, or 1, 2 dichloroethane (Sigma, St. Louis, MO). Solutions were loaded into a handheld air-assisted atomizer and spray coated onto BPCs. Distance from coil targets as well as air pressure was kept constant at 5cm and 20PSI, respectively. SEM was used to assess coating qualities for discrete coil coating, smoothness, and thickness. Adobe Photoshop CS4 Extended was used for measuring coating thickness.

*Robotic Polymer-Atomizing Aneurysm Coil Coating System*

To produce a micron-level polymer coated clinically-deployable aneurysm coils with high precision and reproducibility, a custom atomized polymer dispensing robot was designed using Autodesk Inventor 2014 (Autodesk, Mill Valley, CA). Fabrication was carried out by UCLA’s Department of Physics & Astronomy machine shop. For polymer coating, coils were
stretched linearly and mounted using rubber grips. Coils were then placed within the spray coating chamber. The spray chamber consists of three air-assisted atomizing nozzles arranged concentrically at angles 60° from one another. The nozzles are linked to a stepper motor for vertical translation. Code for computer controlled commands were programmed using Labview (National Instruments, Austin, TX). Parameters which can be modulated include: solution flow rate, speed of vertical translation, time interval between subsequent layers. SCCs used for subsequent testing were coated with 6um of aPLGA-2A using the following parameters: 3mg/s polymer flow rate, 20PSI air pressure, 3cm nozzle-to-coil distance, 20 pass layers, a vertical translational speed of 10cm/s, and heat treated at 50°c for 30 minutes after processing.

*Spiral Coated Coil Coating Assessment*

Polymer friction and wear was assessed for spiral coated coils (SCC) by coating BPCs (Stryker Neurovascular, Fremont, CA) with a 6um layer of aPLGA-2A. A VersaLASER 2.3 laser cutter (Universal Laser Systems, Scottsdale, AZ) was used to create a “U-shaped” pathway on acrylic sheets. To simulate friction and potential polymer loss during storage and clinical use, SCCs were deployed through a dry storage sheath provided by the manufacture or a hydrated Echelon 14 micro catheter (Covidien, Plymouth, MN). An Instron 5564 (Instron, Norwood, MA) was used to deploy and retract SCCs 3 times at varying speeds. Qualitative analysis for polymer loss due to wear was performed using a Nova Nano 230 SEM under low-vacuum (FEI, Hillsboro, OR).

Polymer degradation on SCCs was assessed in vitro. SCCs were cut into 3cm segments and immersed in DPBS and placed in an incubator at 37°c with 5% CO2. Coils were allowed to degrade over 1, 3, 7 and 14 days. Commercially available bioactive coils – braided Polysorb
sutures wrapped around a BPC – were used for comparison. Coils were removed from solutions, briefly rinsed in DI-water and air dried. Low vacuum SEM was used to image degraded coils. Solution pH was measured using a pH meter (Mettler-Toledo International, Columbus, OH).

Cell Culture

Human foreskin fibroblasts (HFF) (ATCC, Manassas, VA) and Human U937 monocytes (ATCC, Manassas, VA) were grown in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Fisher Scientific, Pittsburgh, PA) and 1% Antibiotic-Antimitotic solution (Life Technologies, Grand Island, NY) in a 37°C incubator with 5% CO₂. U937s were activated using 1.0 ug/ml lipopolysaccharide (LPS) (Sigma, St. Louis, MO).

In vitro model

To assess activation of U937s by LPS, cells were suspended in DMEM and supplemented with 0.01, 0.1, 1.0 or 10.0 ug/ml LPS or PMA and allowed to attach to tissue culture plates for 2 days. To ensure only attached U937s were recorded, wells were agitated using a pipette three times, aspirated, and 1ml DPBS was placed on top of cells for imaging. Analysis of U937 activation was performed by optical microscope for attachment and morphology. Activation was further analyzed using a human inflammatory 5-plex magnetic bead assay (MAGPIX) (Luminex Corp., Austin, TX) for cytokine expression.

To determine cell attachment time, HFFs and U937s were plated using 12 well tissue culture polystyrene well plates; media was supplemented with 1.0 ug/ml LPS for HFF and U937 monocultures. Cells were allowed to attach to well plates for 1, 2, and 3 hours. To determine
firm cell attachment to the substrate wells were agitated three times using a pipette, media was aspirated, and 1ml DPBS was placed on top.

Fibrin gels were used for 3D *in vitro* models. Lyophilized fibrinogen and thrombin (Baxter, Deerfield, IL) were reconstituted with aprotinin solution according to manufacturer’s instructions. Fibrinogen was diluted to 10mg/ml using DMEM supplemented with 2.0ug/ml LPS. Thrombin was reconstituted and diluted to 10IU/ml in 40mmol CaCl₂. Equal volumes of fibrinogen and thrombin solutions were mixed well by pipetting three times; final fibrin gels contained 5mg/ml fibrinogen and 5IU/ml thrombin and 1ug/ml LPS.

To determine if cell proximity to proinflammatory PLGA influenced cell response, two 3D culture models were used: diffuse and direct cell exposure. A “Diffuse cell model” was constructed by suspending either 0.1x10⁶ cells/ml HFF in monoculture, 0.01x10⁶ cells/ml U937 monoculture, a 10:1 ratio of HFF-to-U937s embedded, or no cells in 10ug/ml fibrinogen solution. aPLGA-2A coated or non-coated round glass slides were placed into well plates, and fibrinogen suspensions were add directly on top. An equal volume of thrombin was added and mixed well by pipetting multiple times. Gels were allowed to crosslink for 20 minutes prior to the addition of 1.0ug/ml LPS supplemented DMEM. A “Direct cell model” was used to ensure cells were in direct contact with pro-inflammatory materials. The Direct model was created by coating glass slides with 5mg/ml fibrinogen reconstituted in TRIS buffered saline solution for 2 hours. Slides were rinsed twice using DPBS. U937s were added to monoculture and coculture wells and allowed to attach for 3 hours; unattached cells were removed by aspirating media. HFFs were added to monoculture and coculture wells and allowed to attach for 1 hour; unattached cells were similarly removed by aspirating media. 10ug/ml Fibrinogen was added on top followed by10 IU/ml thrombin; final concentration of fibrin was 10ug/ml. Gels were allowed
to gel for 20 minutes prior to the addition of 1.0mg/ml LPS supplemented DMEM. Supernatants were collected at day 1 and 3, and used for MAGPIX cytokine analysis. To detect collagen deposition, wells were stained with Picrosirius red (Abcam, San Francisco, CA) according to the manufacturer’s instructions under polarized light at day 15. Collagen deposition was quantified by red pixel intensity of Picrosirius red images using Adobe Photoshop CS6 (Adobe Systems, San Jose, CA).

**In Vivo Model**

Animal experiments were performed in accordance with policies established by the UCLA’s Chancellor’s animal research committee and Nationa Institute of Health guidelines. Four swine (age range 3-4 months, weight range 30-40 kg) were used in this study. One aneurysm was constructed micro-surgically in each common carotid artery – two aneurysms per animal. Aneurysms were created using methods previously described (ref). BPCs, commercially available 90/10 PGLA bioactive coils, and our prototype spiral coated coil were placed within the aneurysm and harvested at day 14. Specimens were imaged for gross inspection and stained using Masson’s Trichrome (Sigma, St. Louis, MO) for collagen deposition. Macrophages were detected using primary antibodies to anti-human macrophage antibody MCA874G (Serotec, San Diego, CA).

**Statistical Analysis**

Statistical analysis was performed using one-way analysis of variance (ANOVA). Statistical significance was achieved when p-Values were less than 0.05.
2.3 Results

Coating Parameters by Atomized Polymer Deposition

Since coil flexibility is imperative for precise coil deployment within cerebral vasculature, polymer within the center and the grooves of aneurysm coils must be devoid of material; polymer must only be coated on the surface of the coil. To achieve such a coating, atomized droplets must be smaller than adjacent helical turns while also maintain a high solvent content such that they will spread when in contact with the coil surface. Engineering parameters which can influence droplet size include: air pressure, spraying distance away from the substrate, solution concentration, and solvent selection.

To determine the influence of air pressure on droplet size, aluminum foil was sprayed with 2% aPLGA-2A in chloroform using 10 and 20psi driving pressure. At 10psi, many discrete droplets were seen which ranged between 5-100um, however smaller droplets were seen in greater numbers (figure 2-1a). At 20psi, droplets between 5-80um in diameter (figure 2-1b). More landing events were see for 20psi.
Figure 2-1 Droplet size produced from 2% aPLGA-2A in chloroform using a) 10psi and b) 20psi. Smaller droplets are produced with more landing events at higher psi. 500x magnification; scale bar = 200um.
To determine the relationship between droplet size and droplet time-of-flight – i.e. droplet evaporation - 2% PLGA in chloroform was sprayed by hand onto foil sheets at 5, 10, and 15cm away from the spray nozzle. The shortest distance of 5cm produced a semi-continuous coating with merged droplets (figure 2-2a). Intermediate distance of 10cm produced many discrete droplets, approximately 5-30um in diameter (figure 2-2b). Larger droplets were also seen between 80-100um but constituted less than 0.5% of droplets deposited. The longest distance from the spray nozzle produced discrete droplets but significantly less landing events when compared to the 10cm distance. Droplets ranged between 5-40um in diameter at 15cm away from the spray nozzle (figure 2-2c). In addition, polymer spheres less than 5um in diameter were also deposited onto the surface at this distance.
Figure 1-2 Droplets deposited onto aluminum foil by atomized polymer deposition with varied distance from spray nozzle. a) 5cm, b) 10cm, and c) 15cm. Small wetting droplets are produced in higher numbers at 10cm away from target. 500x magnification; scale bar = 200um.
Solution concentration, which modulated solution surface tension and viscosity, were investigated by spray coating BPCs with a 0.5, 1, and 2% solution of aPLGA-2A in chloroform. Discrete coatings were achieved at all concentrations with no discernable differences in coating quality (figure 2-3). However, slightly thicker layers were seen at higher concentrations which influence processing efficiency (data not shown). Reproducibility was less consistent for 2% solutions which highlights a limitation coating efficiency.
Figure 2-3 BPCs coated with aPLGA-2A from a) 0.5%, b) 1%, and c) 2% in chloroform. Higher solution concentration produces slightly thicker coatings. 500x magnification; scale bar = 200um.
To determine the influence of solvent selection on coating quality 2% solutions of aPLGA-2A were created using acetone, chloroform, and 1,2 dichloroethane and spray coated onto BPCs (table 1). When air pressure and target distance are kept constant it was observed that acetone produces a discrete but rough coating on the coil surface (figure 2-4a). Chloroform showed a discrete and smooth coating (figure 2-4b). Whereas, 1,2 dichloroethane produces a smooth coating, but with polymer within the grooves (figure 2-4c). Since 1,2 dichloroethane and chloroform is known to be carcinogenic and therefore undesirable for manufacturing, acetone was optimized by reducing the target distance to 3cm to compensate for faster evaporation rates. This produced an improved coating, however slight surface roughness was still seen due to particle formation during flight (figure 2-4d). To eliminate these particles, coils were heat treated above polymer Tg for 15 minutes after spray coating (figure 2-4e).
Figure 2-4 Discrete coatings can be achieved with different solvents with different evaporation rates, dependent the production of small wetting droplets. 2% solutions of aPLGA-2A deposited onto BPCs using a) acetone, b) chloroform, c) 1,2-dichloroethane. BPC coated using 2% aPLGA-2A in acetone 3cm away from nozzle d) before heat treatment, and e) after heat treatment. 500x magnification; scale bar 200um.
Table 2-1 Physical properties of Acetone, Chloroform, and 1,2-Dichloroethane highlighting different evaporation rates.

<table>
<thead>
<tr>
<th></th>
<th>Acetone</th>
<th>Chloroform</th>
<th>1,2 Dichloroethane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>0.791 g/cm³</td>
<td>1.489 g/cm³</td>
<td>1.253 g/cm³</td>
</tr>
<tr>
<td>Boiling Point</td>
<td>56 °C</td>
<td>61.15 °C</td>
<td>84 °C</td>
</tr>
<tr>
<td>Vapor Pressure</td>
<td>30.6 kPa</td>
<td>25.9 kPa</td>
<td>11.6 kPa</td>
</tr>
<tr>
<td>Viscosity</td>
<td>0.295 cP</td>
<td>0.542 cP</td>
<td>0.84 cP</td>
</tr>
<tr>
<td>Surface Tension</td>
<td>25.2 dyne/cm</td>
<td>32.9 dyne/cm</td>
<td>36.9 dyne/cm</td>
</tr>
</tbody>
</table>
Since processing parameters for coating BPCs by atomized deposition must be controlled with high precision, assessment of coating thickness was not reliable by using the hand-operated spray nozzle. Therefore a polymer-atomizing robot was used for large scale fabrication (see Appendix I for full schematic and code). A 1% solution of aPLGA-2A in acetone was deposited one layer at a time with a translational velocity of 10cm/s. As expected, each subsequent layer produced a greater thickness (figure 2-5). A maximum thickness of 13um was feasible using this system but occasional deposition of polymer between adjacent coil segments reduced coating quality. Therefore final processing of coils were kept at 20 layers; 6um in thickness.
Figure 2-5 Coating thickness on BPCs using automated polymer-atomizing robot showing increasing thickness with the number of layers deposited. *p<0.05
Coating Characterization

To determine if spray coated coils maintained their flexibility after processing, BPCs were either uncoated, coated with 6um aPLGA-2A by simple dip coating, or 6um spiral coated (SCC) by polymer atomization. SEM images showed that SCCs had smooth and even coatings, while simple dip coated coils had smooth coatings, but had polymer within the grooves. Coils were tested using a fixed 3-point bending system and normalized against BPCs for percent change in coil modulus. At 5.7° of deflection, SCCs showed a slight increase in coil stiffness, but was statistically insignificant (figure 2-6e). Simple dip coated coils required a much higher 21.9° of deflection to return to BPC levels.
Figure 2-6 SEM image of a) BPC, b) 6um SCC with discrete coating, and c) 6um Simple Dip coated with polymer within the grooves. Fixed 3-point bending d) diagram of testing system and e) percent change in coil modulus due to coating method. Polymer within the grooves of the BPC significantly increase coil stiffness. 6um coating on BPC slightly increases stiffness, however was not statistically significant. *p < 0.05. 500x magnification; scale bar = 200um.
To observe degradation of our construct, SCCs and a commercially available coil with 90/10 PGLA around a BPC core were degraded in PBS over 14 days. SEM analysis showed distinct degradation characteristic between the two coil types over 14 days. SCCs showed slight swelling during day 1 and 3 as indicated by small bulb like structures on the surface (figure 2-7). At day 7, SCCs show significant swelling which may harbor acidic degradation particles. In addition, large swelling structures indicate delamination from the coil surface, however are still intact around the platinum core. Day 14 shows a reduction of swelling by the absence of bloated structures, indicating possible polymer rearrangement in crystallinity. Braided 90/10 PGLA coils do not show significant changes until day 7 where polymer appears to fracture, but still remain around the platinum core (figure 2-7). At day 14, 90/10 PGLA begins to fragment significantly and appears to form particles away from the coil surface.
Figure 2-7 Degradation of (top) SCC coils and (bottom) braided 90/10 PGLA around BPC core in DPBS over 1, 3, 7, and 14 days. SCCs produce large swelling structures up to day 7, but subside at day 14. 90/10 PGLA braded sutures fragment beginning at day 7. 500x magnification; scale bar = 200um
To assess friction and possible polymer wear during deployment, SCCs with 6um aPLGA-2A were prepared with full detachment system and pusher wire. To simulate an extremely tortuous bend, a 180° “U-curve” with a 3cm diameter was laser cut in acrylic and mounted to an Instron. It is important to note that tortuous bends within cerebral vasculature do not exceed 90°; 180° was used as a stress test. Coils were deployed through either a dry storage sheath used during manufacturing, or a hydrated microcatheter to simulate clinical use. There were then placed inside the U-curve and SCCs were deployed and retracted 5 times at 1000mm/min using an Instron. Qualitative assessment through SEM imaging showed large intermittent and spotted wear marks on the coil surface when deployed through the dry storage sheath (figure 2-8a). These marks spanned 400um in length along adjacent coil segments, with individual oval-shaped spots with a maximum size of 15um x 70um. However, polymer from these wear spots did not appear to be completely liberated from the coil surface (figure 2-8a). In addition, small cracks of the polymer can be seen between adjacent coil segments (figure 2-8b). Coils deployed through hydrated microcatheters showed significantly less polymer wear by the presence of circular spots of 10um in diameter; no cracks between adjacent coil segments were observed (figure 2-8c & d).
Figure 2-8 SEM images of 6um SCC passed through a 180°, 3cm U-curve showing wear. (a) Large abrasive wear spots present within the dry storage sheath but do not liberate polymer particles with (b) cracks between adjacent coils segments. (c & d) Hydrated microcatheter produces adhesive wear spots which may have been liberated from the polymer coating (300x magnification, scale bar = 300um)
Indicators of Accelerated Healing In Vitro

To determine conditions for inflammatory processes in vitro, human U937 monocytes were investigated for activation. U937s were activated with either LPS or PMA using concentrations of 0.01, 0.1, 1.0, or 10.0 ug/ml. LPS activated U937s showed increased attachment and spreading with increasing concentrations of LPS (figure 2-9 top row). However, lower concentrations did not show clear colony formation due to the absence of clustered cells. U937s began to form colonies at 1.0 and 10.0 ug/ml LPS only (figure 9 top row). PMA activated U937s firmly attached, produced extended projections, and formed colonies for 0.01, 0.1, and 1.0 ug/ml concentrations. Very few cells were seen after activation with 10ug/ml PMA; cells that were present showed no cell attachment, spreading, or colonie (figure 2-9 middle row). Control wells did not show an increase in size or attachment of cells. Since LPS activated U937s produced colonies, cell spreading, and a regular response with respect to concentration, 1.0 ug/ml LPS was as the activating agent for future tests.
Figure 2-9 U937 activation after two days using (top row) LPS, (middle row) PMA, or (bottom) no activating agent. Activation by LPS shows cellular protrusions starting at 0.1ug/ml, however colonies only form after higher concentrations of 1.0ug/ml. PMA shows cellular projections and colony formation at lower concentrations, but concentrations are drastically reduced at 10ug/ml (40x magnification; scale bar 50um)
To ensure adequate cell attachment time was provided for slowly attaching U937s after activation, HFFs and U937s in monoculture at 0.1x10^6 cells/ml. Cells were allowed to attach for 3 hours, media was agitated to remove loosely attached cells, aspirated, and then 1ml DPBS placed on top for imaging. One hour appeared to be sufficient for HFF attachment, however, 3 hours were required for U937s to activate and attach (figure 2-10). It was observed, however, that U937s did not attach to the well plates in similar numbers as originally seeded. A 5.0mg/ml fibrinogen coating prior to cell seeing was required to achieve cell attachment in consistent and uniform quantities (data not shown).
Figure 2-10 Cell attachment time for (top) HFF and (bottom) U937 activated with 1.0ug/ml LPS. HFFs begin to attach after 1hr; U937s require 3 hours for attachment.
To investigate the potential for accelerated healing and the possibility of a dose
dependent relationship, collagen deposition was observed through Picrosirius Red Staining under
polarized light. Monocultured HFF, monocultured U937, and Co-Cultured 10:1::HFF:U937s
were directly exposed to either 0.75mg, 1.5mg, 4.5mg, or no aPLGA-2A coated on round glass
slides and encapsulated with a top layer of fibrin. The experiment was stopped at day 15 since
most of the fibrin was degraded for HFF and Cocultured conditions. Wells with U937s did not
degrade the fibrin matrix and were not stained. Wells with glass slide alone did not show staining
under polarized light for both HFF and cocultured conditions (figure 2-11). Monocultured HFFs
showed increased alignment with increasing amount of aPLGA-2A. Cocultured wells showed
strong staining as well, however collagen appeared to be more fragmented but still aligned with
increasing amount of aPLGA-2A. Quantification of collagen deposition through red pixel
intensity showed HFF monoculture produced higher collagen deposition with the amount of
aPLGA-2A, however statistical significance was only seen between glass and 0.75mg aPLGA-
2A, as well as between glass and 4.5mg aPLGA-2A. No significance was found within aPLGA-
2A groups suggesting no dose dependency (figure 2-12). Red pixel intensity showed aPLGA-2A
produced more collagen deposition in Coculture when compared to glass among all aPLGA-2A
groups, however, statistical differences were not seen within aPLGA-2A treated groups (figure
2-12). Collagen deposition in Coculture for 0.75mg aPLGA-2A groups showed more collagen
deposition within this group when compared to HFF monoculture (figure 2-12, green bracket).
Figure 2-2 Picrosirius Red staining at day 15 after cell exposure to increasing amounts of aPLGA-2A. (top row) HFF monoculture and (bottom row) coculture. Collagen deposition is observed for aPLGA-2A treated groups, but not glass treated (magnification 10x, scale bar 200um)
Figure 2-3 Quantified collagen deposition of figure 11 through red pixel intensity. Collagen deposition in HFF monoculture shows an increasing trend, however was only statistically significant between glass and 0.75mg, and glass and 4.5mg aPLGA (*p<0.05 blue bracket). Collagen deposition in Coculture is enhanced through the use of aPLGA-2A when compared to glass, however no trends were seen within the aPLGA-2A groups (*p<0.05 orange bracket). Collagen deposition within 0.75mg aPLGA-2A showed higher collagen deposition in Coculture when compared to HFF monoculture (*p<0.05 green bracket)
The role of IL-8 is chemotactic factor for inflammatory cells and leads to the induction of angiogenesis from other cell types. U937 exposure to aPLGA-2A in the direct model showed a decrease in IL-8 production with increasing mass of aPLGA-2A at day 1, however an increase in cytokine expression with increased amount of aPLGA-2A at day 3; statistical significance was found for all groups and over both days with the exception of 0.75mg and 1.5mg aPLGA-2A (figure 2-13). HFF exposure to aPLGA-2A in the direct model showed no trend over the groups at day 1 but were found to be statistically significant between 0.75mg and 4.5mg aPLGA-2A, as well as 0.75mg and 4.5mg aPLGA-2A. An Increased IL-8 expression was found with increasing amount of aPLGA-2A at day 3 but was not significant. IL-8 expression in Cocultured conditions did not show trends over day 1 in the direct model. Day 3 expression of IL-8 showed an increasing trend with the amount of aPLGA-2A, however was not statistically significant. When the diffuse model was used, no trends were found for U937 conditions when exposed to aPLGA-2A. HFF in the diffuse model showed an increasing trend with the amount of aPLGA-2A, but was only statically significant at day 3 between glass treatment and 1.5mg aPLGA-2A. Cocultured conditions in the diffuse model showed an increasing trend with amount of aPLGA-2A for day 3 only; statistical significance was achieved between glass and 1.5mg aPLGA-2A, and between 0.75mg aPLGA-2A and 1.5mg aPLGA-2A. Comparison of the absolute maximum of IL-8 between the direct and diffuse model showed comparable levels. In addition, IL-8 expression was the highest of all cytokines detected.

The role of IL-6 is known to regulate survival, proliferation, and differentiation of monocytes. Expression of IL-6 in U937 conditions were below detection limits for glass and 0.75mg direct model (figure 2-14). IL-6 expression in the direct model for HFF conditions showed statistical significance between glass and 0.75mg aPLGA-2A, where expression was
lower for aPLGA-2A treated groups. In addition, statistical significance was achieved between 0.75mg and 4.5mg aPLGA-2A, with higher amounts of aPLGA-2A producing more IL-6. IL-6 expression under Cocultured conditions did not show a trend at day 1 in the direct model. However, an increasing trend was observed for day 3, but was not statistically significant. U937s in the diffuse model showed a statistically significant increase in IL-6 with amount of aPLGA-2A at day 1, between 0.75mg and 1.5mg aPLGA-2A only. IL-6 expression of HFF conditions showed an increasing trend over both days, however was statistically significant for day 1 only, between aPLGA-2A groups. Cocultured conditions of the diffuse model also showed an increasing trend for day 3, however was not statistically significant. IL-6 expression levels between the direct and diffuse model were comparable.

IL-1b is known to modulate the production of inflammatory cytokines and extracellular matrix proteins. U937s in the direct model did not show a trend in IL-1b expression (figure 2-15). HFF expression of IL-1b in the direct model showed a slight increasing trend with amount of aPLGA-2A at day 1 and 3, however statistical significance was achieved at day 3 between glass and 4.5mg aPLGA-2A, and 1.5mg and 4.5mg aPLGA-2A. Coculture in the direct model showed an increasing trend with aPLGA-2A, however was not significant over both days. The diffuse model showed an increasing trend of IL-1b at day 3, however statistical analysis was not possible due to no variance within groups. HFF and Cocultured conditions in the diffuse model for IL-1b expression did not produce any trends. The maximum expression of IL-1b in the direct model was almost 3 times higher than the diffuse model.

Granulocyte macrophage colony-stimulating factor (GM-CSF) induces proliferation and generates colonies of macrophages. GM-CSF expression in the direct model of U937 conditions did not show trends over 3 days (figure 2-16). GM-CSF expression in the direct model with
HFFs showed an increasing trend with the amount of aPLGA; was not statistically significant for either day 1 or 3, with the exception of decreased expression in 0.75mg aPLGA-2A compared to glass. GM-CSF expression in the direct model from Cocultured conditions did not show a trend at day 1, however showed a statistically insignificant increasing trend with amount of aPLGA-2A at day 3. The diffuse model produced a slight but significant decreasing trend with amount of aPLGA-2A at day 1 between glass and 1.5mg aPLGA-2A in U937 conditions, but no trend was found at day 3. GM-CSF for HFF produced increasing trends with aPLGA-2A, but was statistically insignificant. Cocultured conditions of the diffuse model showed a slight increasing trend at day 3, but was not statistically significant. The maximum expression of GM-CSF in the direct model was almost twice as much for HFF and Cocultured conditions, but one third less in U937 conditions.

Tumor necrosis factor alpha (TNF-a) is known to stimulate cell proliferation in both monocytes and fibroblasts, as well as increase the production of IL-6, IL-1 and collagen synthesis. TNF-a expression using the direct model from U937s were below detection limit for all aPLGA-2A treated groups (figure 2-17). TNF-a expression from the direct model when exposed to HFFs produced an increasing trend for aPLGA-2A at day 1, but was only statistically significant between 0.75mg and 4.5mg aPLGA-2A. Expression at day 3 showed an increasing trend with the amount of aPLGA-2A and was statistically significant. Cocultured conditions in the direct model showed increased expression at day 1 with amount aPLGA-2A, but was not significant. At day 3 no trend was observed for Cocultured conditions in the direct model. In the diffuse model no trends were observed in either U937, HFF, or Cocultured conditions. Comparison of the maximum expression of TNFa between the direct and diffuse model showed
4.5 fold increase in TNFa expression in the direct model at similar conditions. In addition, TNF-a showed the least expression among all cytokines tested.
Figure 2-13 IL-8 release profile for Direct and Diffuse In vitro models. Statistical significance was clearly seen for U937s in the direct model. Other conditions produced very few differences in expression levels (*p < 0.05, blue brackets day 1, green brackets day 3).
Figure 2-14 IL-6 release profile for Direct and Diffuse In vitro models. No clear trends were presented within the direct model or the diffuse model. (*p < 0.05, blue brackets day 1, green brackets day 3)
Figure 2-15 IL-1β release profile for Direct and Diffuse In vitro models. No clear trends were presented within each model. Expression of IL-1β was higher in the direct model for HFF and Cocultured conditions (*p < 0.05, blue brackets day 1, green brackets day 3)
Figure 2-16 GM-CSF release profile for Direct and Diffuse In vitro models. No clear trends were presented within the direct or diffuse model. Expression of GM-CSF was higher in the direct model. (*p < 0.05, blue brackets day 1, green brackets day 3)
Figure 2-17 TNF-a release profile for Direct and Diffuse In vitro models. HFF in the direct model produced a statistically significant increase in TNF-a expression with aPLGA-2A at day 3, however was not consistent in Coculture. Expression of TNF-a was much higher when compared to the diffuse model (*p < 0.05, blue brackets day 1, green brackets day 3)
To determine effects of the 3D ECM around cells in response to pro-inflammatory aPLGA-2A, a direct collagen model was used; U937 monoculture was not explored since values from the previous fibrin study showed values close to background levels. IL-1b expression from HFF monoculture showed no significant differences between groups at day 1 (figure 2-18). At day 3, however, a slight yet statistically significant increase in IL-1b between aPLGA-2A treated groups was seen between 0.75mg and 1.5mg aPLGA-2A, and 0.75mg and 4.5mg aPLGA-2A. Unexpectedly, day 5 showed a statistically significant decrease between glass and 4.5mg aPLGA-2A, as well as 0.75mg and 4.5mg aPLGA-2A. IL-1b in Coculture showed statistical significance between all groups tested at day 1, however did not exhibit trends with aPLGA-2A. A decrease in IL-1b was seen at day 3 and 5, but was not statistically significant. In comparison to the direct and diffuse fibrin models, collagen showed slightly higher expression in IL-1b than the diffuse in-fibrin model, but significantly lower than the direct in-fibrin model.

IL-6 expression for HFF monoculture did not show distinct trends among the conditions tested at day 1 or 3, however statistical significance showed expression levels were different (figure 2-18). Expression for glass treated groups showed higher expression than 4.5mg aPLGA-2A at day 5 and was statistically significant. IL-6 expression in Coculture showed no trends at day 1 and 5, however statistical significance was achieved at day 3 with a decreasing trend with increasing amount of aPLGA-2A; glass treated conditions were significantly lower than all aPLGA-2A treated groups. IL-6 expression in collagen was lower than both fibrin models used.

IL-8 expression in HFF monoculture and Coculture conditions did not show discernable trends between all groups tested for days 1, 3, and 5 (figure 2-18). Day 3 did show statistical significance in Cocultured conditions, however no trend with the amount of aPLGA-2A was
observed. IL-8 expression was the highest recorded cytokine expressed within the collagen model. However, IL-8 expression in collagen was lower than both fibrin models.

GM-CSF expression for HFF monoculture was below detection limits for day 1, and no trend was seen at day 3 (figure 2-18). However at day 5, glass treated conditions showed almost twice as much expression when compared to aPLGA-2A groups, however, statistical significance was not seen within aPLGA-2A groups. GM-CSF expression in Coculture showed a slight positive trend with aPLGA-2A treatment compared to glass at day 1. In addition, a slight negative trend was seen for day 3 and 5, but was not significant over all aPLGA-2A groups. GM-CSF expression was lower in collagen than both fibrin models.

TNF-a expression in HFF monoculture did not show trends at day 1 or 3 (figure 2-18). However, TNF-a expression was statistically significantly higher for glass when compared to 1.5mg aPLGA-2A only at day 5. Expression in Coculture did not produce trends within aPLGA-2A groups at day 1. However, aPLGA-2A treated groups were lower than glass condition and were statistically significant. Day 3 and 5 in Coculture produced negative trends with aPLGA-2A but only day 3 was found to be statistically significant. TNF-a expression was the lowest detected cytokine in collagen. TNF-a expression in collagen was comparable to the diffuse fibrin model.
Figure 2-18 Inflammatory cytokine expression of IL-1b, IL-6, IL-8, GM-CSF, and TNF-a. (*p<0.05, blue brackets day 1, green brackets day 3, red brackets day 5)
Biological Efficacy of a Novel Spiral Coated Coil In Vivo

To determine if a thin 6um SCC could produce accelerated healing in vivo, prototypes were implanted into an artificially created venous pouch model in swine and harvested at day 14. Coil types for this experiment included: BPCs, commercially available braided 90/10 PGLA suture around BPC, and 6um coated SCC. At day 14 gross inspection of resected samples showed large amounts of immature thrombus for BPC treated samples (figure 2-19). 90/10 PGLA treated samples showed more mature thrombus, however still had unhealed portions (figure 2-19). SCCs showed the most mature healing with no immature thrombus seen (figure 2-19). Masson’s Trichrome staining supported these findings with the most collagen deposition and organization in SCC treated, followed by 90/10 PGLA treated showing less, and BPCs showing the least. In addition macrophage staining showed increasing amounts of inflammatory cells proximal to coil materials with BPC having the lowest, 90/10 PGLA as intermediate, and SCC with the highest. This suggests a stronger inflammatory response to a 6um aPLGA-2A coating than either 90/10 PGLA or BPC.
<table>
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<th>Gross Inspection</th>
<th>BPC</th>
<th>90/10 PGLA</th>
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<td>Masson’s Trichrome</td>
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<td>Macrophage Stain</td>
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*Figure 2-19* Aneurysm coils implanted in side-wall swine model at day 14 showing (top row) gross inspection, (middle row) Masson’s Trichrome, and (bottom row) Macrophage stain of (left column) BPC, (middle column) 90/10 PGLA coil, and (right column) 6um SCC.*
2.4 DISCUSSION

In many cases, the motivation behind scientific research is to further knowledge of specific mechanisms, with the hope that this knowledge will be translated to improved patient outcomes. However, translation of these findings can often be difficult since the experimental settings in the laboratory do not consider the clinical setting or manufacturing capabilities; in some cases they cannot be translated at all. Therefore, the focus of this project was to develop a novel endovascular device with user adoption and manufacturability such that this technology may be translated to patients.

Coating BPCs with a smooth micron-level polymer relies heavily on droplet size for discrete coatings. There are many equations which model droplet formation and evaporation. Theoretical parameters which influence droplet formation include: surface tension, drag of the droplet in gas, density of the liquid, and the relative velocity of the droplet to the gas phase (10). In addition, polymer droplets are further influenced by the initial droplet size, the evaporation rate of the solvent, and the mass fraction of the polymer phase to solvent phase with respect to time (11). In practice, however, these theoretical parameters must be translated to engineering parameters which can be used by real equipment. In this case, engineering parameters included: air pressure, the distance from the BPC target, solution concentration, and the solvent phase selection.

The key to achieving discrete coatings on BPCs is through droplet control, where small wetting droplets only cover the surface. In agreement with theoretical equations, increasing air pressure produced smaller droplets. While the size distribution of droplets did not change significantly – 5-100um at 10 PSI, compared to 5-80um at 20 PSI – more landing events were observed at the same distance. In turn, coating efficiency is increased, and time for processing is
reduced using higher air pressure. However, since droplets at higher pressure are moving faster, solvent evaporation rate and target distance should be considered for translation to other coating systems.

The target distance from the spray nozzle also influences droplet size. As the target distance increases, the droplet time of flight is extended, and in turn the mass fraction of polymer within droplets increases. As a result, this parameter influences the wettability of polymer droplets which begin as primary droplets with large high-solvent content, secondary droplets which are smaller and wetting, and finally a tacky polymer spheres which do not wet. For discrete coating on BPCs, secondary droplets are utilized. Primary droplets with high solvent content allow polymer to flow along the platinum substrate which can occlude helical coil movement. Tacky polymer spheres are also undesirable since they create rough surfaces and also prevent helical flexation. This parameter may be modulated for other systems by increasing target distance for slowly evaporating solvents, or decreasing target distance for faster evaporating solvents.

Solvent selection influences droplet evaporation rate and viscosity. Of the three solvents tested, even coatings were achieved. However discrete coatings were not maintained using slowly evaporating solvents, and rough surfaces formed when fast evaporating solvents were used. These features were easily rectified by increasing or decreasing target distance. Therefore, the decision for the final solvent selection was based on environmental and biological safety; manufacturing concerns.

Theoretical equations for droplet formation included solution surface tension, viscosity, and density of the solution. In practice, these parameters were modulated by solution concentration, however, was not found to significantly affect coating quality. Smooth discrete
coatings were achieved from all of the three concentrations tested. The impact of solution concentration was found in the amount of polymer deposited per number of coating passes, whereby higher concentrations deposited more polymer with less passes. Therefore higher concentrations are more efficient. However a limitation is reached when solution concentration increases surface tension, viscosity, and density, which produce larger droplets.

Significant friction and ratcheting perceived by physicians has had profound impact on clinical adoption and patient outcomes; these problems have been reported through the use of bioactive 90/10 braided PGLA on BPC (8, 9). Therefore utilizing a discrete, smooth, and thin polymer coating has significant impact on the friction perceived during delivery. Tests here have shown that minimal impact on coil flexibility is imparted to the BPC with a 6um aPLGA-2A coating. In addition, friction during deployment and retraction showed no significant difference against BPC controls.

A requirement for endovascular devices is to assess particle or debris liberated during deployment. Assessment of the novel SCCs showed that slight wear of the polymer layer. However, due to the size of these wear spots observed here, and SCCs tested by other groups for particulate formation, these novel coils passed required industry benchmarks. In addition, observation of degradation through SEM showed that the integrity of the polymer coating was maintained throughout 14 days. Had the polymer coating fragment during degradation, further investigation would have been required to ensure that particles downstream were small enough to prevent obstruction of the vasculature; this experiment was not required for SCCs. Additionally, methods for improving polymer integrity and limiting wear – i.e. the addition of lubricants or increasing polymer retention by surface modification – were investigated but not implemented.
Biological assessment *in vitro* showed no clear dose response for the mass or thickness of aPLGA-2A used. Pixel intensity quantification of picrosirius red staining showed no significant differences among aPLGA-2A groups in coculture, however collagen deposition was enhanced over glass treatment control. In addition, no clear evidence points to a dose dependent nature for cytokine expression with the amount of aPLGA-2A used in the two fibrin models or collagen model. Inflammatory responses to these materials appear to be surface mediated for aPLGA-2A. Previous research has reported an increased inflammatory response due to aPLGA in coculture, however, these experiments were performed using microparticles at a single dose (12). It is well established in the literature, that the material used in 3D cultures can produce profound differences in cellular response (13, 14, 15). In addition, since glass treated groups produced elevated cytokine release above 0.75mg aPLGA-2A in specific conditions, there may be a threshold value to which an inflammatory response is induced to aPLGA-2A (15, 16). Glass slides were used in this case, however, to observe collagen deposition under polarized light; TCPS well plates diffracted polarized light which prevented further analysis.

It should be noted that there are limitations to the two fibrin models used. First, the dependency of cell viability and cytokine expression in relation to fibrin thickness was not investigated. The fibrin and collagen gels used here were not varied for thickness, and therefore it is possible that hypoxia and limited diffusion of nutrients to cells in the direct models resulted in differential results from previous work. Future work should be performed to validate these experiments.

In accord with the wound healing cascade, where collagen is deposited temporally after fibrin, inflammatory cytokine expression was less in collagen than both fibrin models. This reflects the importance of the ECM to which *in vitro* studies are performed. This model may
have utility to investigate phenomena of the wound healing cascade during the proliferative phase or angiogenesis. However, additional cytokines would be required which reflect proliferation, rather than inflammation.

Final assessment \textit{in vivo} of BPCs, 90/10 PGLA braided suture on BPC, and SCCs showed significantly different vascular wound healing in the low-flow swine model. These prototype SCCs showed complete healing at day 14, with well-organized collagen deposited, and the presence of inflammatory cells. Due to these findings and the fact that larger aneurysms take significantly longer to heal, SCCs may extend endovascular coil treatment and improve patient outcomes for small saccular aneurysm as well as larger aneurysms.

While extensive research has been performed to understand wound healing, the precise mechanism for biomaterial-induced accelerated healing is still poorly understood. However, literature in this field supports a hypothesis based on the speed of degradation as well as the chemical composition of the polymer (6, 7). In addition to these factors, work here has contributed to the scientific field by showing that inflammatory reactions may be influenced by surface phenomena as well. Therefore, the degradation rate of polymers may not have a significant role as previously thought. Furthermore, the form factor to which biomaterials are delivered \textit{in vivo} also can have implications in inflammatory reactions (12). While the mechanism of accelerated wound healing remains to be elucidate, the aPLGA-2A polymer used here has shown significantly accelerated vascular wound healing. Thus, future work should be performed to determine mechanisms of aPLGA action on inflammation. Modulation of molecular weight, acid vs ester end-groups, and surface charge are of key interest.
2.5 CHAPTER TWO REFERENCES


CHAPTER THREE - DEGRADABLE METALS FOR ACCELERATED WOUND HEALING

3.1 INTRODUCTION

Metals have been used in medical devices since the inception of implantable materials (1). Extensive studies have been performed to understand the biocompatibility of these materials, however, little investigation has been made towards utilizing inflammatory reactions for beneficial purposes; specifically accelerated wound healing. Furthermore, even with the advent of biodegradable metallic stents, subsequent studies have been published with the primary concern with long term outcomes rather than utilizing material properties to influence the wound healing cascade.

As a class of materials, metals are universally pervasive in medical devices. In many cases, metals are selected for structural purposes alone. However, there is growing interest in biodegradable metals for endovascular devices since they can provide structural support, and leave no foreign materials after the body has healed. Iron stents have been tested in vivo for long term outcomes. Investigators reported that endothelialization was achieved at 6 months, however remnant iron struts were still seen at 18 months with large amounts of inflammatory cells proximal to stent materials (2). Since biodegradable iron has been shown to have safe outcomes and the presence of inflammatory cells, further investigation is warranted.

It is known that certain metals produce an inflammatory response. Determining factors which influence these reactions include the presence or accumulation of wear particles, biodegradation of the metal, the surface topography, and of course the metallic species itself (3, 4). In this work, we have focused on two metals - zinc and iron – based on biocompatibility, ability to degrade in vivo, and the ease of processing.
Previous studies have shown that degradable metals produce an inflammatory response. Work here has been performed to induce this response by a thin biodegradable metal coating. Rather than focusing on biological efficacy, however, our main concern was the development and translation of technology to the clinic. Therefore, our primary goal was to demonstrate proof-of-concept that a BPC could be coated with a thin metallic coating for endovascular clinical delivery with limited friction. The criteria for “high quality” coating was defined by the presence of 1) a smooth surface layer and 2) a discrete coating only on the surface of the platinum coil. Our secondary goal was to determine if biodegradable metallic coatings could induce accelerated healing in saccular aneurysms.

As proof-of-concept, metal deposition on BPCs by electroplating was explored. Components of electroplating include an electrolyte bath which contains metal ions to be plated, a non-plating counter ion, a cathode immersed in the electrolyte where deposition occurs, an anode, and a power source which delivers voltage and direct current to the system. Electroplating is the deposition of metal onto a conductive surface by an electrochemical process governed by Faraday’s law (5):

\[ W = \frac{I \times t \times E_q}{F} \]

Where W is the weight of the deposit, I is current delivered to the system, t is time, \( E_q \) is the equivalent weight of the deposit, \( F \) is Faraday’s constant. Rearranging this equation provides:

\[ I \times t = \frac{W \times F}{E_q} \]

By measuring the current delivered to the system, the amount of chemical change and, in turn, the equivalent weight of metal can be determined. In practice, additional factors influence
smooth coatings which include ionization of metallic species, throwing power of the solution, and current density (1). These factors directly influence the flux of ions to the plating surface, crystalline structure, which affect the resulting coating. Even and thin coatings were achieved through modulation of plating parameters. Prototype coils were then tested for wear, degradation, and biological efficacy in vitro.

3.2 MATERIALS & METHODS

Sample Preparation by Electroplating

To determine optimal solutions for electroplating complex helical geometry of BPCs, electrolyte concentration, electrolyte composition, current density, and solution pH were modulate. Zinc chloride (ZnCl₂), zinc sulfate heptahydrate (ZnSO₄), and iron sulfate heptahydrate (Sigma Aldrich, St. Louis, MO) were dissolved in DI-H₂O in at 1.0M, 2M, 5M solutions. Solution pH was adjusted using either 1M sulfuric acid or 1M hydrogen chloride (Sigma Aldrich, St. Louis, MO).

A custom 3D printed (3DP) holder was designed and fabricated from ABS plastic. Silicone rubber was used as grips to secure either stainless steel wires or BPCs in a linear fashion. A platinum wire was used as an electrode and placed in contact with the substrate to be coated. Once substrates were mounted, they were immersed directly into electrolyte bath solutions and immediately plated using an EC1000-90 ThermoEC power supply (Thermo Savant/Thermo EC Holbrook, NY, United States). After plating was complete, samples were removed from electrolyte baths and immediately rinsed in DI-H₂O for 30 seconds. Samples were removed from sample holders and air dried.

Sample Preparation by Sputter Deposition
Round 18mm glass slides were coated using a Denton Vacuum Discovery-550 Sputterer (Mooresstown, NJ) for 30 minutes using 0.3 A. BPCs were elongated and taped to the sputtering stage and coated on each side for 30 minutes each to achieve complete coil coating.

**Material Analysis**

Electroplated coils were degraded in either DPBS or DMEM supplemented with 10% FBS and 1% antibiotic antimitotic solution for 1, 3, and 7 days in a 37°C incubator with 5% CO₂. Coils were removed from solution at time points, rinsed in DI-H₂O, and air dried for SEM. Solution pH was measured by a pH meter (Mettler-Toledo International, Columbus, OH).

Particle wear and friction was assessed using an Instron 5564 (Instron, Norwood, MA) with a custom laser cut “U-shaped” pathway from a VersaLASER 2.3 laser cutter (Universal Laser Systems, Scottsdale, AZ).

Samples were imaged and analyzed for elemental analysis using Nova Nano 230 SEM-EDS (FEI, Hillsboro, OR) under high vacuum.

**Cell Culture**

Human foreskin fibroblasts (HFF) (ATCC, Manassas, VA) and Human U937 monocytes (ATCC, Manassas, VA) were grown in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Fisher Scientific, Pittsburgh, PA) and 1% Antibiotic-Antimitotic solution (Life Technologies, Grand Island, NY) in a 37°C incubator with 5% CO₂.

To detect collagen deposition, wells were stained with Picrosirius red (Abcam, San Francisco, CA) according to the manufacturer’s instructions under polarized light at day 15.
Collagen deposition was quantified by red pixel intensity of Picrosirius red images using Adobe Photoshop CS6 (Adobe Systems, San Jose, CA).

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA). Statistical significance was achieved when p-Values were less than 0.05.

3.3 RESULTS

Demonstration of Uniform Biodegradable Metal Coatings on Bare Platinum Coils

To demonstrate feasibility for degradable metallic coatings on BPCs, solutions of zinc and iron were used to elucidate parameters for discrete coatings. Factors which influence smooth even coatings include: current density, electrolyte composition, solution pH, and solution concentration. Additional parameters found in literature, which include complexation of ions through the use of cyanide species, were not investigated due to safety and a possibility for a barrier to entry.

To determine the influence of solution concentration on coating quality stainless steel wires were electroplated from a 1, 2, and 5M ZnSO₄ solution at 7.7A/dm² for 2 minutes. 1M solutions produced smooth coatings which are predominantly continuous with only slight pitting (figure 3-1a & d). 2M solutions produce predominantly continuous, yet uneven surfaces (figure 3-1b & e). 5M solutions produced extremely uneven surfaces with large portions devoid of zinc (figure 3-1c). In addition, rows of zinc can be seen which follow surface imperfections of the stainless steel wire (figure 3-1f).
Figure 3-1 SEM images of electroplated stainless steel wires from ZnSO\(_4\) solutions showing increasing concentration resulting in reduced coating quality. Electroplated at 7.7A/dm\(^2\) for 2 minutes on stainless steel wires from ZnSO\(_4\) at concentrations of a & d) 1M, b & e) 2M, and c & f) 5M. (magnification 500x; scale bar = 200um, magnification 300x, scale bar = 40um)
To determine the influence of competing ions within the electrolyte solution, non-depositing calcium ions were incorporated by creating 1M solutions of either ZnCl₂, or ZnCl₂ + CaCl₂. BPCs were immersed in solution and electroplated at 15 A/dm² for 1 minute. Coils electroplated from ZnCl₂ produce uneven surfaces which cover the outer surface, but not significantly within the grooves (figure 3-2a & b). Elemental analysis through SEM-EDS shows atomic percentage of 48.5% Zn, 34.9% O, 16.6% Cl and 0% Ca (figure 3-2c & g). These percentages can be attributed to a metallic coating comprised mostly of ZnO with residue or entrapment of chlorine ions from the salt bath. Coils electroplated from 1M ZnCl₂ + CaCl₂ produce uneven and rough cluster-like surfaces which stem predominantly from coil surfaces, but also contain metal within the grooves (figure 3-2d & e). In addition, opaque residue can be seen under high magnification which is not part of the deposited layer. Elemental analysis of coils coated form ZnCl₂ + CaCl₂ constitute 48.3% Zn, 31.2% O, 18.5% Cl, and 2% Ca. These values show that the coating consists mostly of Zn or ZnO. Calcium deposition is also possible but more likely due to entrapment of ions during the plating process.
Figure 3-2 SEM-EDS analysis of BPC electroplated with competing electrolyte composition of Zn and Ca ions, showing pronounced differences in coating quality. SEM and EDS spectra of BPC electroplated at 15A/dm$^2$ from (a-c) 1M ZnCl$_2$, (d-f) 1M ZnCl$_2$ + 1M CaCl$_2$, and (g) atomic percentages by EDS analysis. (A and d, magnification 500x, scale bar = 200um. b and e, magnification 3000x, scale bar = 30um)
To determine the influence of ion availability on coating quality BPCs were electroplated at 2A/dm² for 5 minutes with a 1M ZnCl₂ solution or 1M ZnSO₄ solution. These solutions were chosen based on their solubilities in water, and in turn reflect the high availability of Zn in ZnCl₂ solution, compared to the low availability of Zn in ZnSO₄ solution. In addition, solution pH was adjusted using HCl or H₂SO₄, respectively, for a final pH of 3.7. Electroplated samples from ZnSO₄ solutions produce even surfaces (figure 3-3a & b). BPCs coated from 1M ZnCl₂ at pH 3.7 produced coatings which were strictly on the outer most surface of the platinum surface, with larger crystalline structures (figure 3-3c & d). Differences in coating structure between the two electrolyte solutions are the direct result of increased or decreased ion throwing power.
Figure 3-3 SEM image of Zn electroplated on BPC from different electrolyte solutions composed of (a & b) ZnSO₄, and (c & d) ZnCl₂. Differences in coating structure are the result of electrolyte throwing power; high throwing power from sulfate solutions, low throwing power of chloride solutions. (Left: magnification 500x, scale bar = 200μm. Right: magnification 5000x, scale bar = 20μm)
To determine the influence of current density for electroplating Zinc on BPC, a 1M ZnSO₄ was used and electroplated for 2 minutes at 2, 5, 10, and 15 A/dm². SEM images showed improved coating quality with lower current density. At 2 A/dm² an extremely smooth and even surface is achieved (figure 3-4a). Increasing current density to 5 A/dm² maintains surface quality, however surface irregularities are still present due to localized charge concentration (figure 3-4b). Increasing current density to 10 A/dm² reduces surface quality, by the presence of rough surfaces with localized growth (figure 3-4c). At 15 A/dm² very rough but discrete surfaces are produced on the BPC; large crystals can be seen (figure 3-4d). In addition, to surface roughness, more Zn was deposited with higher current densities.
Figure 3-4 SEM image of Zn electroplated on BPC with increased current density showing larger surface features and increased thickness. BPC electroplated using 1M ZnSO₄ solution for 2 minutes at (a) 2, (b) 5, (c) 10, and (d) 15 A/dm². (magnification 500x, scale bar = 200um)
To determine the feasibility of alternative metals deposited on BPCs by electroplating, iron was investigated. A solution of 1M FeSO₄, with adjusted pH of 2.1 using 1M H₂SO₄, was used for electroplating BPCs at 1, 2, or 5 A/dm². Adjusting solution pH was necessary since FeSO₄ quickly oxidized within 10 minutes after dissolution in water; solutions turned from clear green to turbid brown. During the electroplating process, small gas bubbles formed on the coil surface, most likely due to the formation of H₂ gas. After coils were electroplated, rinsed in DI-H₂O, and air dried coils quickly changed color from dull gray to slightly brown, suggesting oxidation of the Fe layer. BPCs electroplated using low current density produced extremely thin coatings which were not clearly visible through SEM (figure 3-5a & b). However, elemental analysis by SEM-EDS showed an atomic percentage of 60% Fe and 40% O which indicates that an iron oxide layer was indeed deposited (data not shown). Increasing the current density to 2A/dm² also produced a very thin and smooth layer, however small white particles can be seen under high magnification (figure 3-5c & d). The highest current density of 5 A/dm² produced a clearly visible iron coating which was discrete and even on the platinum surface (figure 3-5e). Under high magnification the surface appears rough with the presence of the same particles seen from lower current densities (figure 3-5f). These particles appear white under SEM which suggests lower atomic weight under high vacuum imaging. Elemental analysis through SEM-EDS showed that the particles were composed of 23% Fe and 77% O (data not shown). In addition, a significant iron layer was deposited, at higher current density, however was still too thin to measure.
Figure 3-5 SEM image of Fe electroplated on BPC from FeSO₄ solutions with increased current density, resulting in more deposition with increased current density. BPC electroplated from 1M FeSO₄ for 1 minute using (a&b) 1A/dm² (c&d) 2A/dm², and (e&f) 5A/dm². (Left column: magnification 500x, scale bar = 200um. Right column: 5000x, scale bar = 20um)
Coating Assessment

To assess coating stability, coils were deployed through a dry storage sheath used for packaging for BPCs. Coils were deployed through an 180° turn with diameter of 30mm at a rate of 1000 mm/min to simulate an extreme bend which is far beyond what is seen within physiological vasculature. Coils with full detachment junction and pusher wire were electroplated from a 1M solution of either FeSO₄ or ZnSO₄ at 2 A/dm² for 1 minute. Zinc coated coils showed large periodic scaring after deployment through the dry storage sheath (figure 3-6a). Iron coated coils did not show significant wear, however a significant amount of circular structures were seen, but were attributed to gas evolution during the electroplating process (figure 3-6b). Differences in surface features of these Fe electroplated coils used here and previous electroplated samples were attributed to the contribution of charge fluctuation due to pusher wire and detachment zone.
Figure 3-6 SEM image of Zn or Fe electroplated coils after deployment through dry storage sheaths for manufacturing. Electroplated BPCs using 2A/dm² for 1 minute from 1M solutions of (a) ZnSO₄ show clear wear from dry storage sheath, and (b) FeSO₄ show rough surfaces with round spots attributed to electroplating process but no clear indication of wear. (magnification 500x, scale bar = 200μm)
To confirm biodegradation of these metallic coils, BPCs, Zn, or Fe electroplated coils were immersed in DPBS or DMEM media with 10% FBS, 1% antibiotic-antimitotic solution, and placed in a sterile incubator with 5% CO₂. Either DPBS or DMEM alone were used as a control; solution pH was recorded at 1, 3, and 7 days. Zn coated coils degraded in DPBS showed large crystal like structures starting at day 1 which increase in size at day 3 (figure 3-7b & c). However, these structures begin to decrease in size at day 7, most likely due to Zn ion degradation into solution (figure 3-7d). Zn coated coils degraded in DMEM produce structures which appear significantly different; bulb like protrusions form in localized areas on the coil surface (figure 3-8b-d). At day 7, the Zn coating appears to delaminate and fragment from the underlying platinum surface (figure 3-8d). Fe coated coils degraded in DPBS produced small spherical particles starting at day 1 and persists until day 7 (figure 3-7f-h). In addition, the Fe coating appears to form plate like structures between coil segments within the grooves at day 3 and 7 (figure 3-7g&h). Fe coated coils degraded in DMEM show large plate like structures which appear brittle at day 1, and appear to delaminate at day 3(figure 3-8g). At day 7, the Fe coating can no longer be seen by the absence of large degradation products, however, large amounts of debris were found in solution (figure 3-8h). Solution pH from DPBS showed a decrease in pH at day 1 for DPBS alone and BPC groups (figure 3-9a). Degradation solutions from Zn and Fe coated coils showed higher pH, which overcame DPBS buffering capacity (figure 3-9a). This trend was maintained throughout the 7 days. Degradation in DMEM did not show significant changes in solution pH over the 7 days (figure 3-9b).
Figure 3-7 SEM images showing degradation in DPBS for Zn and Fe electroplated coils. Degradation in DPBS of BPCs coated with Zn at (a) day 0, (b) day 1, (c) day 3, and (d) 7 days showing large crystalline like structures. Degradation in DPBS of BPCs coated with Fe at (e) day, (f) day 1, (g) day 3, and (h) 7 days showing the generation of small surface particles. (magnification 500x, scale bar 200um)
Figure 3-8 SEM images of Zn and Fe electroplated coils in cell culture media. Degradation in DMEM media of BPCs coated with Zn at (a) day 0, (b) day 1, (c) day 3, and (d) 7 days showing localized degradation spots along the coil surface. Degradation in DMEM media of BPCs coated with Fe at (e) day, (f) day 1, (g) day 3, and (h) 7 days showing increased surface roughness and large degradation particles up to day 5. (magnification 500x, scale bar 200um)
Figure 3-9 Solution pH from negative control solution alone, BPC, Zn coated, and Fe coated coils in (a) DPBS and (b) DMEM media. *Statistical significance of p<0.05 achieved for Zn over all days, Fe for day 1 and 7 only when compared to DPBS. No significance observed for DMEM solutions.
Biological Efficacy In Vitro

To determine if degradable metals have the capacity to induce accelerated healing, collagen deposition was observed through Picrosirius Red staining under polarized light. Glass slides were coated with either Fe or ZnO by sputter deposition; glass slides without coating were used as controls. Monocultured HFFs and Co-Cultured 10:1 ratio of HFFs:U937s were seeded directly to materials and encapsulated with a top layer of crosslinked fibrin. The experiment was stopped at day 15 since the fibrin gels had completely degraded. HFFs when exposed to glass slides alone formed continuous cellular layers, however did not show significant collagen deposition by picrosirius red when observed through polarized light (figure 3-10a). Cocultured conditions exposed to glass slides showed similar results (figure 10d). HFFs exposed to ZnO showed a very low cell density at day 15 suggesting cell death due to the material, however cells that were present showed slight red staining under polarized light suggesting very small amounts of collagen being deposited (figure 3-10b). Cocultured conditions when exposed to ZnO showed slightly higher cell density, but no collagen deposition (figure 3-10e). Fe sputtered glass slides showed significant amounts of collagen with a high degree of alignment for HFF conditions (figure 3-10c). Cocultured conditions exposed to Fe sputtered samples also showed significant collagen, however images appeared much more localized, but more organized (figure 3-10f). It should be noted that the fibrin layer in Cocultured delaminated from the Fe coated slide at day 10. However, cells that remained attached still showed collagen deposition. Quantification of picrosirius red staining was performed through red pixel intensity. ZnO treated groups showed the least collagen and was statistically significant between glass and Fe treated groups (figure 3-11). Glass treated samples showed twice as high pixel intensity as ZnO treated groups, with HFF monoculture producing slightly less than Coculture but was not statistically significant. Fe
treated groups produced twice as much collagen deposition when compared to glass treated groups, with HFF condition producing more than Coculture; statistical significance was found between HFF and Cocultured conditions of Fe treated groups. However, collagen deposition between glass and Fe in Coculture were not statistically significant, most likely due to partial delamination at day 10.
Figure 3-10 Picrosirius Red stain under polarized light at day 15 of (a) HFF on glass slide, (b) Cocultured 10:1 HFF:U937 on glass slide, (c) HFF on ZnO sputter coated slides, (d) CoCulture on ZnO, (e) HFF on Fe sputtered slides, and (f) CoCulture on Fe sputtered slides (magnification 10x, scale bar 200um)
Figure 3-11 Quantified red pixel intensity from picrosirius red staining at day 15 comparing glass, ZnO, and Fe treated HFF monoculture, and Coculture. Fe produced the greatest amount of collagen deposition in HFF monoculture and Coculture. Statistical significance was achieved across all groups, with the exception of collagen deposition between glass and Fe conditions in Coculture. (*p < 0.05 for HFF, #p < 0.05 for Coculture, §p<0.05 HFF vs Coculture)
3.4 DISCUSSION

Work here has shown that collagen deposition in vitro can be induced at higher levels with iron when compared to control. The mechanism of action of iron to the wound healing cascade is due to the importance of iron in biological systems. Adult humans require a daily intake of iron of 1-2 mg/day, however, hemoglobin synthesis require much higher amounts of 20 mg/day (7, 8). Foreign pathogens – i.e. bacterium – also require iron for cellular functions (6). Host immunity has evolved such that iron is actively sequestered from opportunistic pathogens, which results in enhanced inflammation due to free iron (10). Therefore, as a tool for accelerated healing, and in combination with data presented here, iron has the potential to utilize endogenous mechanisms for accelerated vascular healing.

Technological development towards bioactive endovascular devices for intracranial aneurysms has focused predominantly on utilizing polymers to induce accelerated healing. However, little effort has been made towards investigating the same potential for degradable metals. In this work we have shown that it is feasible to coat BPCs with a thin zinc or iron coating through electroplating. To achieve smooth discrete coatings only on the platinum surface, the deposition of metal ions must be controlled at an even rate to limit uncontrolled growth.

Controlling the flux of metal ions to the substrate is of fundamental importance to electroplating (5). Of the parameters tested – solution concentration, solution pH, solution composition, and current density – current density appeared to have the most significant influence on coating quality on BPCs. Electroplating from high concentration solutions allowed for faster deposition from solution due to the availability of ions to organize into a crystal lattice. These resulted in rough surfaces which suggest larger grain size at the surface. Ion dissociation
from salt solutions had a similar effect, whereby higher dissociation from salt solutions allowed depositing metal ions to move freely in solution and to the substrate, which showed larger surface grains (5). In theory, smooth coatings can be achieved with high current densities by reducing solution concentration or complexing depositing metal ions with other molecules to reduce deposition rate. However, since the helical geometry of the BPC must be maintained, discrete coatings on the coil surface may be lost due to charge concentration.

It should be noted that SEM analysis performed here cannot definitively report grain size since only surface features can be observed; grain size is better observed when sample are viewed under cross-section. However, since this work was to demonstrate proof of concept, grain size is not explicitly the objective. Rather, discrete and smooth coatings on the BPC surface was the goal. In addition, literature on electroplating have produced protocols and specific electrolyte compositions.

Experiments from theses prototype coils showed significant wear, however, it is important to realize that these tests were performed for proof-of-concept investigation only. Electroplating was used for these tests for four reasons: 1) electroplating is safe and facile for laboratory tests, 2) cost and time for fabrication are low for electroplating at the laboratory scale, 3) the goal of this work was to demonstrate feasibility of metallic coatings on BPCs, and 4) construction of full length coils with deployment system and pusher-wire intact were not feasible in the lab.

Alternative methods for metal coatings on BPCs such as sputter deposition are also possible. Coatings produced from sputter deposition produced extremely smooth surfaces with discrete coatings on BPCs (figure 3-12). In addition, minimal preparation to coils, little to no environmental waste, and the ability to easily control coating thickness make this technology
extremely attractive from a manufacturing perspective. Fabrication costs and long processing
time for small batches prohibited extensive experimentation on BPCs. However, since metals
possess significant beneficial qualities for this application – i.e. less friction, less wear, and
longer shelf life – in addition to data which suggests that iron coated glass slides induces
collagen deposition, prompts further investigation.
Figure 3-12 BPC coated by sputter deposition with (a & b) ZnO and (c & d) Fe showing sub-micron level coating with extremely smooth surfaces. (A & c magnification 500x, scale bar 200um. B & d magnification 3000x, scale bar 40um)
Metallic coatings are extremely appealing since they possess superior material properties and have the potential for longer shelf life. Metals in general will have higher material hardness when compared to polymers, but are more easily processed than ceramics for small complex geometries.

Previous experimentation, as well as commercial products, have shown that polymers have the capacity to produce accelerated healing \textit{in vivo}. Since this work successfully showed proof-of-concept, immediate next steps should be made to determine the biological efficacy of these biodegradable metal coated coils, \textit{in vivo} in previously established swine models. In addition, since this work focused on only two degradable metals, other metals and alloys should also be considered based on the speed of degradation and known inflammatory reactions. However, it is imperative to make the distinction between beneficial inflammation for accelerated healing, and patient allergy when candidate metals are chosen. Lastly, since metals have higher materials strength, a fully degradable coil may be possible in the future.

3.5 CHAPTER THREE REFERENCES


CHAPTER FOUR: CONCLUSIONS AND FUTURE DIRECTION

FUTURE DIRECTION

4.1 LIST OF NOVEL FINDINGS

Parameters for Discrete Coatings on Bare Platinum Coils

- Small wetting droplets are required for discrete coatings on helical structures through atomized polymer deposition
- Current density is the most crucial factor for discrete coatings through electroplating

Materials Assessment for Thin Bioactive Coatings on Bare Platinum Coils

- Smooth surfaces limit friction perceived during deployment
- Inherent material properties

Biological Efficacy In Vitro

- Cytokine expression in vitro is highly dependent on proximity to inflammatory materials
- Cytokine expression in vitro is highly dependent on the 3D matrix used
- Cytokine expression has a threshold response to aPLGA-2A in vitro
- Collagen deposition in vitro can be observed through 3D fibrin gels

Biological Efficacy In Vivo

- Prototype polymer coated SCCs can achieve accelerated above two commercially available coil systems in vivo

4.2 CONCLUSION AND FUTURE DIRECTION

This research has successfully demonstrated the feasibility of coating bare platinum coils with two different classes of biomaterials for accelerated ICA healing with direct implications for commercial manufacturing. In addition, the work here has shown that accelerated vascular
wound healing can be achieved through inherent material properties alone. The ramifications of this research on biomedical device design is that biomaterials themselves may be used for therapy; previously this was exclusive to pharmaceutical drugs and growth factors alone.

To complete assessment of these two classes of materials, long term shelf life assessment should be performed. However, since equipment used for large scale fabrication, further processing such as sterilization, and packaging will be dependent on specific manufacturing capabilities of each medical device company, these parameters should be tested with specific processes in mind.

Future research in this area should be towards determining the full potential of inherent biomaterial properties. In this work we have shown that it is possible to induce accelerated vascular wound healing through either: 1) stress induced acidosis via aPLGA-2A, and 2) perturbation of cellular hemostasis to two essential metal ions. Further research should be performed to investigate the potential of other inherent biomaterial properties to modulate the wound healing cascade, beyond inflammation.
APPENDIX A: POLYMER ATOMIZING ROBOT DESIGN

(A) Picture of assembled polymer atomizing robot. (B) top plate and (C) bottom plate schematic
4 (A) Mounting bracket for air brush nozzles. (B) mounting bracket for part A.