Year 10: Research Project Highlights

Project Title: Processing of Bulk Amorphous and Polycrystalline Magnesium, Zinc and Iron-based Alloys
Principle Investigators: Prashant N. Kumta, PhD (University of Pittsburgh)
Thrust Area: ES-I Craniofacial and Orthopedic Applications (Materials Development and Processing)
Faculty Participants: Abhijit Roy (UPitt). Oleg Velikokhatnyi (UPitt); Zhigang Xu (NC A&T)
Student and Postdoc Fellow Participants: University of Pittsburgh – Jingyao Wu (MS, PhD candidate)
Industry Participants: Dr. Howard Kuhn, ExOne Company, LLC.

Year 10 accomplishments: The goal of this project was to process and manufacture near net shaped biocompatible and biodegradable novel patent pending and proprietary magnesium (Mg) and iron based alloys that exhibit controlled corrosion without eliciting any toxic responses, while possessing excellent mechanical properties, and enabling regeneration of bone for orthopedic and craniofacial applications. It is well-known that magnesium based alloys are very reactive and undergo rapid electrochemical dissolution in the presence of body fluid and physiological conditions. Due to this ubiquitous adverse attributes of Mg, we have selected a series of biocompatible alloying elements including Zn, Y, Zr, Ca, Sr, etc. determined by theory and empirical assessments to enhance the corrosion resistance while also achieving high strength of the Mg alloy without compromising the biocompatibility. Over the past years, we synthesized various Zn, Sr, Zr, Ca and Y containing novel Mg alloys by melting and casting followed by extrusion. Pure elements and master alloys were melted in a mild steel crucible under 0.1% SF_6 + Ar protective atmosphere and cast into heated molds. The as-cast ingots obtained were then solution treated (T4) and then quenched into water at room temperature. Extrusion was used to further refine the alloy microstructure and modify the mechanical and corrosion properties. Thus far, several alloys have been extruded in collaboration with Dr. Xu and others at NCAT resulting in significant reduction in grain sizes in all of the extruded alloys. The as cast and extruded materials were characterized by X-ray diffraction. The as cast and extruded alloys demonstrated single phase of Mg according to the X-ray diffraction pattern without indicating presence of secondary phases, which clearly confirmed the formation of single phase solid solution. However, scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS) revealed the presence of secondary phases containing Mg, Sr, Zn, Y, and Zr. Young's modulus, tensile strength, and elongation of the strontium-containing magnesium alloys were comparable to commercially available pure Mg and Mg-Al-Zn (designated AZ31B) magnesium alloy (Goodfellow Corporation, USA). The invented proprietary and patented alloys were found to exhibit higher yield and ultimate tensile strength compared to pure Mg and AZ31. Immersion corrosion rates of these alloys in Hank's Balanced Salt Solution (HBSS) at 37 °C were measured and compared to pure Mg at various time points. The corrosion rate of Zn and Sr containing alloy was comparable to that of pure Mg whereas Sr and Y containing alloys displayed much higher corrosion rates compare to pure Mg. The in-vivo corrosion rates of these alloys were tested in rat gluteal muscle over a period of 4, 8, 16 and 26 weeks. Interestingly, the in-vivo corrosion rate of the Sr and Y containing alloy was lower than that of the Zn and Sr containing alloy at all these time points. Moreover, the in-vivo corrosion rates of both these alloys were higher than that of pure Mg and may be due to the presence of secondary phases in these alloys. The local and systemic toxicity of various alloying elements present in alloys were also tested in the rat gluteal muscle and the results demonstrated absence of any detectable toxicity in various organs analyzed. Iron based alloy films also showed good cytocompatibility. Further, using the density functional theory computational methods many Zn alloys with cubic symmetry were identified with improved ductility and ultimate tensile strength. The roles of many biocompatible elements added to the primary metal was assessed with the primary goal to improve the desired mechanical properties of the resultant alloy. The study indicated that several novel biodegradable Zn based alloys can be identified.

Project Title: Processing of Porous and Nonporous 3D Biodegradable Metallic Alloys and Composites
Principle Investigators: Prashant N. Kumta, PhD (University of Pittsburgh)

Thrust Area: ES-I Craniofacial and Orthopedic Applications (Materials Development and Processing)

Faculty Participants: University of Pittsburgh - Abhijit Roy, PhD – Research Assistant Professor

Students: University of Pittsburgh - John Ohodnicki – (BS), Matthew Criado– (MS)

Industry Participants: Howard Kuhn, Ex-One, Pittsburgh; Oberg Industries, Pittsburgh; Refrac, AZ.

Year 10 accomplishments: The broad goal of this project is to design and develop patient specific, customizable and complex 3D anatomical shapes from biodegradable metals and biodegradable metal-polymer composites with hierarchical porous structures mimicking the macroscopic and internal microstructure of various and specific organs and tissues while also providing temporary mechanical function and mass transport properties using primarily the binder-jetting 3D printing technique. The flexibility in printing various parts from CAD/CAM data acquired also addresses an overarching goal of the ERC-RMB to develop prototype devices without the need for developing specific tooling as may be needed and considered mandatory in conventional machining techniques. In the past years, the research has been focused on assessing in vitro degradation, mechanical, and cytocompatibility properties of 3D-printed samples fabricated using novel patent pending proprietary biodegradable ERC-Fe based alloy powders. The approach was extended to fabricating 3D porous structures for in-vivo animal studies as well as generating a goat mandible in 3D using computerized tomography images. Moreover, atomized Fe-based and pure Mg powders were obtained in collaboration with Hoeganaes Corporation and Magnesium Elektron. These powders were then used to pursue selective laser melting (SLM) of Fe-based alloys, binder-jet 3D printing of biomimetic plywood designs, and electron beam (EB) melting (EBM) of pure Mg powder. In the last 12 months, we have focused on printing biodegradable metal-biopolymer scaffolds using the fused deposition modeling (FDM) 3D printing technique. We have studied in detail various printing parameters to successfully print porous and non-porous scaffolds of poly-caprolactone-biopolymer-Mg composites with varying geometry and Mg contents. The results showed that printing temperature, effect of shell thickness and printing speed all strongly influence the various geometrical dimensions of the printed scaffolds. The results also demonstrated that higher printing temperatures resulted in more fusion of the printed layers thereby generating more mechanically stable scaffolds. Moreover, the amount of Mg content in the composites also played a significant role on the mechanical properties of the printed constructs. Higher Mg content resulted in a less ductile 3D scaffold, with an increase in Young's modulus but lowering the % strain at failure. In addition, we are also developing diffusion bonding based additive manufacturing techniques to fabricate porous 3D magnesium alloy based scaffolds. The technology offers an economic approach with much to gain in terms of physical, chemical and biological properties and outcome with minimal compromise of the desirable attributes. In this process micro to millimeter thick Mg alloy plates were pressed inside a hot press applying a a precise range of desirable pressure for various specific times at a particular engineered and desirable temperature. Our initial feasibility studies show that complete joining of two or more Mg plates is possible and the nature and strength of the bonded layers depends, in addition to the intrinsic physicochemical nature of the alloys, on time, temperature, pressure, and surrounding atmosphere. Results of these studies offer insights into the mechanisms of the bonding process providing a path to increase the shared contact area of the structure increasing the bonding. In the future, in vitro and in vivo controlled release of regenerative or pharmacologically relevant molecules using hydrogel coatings on the 3DP constructs will be investigated to fabricate functional 3D printed scaffolds while also exploring possibility to commercialize and license the technology to a potential industrial partner.

Project Title: Processing of Novel Porous and Nonporous Biodegradable Magnesium Alloys
Project leader: Zhigang Xu, North Carolina A&T State University
Thrust Area: ES-1 Craniofacial and Orthopedic Applications (Materials Development and Processing)
Faculty Participants: Sergey Yarmolenko, Yeoheung Yun (NCAT) Prashant Kumta (UPitt), Zhongyun Dong, V. Shanov (UC)
Students: Honglin Zhang (PhD), Natalia Guarnizo Mendoza (BS), Chris Plott (BS), Alex Arriaga-Atwater (BS), Justin Chandler (BS)
Industrial participants: Luminal Solutions, Fort Wayne Metals

Project Overview: In last year, we started a project to develop Mg-based sternotomy wires in collaboration with Luminal Solutions. Mg-alloy wires in diameters from 0.7mm to 1.2 mm from one typical ZXK alloy and a few ZXKWE alloys were successfully produced through cold drawing. Through post heat treatment, ductility of the wires was greatly improved in different extents from different alloys. Tensile properties showed that the ZXKWE alloy wires had lower yield strength and higher strain-hardening rate than ZXK alloy after post heat treatment. This feature is particularly valuable, which provides wires with ease in twisting and strength for tightening during median sternotomy.

In this year, our focus of the research has been placed on exploring the refinement of heat treatment conditions for wires from different alloys and the development of innovative alloys by adding lithium (Li) to ZXKWE alloy, which are expected to provide better ductility and load carrying capacity.

The research achievements in this alloy development and processing team are summarized as follows:

(1) Refine post heat treatment condition for the sternotomy wires with better ductility and strain hardening characteristics;
(2) Developed a new series of alloys by adding Li to ZXKWE alloy targeting moderate-to-high mechanical strength, ductility, load carrying capacity in twist-tightened form.

Year 10 Accomplishments:

Optimization of Post Heat Treatment Conditions: Magnesium alloys were first extruded into thick wires of 1.7 mm in diameter with an extrusion ratio ~80, then were further drawn into thin wires of 1.2 mm in diameter by cold drawing with a cold work ratio of ~10% for each pass. Since the existence of internal stress and intensive texture generated by extrusion and multiple-step cold drawing, the as-drawn wires have very limited ductility such that they break quickly during twist-tightening procedure which is a typical step in median sternotomy. Post heat treatment or annealing was desired to improve the ductility of the cold-drawn wires. We employed grain growth observation method to determine the refined annealing condition domain to release the internal stress and maintain the fine grain structure. As shown in Figure1, the as-drawn wire has crushed and elongated grains in the drawing direction which represents non- or partial-recrystallization; after annealing, at 350°C for 0.5h, bimodal grain structure can be seen, grain elongation is alleviated; with annealing at higher temperatures, grain growth can be observed and grains become more equiaxial. Therefore, temperatures 350°C and 390°C were chosen to anneal this alloy wires. Experiments showed the wires annealed at 390ºC possess lower tensile yield strength (321.9 MPA), higher strain-hardening rate and higher load (241.6 N) in twist-tightened form. Similar process was applied on wires made from other alloys.

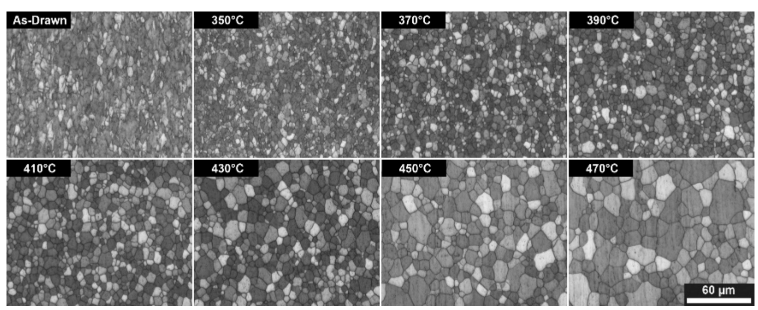


Figure 1. Microstructures of a ZXKYE alloy after annealing at different temperatures for 0.5 h.

Alloy Development: To further improve the performance of the sternotomy wires, different amounts (2, 3, and 4%) of Li element were added to the above mentioned ZXKYE base alloy. Among them, addition of

2% Li resulted in wires with maximum ultimate tensile strength 265.2 MPa and load-carrying capacity 260.9 N, respectively.

More alloys in extruded forms have been sent to our industrial collaborator, Fort Wayne Metals, to be drawn thin wires. This collaboration will produce enough amount for further annealing optimization, mechanical evaluation, corrosion evaluation and protection. Some thinner wires in 0.4 mm diameter will be made into 7x1 cable for evaluation.

Other progresses: We have finished the trial run of Mg-alloy processing with a new differential speed rolling mill from International Rolling Mill, Inc. in RI, USA. We will fabricate Mg-alloy sheet metals with control the texture in the sheet metals, either tilted textures or more randomized texture, which will lead to better deformability of the sheet metals.

Project Title: Biodegradability and platelets adhesion assessment on coated Mg surface

Lumei Lui, Youngmi Koo, Sangho Ye, **Yeoheung Yun** (PI), William Wagner

Abstract: We compared and measured the surface degradation behavior of coated Mg alloys and stainless-steel (control) under different flow conditions. We fabricated a microfluidic system, containing poly(carbonate urethane) urea (PCUU) coated Mg alloys, in which we measured degradation behavior and cell adhesion in terms of rates, morphology, attached products on surface, particulates, and types of degradation of Mg alloys. We found that particulates consists of Magnesium oxide, Calcium/Zinc Phosphates complex of $(Ca_{1-x}Mg_x)_{10}(PO_4)_6OH_2$. We also found that varying flow-induced shear stress (calculated by computational fluidic dynamic simulation) and static culture in microfluidic chips affect degradation behavior of Mg alloys. The results showed that brain endothelial cells number on PCUU-coated alloys surface was significant higher than that on non-coated alloys surface (P<0.05). PCUU coating alloys improved the live endothelial cells compared with bare alloys (**Figure 1**). Two students gave presentation on national conferences, TERMIS AM and NCTERMS and published in peer review journals.

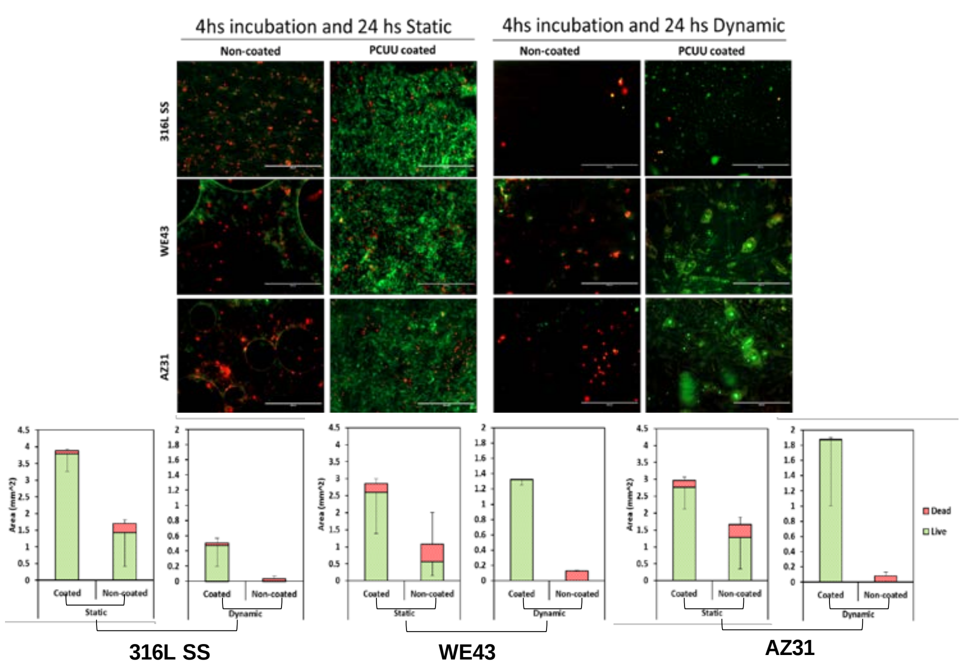


Figure 1. (A) Live and dead fluorescence of brain endothelia cells on PCUU coated and non-coated alloys surface at both static and dynamic conditions. Scale bars: 400= µm. (B) The areas of live (green) and dead (red) on PCUU coated and non-coated alloys surface at both static and dynamic conditions. Parenthesis represent significant difference at P<0.05.

Project Title: Characterization and Testing of Prospective Magnesium Alloys

Principle Investigator: Sergey Yarmolenko (NCAT)

Thrust Area: ES-I Craniofacial and Orthopedic Applications (Materials Development and Processing)

Faculty Participants: Zhigang Xu (NCAT), Boyce Collins (NCAT), Svetlana Fialkova (NCAT), Sudhir Neralla (NCAT), Prashant Kumta (UPitt), Abhijit Roy (UPitt), Vesselin Shanov (UC)

Students: Paul McGhee (PhD), Christopher Plott (BS), Kevin Galdamez (BS), Isaac Robinson (BS), Natalia Guarnizo-Mendoza (BS), Justin Chandler (BS) – all NCAT

Project Goals: The goal of this project is to support new materials development with set of property evaluation and characterization techniques, systematic accumulation property data for prospective ERC-RMB materials and maintaining materials property database. Previous years the characterization and testing of new materials were performed within dedicated projects of Materials Development and Processing thrust (Red Team), however during last year of program the need of systematic integration of property data become evident.

Highlights of 10th Year Accomplishments: During reporting period the following tasks were:

<u>*Task 1. Method development and standardization:*</u> In this task we performed SOP development for texture analysis (XRD and EBSD) and elemental analysis by μ-XRF method. For optimization of cold drawing process, we set up laboratory procedure for evaluating the effect of annealing temperature on microstructure and mechanical properties (Fig. 1). Fixtures for mechanical property testing of magnesium wires were designed and evaluated.

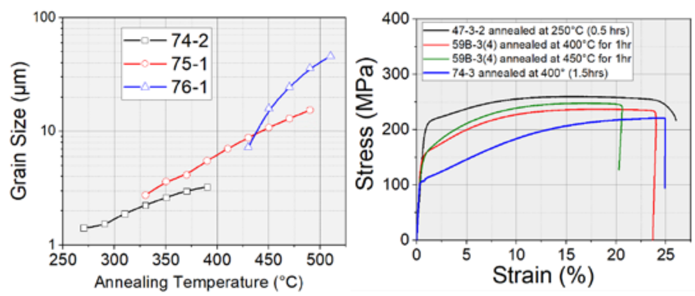


Fig. 1: Effect of annealing temperature on grain size and mechanical properties of Mg wires.

<u>*Task 2. Effect of processing parameters on materials properties:*</u> (a). Optimization of cold drawing and testing of Mg wires were done with BS students C. Plott, N. Guarnizo Mendoza and J. Chandler and presented at ASME Conference. PhD student P. McGhee successfully completed his study on effect of extrusion temperature and aspect ratio on strength, texture and fatigue properties of Mg alloys (Fig. 2).

<u>*Task 3. Structure-property relationships:*</u> (a) Effect of structure modulating of thin films by small amounts of Gd, Zn and Cu on mechanical properties was studied with BS students K. Galdamez and I. Robinson and presented at ASME conference; (b) Nanoindentation method was proposed for the incipient plasticity evaluation along crystallographic directions in Mg and Mg alloy grains. (c) Effect of annealing on ductility of Mg alloys was demonstrated on the series of extruded rare-earth containing Mg alloys.

<u>*Task 4. Materials Property Database:*</u> mechanical and corrosion properties of ~200 magnesium alloys were accumulated in database.

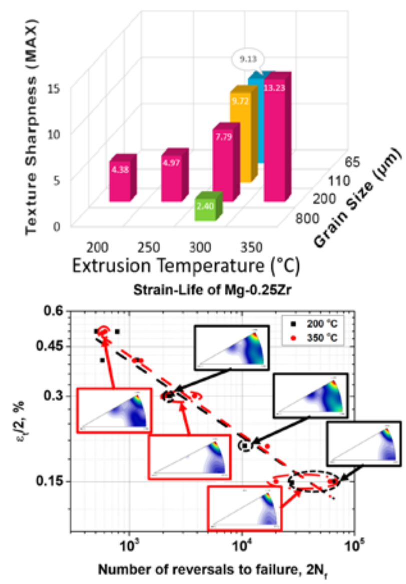


Fig. 2: Effect of extrusion temperature and grain size on sharpness of texture of Mg-Zr alloys (top); Texture evolution during fatigue of Mg-Zr alloys extruded at different temperatures (bottom).

Project Title: In Vitro and In Vivo Evaluation of Magnesium and Zinc alloy based Nasal Dilator for Nasal Valve Stenosis
Principal Investigators: Prashant N. Kumta, PhD, Department of Bioengineering, University of Pittsburgh.
Thrust Area: ES2: Cardiovascular and Thoracic Devices
Faculty Participants: Abhijit Roy, PhD; Oleg I. Velikokhatnyi PhD; D. Chi, MD
Student: Puneeth Shridhar MD MS, PhD student; Jingyao Wu, BS, PhD Candidate
Industry Participants (if applicable): Philips Respironics

Year 10 accomplishments: In Year, 10, we successfully manufactured nasal dilator (nasal stent) using our proprietary ultra-high ductility (UHD) Mg alloys. In addition, new designs of the nasal dilators were developed and prototype nasal dilators were manufactured from the extruded UHD Mg alloy rods. Magnesium rod was machined into tubes measuring 5 mm in diameter, 300 μm in wall thickness, 60 mm in length using wire-EDM (EDM: Electrical discharge machining). These tubes were then laser cut (with Nd:Yag Laser) into nasal dilators (nasal stents) using the most appropriate dilator design. To achieve a better surface condition, all the dilators will be electrochemically polished. As shown in **Figure 1**, the dilator design for nasal application suitable for pig nasal passage (nostrils) was developed and a smooth surface after our electrochemical polishing process will be obtained. In year 10, we also fabricated balloon expandable dilators made from our zinc based alloys for implantation in pig nasal model **(Figure 2).** Zinc alloy dilator with 5 mm diameter, 300 μm wall thickness and 10 mm length was designed and laser cut for the left pig nostril with right (non-implanted) pig nostril acting as the control group in this study. Moving forward, we intend to initiate porcine studies at UPMC animal facility. Two groups will be studied: (1) <u>Experimental Group</u>: UHD Mg or Zinc alloy dilator group in right nostrils; (2) <u>Control group</u>: No dilator group in left nostrils. Both UHD Mg alloy and Zinc alloy dilators will be implanted for 1 week, 2 weeks and 4 weeks, respectively, and the pigs will be sacrificed at the end of each time point. The implantation site of the dilator will be verified using nasal endoscopy, nasal inspiratory flow meter and acoustic rhinometry. Optical coherence tomography (OCT) with high resolution will also be conducted to study the changes in the nasal lumen following the implantation study planned in the porcine nasal models. Micro-CT analysis will be performed to analyze full nasal dilator degradation expected after 4 weeks of implantation. The dilated nasal mucosal section will be harvested and compared with nasal mucosa of control nostrils by immunohistochemistry (H&E staining for histology and CD68 staining for inflammatory response evaluation). Also, density functional theory calculations of mechanical properties of the Mg-based alloy compositions validate the observed improved ductility of the novel UHD Mg and Zn alloys. We are closely working with FDA regulatory experts at Philips for FDA approval in the Class I category. Recently, our team completed NSF I Corps customer discovery interviews to validate the adaptability and usability of biodegradable UHD Mg alloys for various stent applications.


Figure 1. Design of UHD Mg alloy nasal dilators.

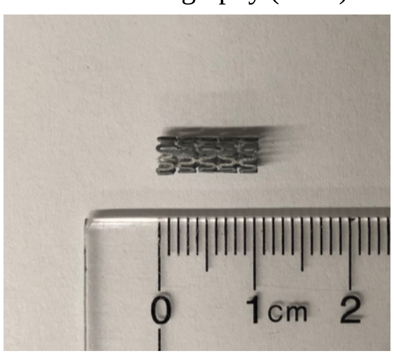


Figure 2. Nasal Dilator from zinc based alloys.

Project Title: Smart Stent for the Arteriovenous Fistula
Thrust Area: ES-II Cardiovascular and Thoracic Devices
Principal investigators: Mark Schulz
Faculty Participants: Prabir Roy-Chaudhary (Az) Vesselin Shanov (UC), Diego Celdran (U. of AZ), Begona Campos-Naciff, (UC), Sang-Ho Ye (Pitt), Bill Wagner (Pitt), Yeohueng Yun (NCAT)
Student and Post-doc Fellow Participants: Chenhao Xu, PhD (UC), Michelle Goh, BS (UC)
Clinicians: Prabir Roy-Chaudhary (Az)
Industrial participants: Inovasc LLC, Begona Campos

Project Goals and Description: Conventional stents stay in the body permanently and are prone to develop aggressive in-stent restenosis which limits inflow. A biodegradable maturation enhancing stent (*b*MES) could provide initial scaffolding allowing optimized hemodynamic shear stress profiles enhancing outward remodeling and also inhibit neointimal hyperplasia. The key feature of a biodegradable stent is that the stent degrades in the body and eventually disappears after the AV fistula matures. It avoids the long-term problems such as in-stent restenosis, thrombosis, and the prolonged use of anti-platelet agents, which characterize traditional non-biodegradable stents. However, past study and in-vivo experiment showed a design problem with the *b*MES. The stent starts to degrade at the moment of stent implantation. With time, the strength decreases and may reduce vascular maturation. The ideal situation is that the stent will support the full scaffolding while the fistula matures in about 4-6 weeks, and then the stent degrades on command within about 4 weeks. A new generation of coatings may better control stent degradation to start when the maturation is accomplished. The time at which coating degradation occurs can be controlled using a smart stent. A smart stent can be heated externally using induction heating. Heating the metal stent also heats the coating causing it to dissolve on command. A second version of a smart stent can prevent corrosion and then accelerate corrosion on command based on electrical potential applied to the stent. These two versions of smart stents have not been investigated in the literature to our knowledge and give the ERC a small lead internationally in this research. This project is interdisciplinary with contributors from all three universities.

Study Procedure: The induction heating (IH) smart stent was tested in vitro, Fig-1a,b. A Mg or Fe wire to simulate a stent was placed inside of a plastic tube that was routed through a copper coil of an induction heater. Saline water was pumped through the tube. A thermocouple recorded the water temperature downstream of the wire. Different coatings were tried on the wires and the current in the heater and the heating times were varied. It was determined that the coating on the Fe wire was easy to melt at low current (109A) and short time (100 sec). The temperature rise of the downstream water was less than 1 deg C. The coating on the Mg wire was difficult to melt and a higher frequency induction heater may be needed to heat it. In vivo testing is needed. The electrochemical corrosion smart stent was tested in a pig AVF for one week, Fig-1c. The electrical current corroded the stent faster relative to a reference stent but a problem with the electronics shortened the test. Further in vivo testing is needed. The electronics also need to be made biodegradable, Fig-1d. Another approach for the smart stent is to have a non-degradable Fe stent that is heated by the IH to remove tissue from the stent as needed. Smart stent design extends to other implants.

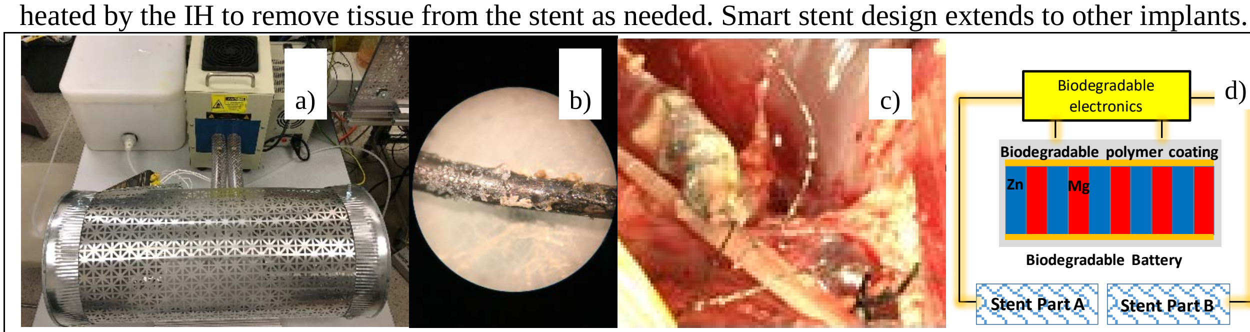


Figure 1. Smart Stent concepts: (a) Induction heating (IH) smart stent experimental setup. (b) Polymer

Project Title: Evaluation of Magnesium-based alloys for airway stenting

Principal Investigator: Prashant N. Kumta, PhD, Department of Bioengineering, University of Pittsburgh.

Thrust Area: ES2: Cardiovascular and Thoracic Devices

Faculty Participants: Abhijit Roy, PhD, Oleg I. Velikokhatnyi, PhD, D. Chi, MD.

Student: Jingyao Wu, BS, PhD Candidate; L. Mady, MD, Puneeth Shridhar MD MS, PhD student.

Industry Participants (if applicable): Acell, Inc.

Year 10 accomplishments: In Year, 10, proprietary ultra-high ductility (UHD) Mg alloy stents were successfully fabricated. Prototype Mg stents were manufactured from extruded magnesium alloys rods. The stent design was determined prior to fabrication. The magnesium rod was machined into tubes, 4.2 mm in diameter, 300 μm in wall thickness, and 600 mm in length using wire-EDM (EDM: Electrical discharge machining). Following this, the wire-EDM tubes were laser cut into stents based on the stent design. Two stents were manufactured from each 600 mm long tube. To achieve better surface conditions, all the stents were electrochemical polished. As shown in **Figure 1**, the stents demonstrated smooth surface finish after they were subjected to electrochemical polishing.

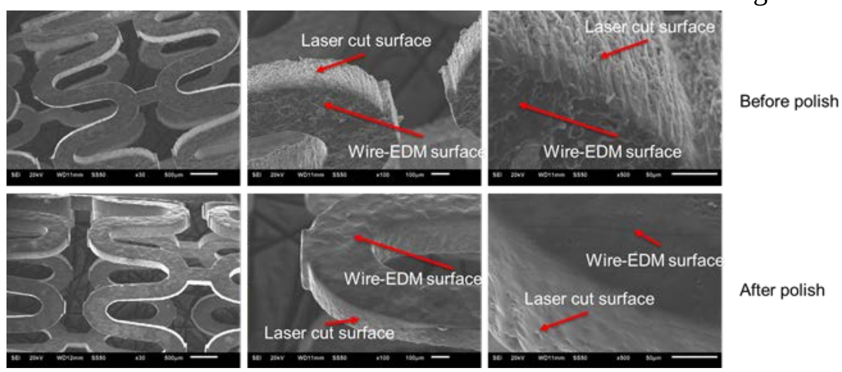


Figure 1. Effect of electrochemical polishing on surface of Mg stents.

In year 10 balloon expandable stents made from our proprietary UHD Mg alloys were implanted into the rabbit tracheal model. 316L stainless steel (SS) stent with identical dimension and design served as the control. The stents were implanted for 4, 8 and 12 weeks, respectively and the rabbits were sacrificed at the end of each time point. The implantation site of the stent was verified using tracheobronchial endoscopy. Mg stents were visible at 4 weeks, but fully degraded in trachea between the 4 and 12 weeks' time point. Tracheobronchial endoscopy showed that the airway patency was maintained throughout the study for the Mg stent group. However, the 316LSS stent group displayed thick granulation tissue around the stent after 4 weeks of implantation. At 8 and 12 weeks, the granulation tissue grew with airway narrowing seen in the 316L SS stent group. In contrast, the Mg stent group exhibited normal healthy trachea after full degradation. OCT imaging of the stented airway (**Figure 2**) verified this observation. The lumen size of the rabbit airway continued to grow for the Mg stent group, while the lumen size remained unchanged for 316L SS and stopped growing with even reduction seen due to granulation. Micro-CT analysis showed that about 35% of the Mg tracheal stents degraded after 4 weeks of implantation, and completely degraded between 4 weeks and 12 weeks. The stented airway tissue was harvested for histology analysis. H&E staining showed encapsulation of stents at 4 weeks while CD68 staining showed less inflammatory response for the UHD Mg stents compared to 316L SS stents. Further, density functional theory (DFT) calculations of mechanical properties of the Mg-based alloy compositions validate the observed improved ductility of the novel UHD magnesium alloys.

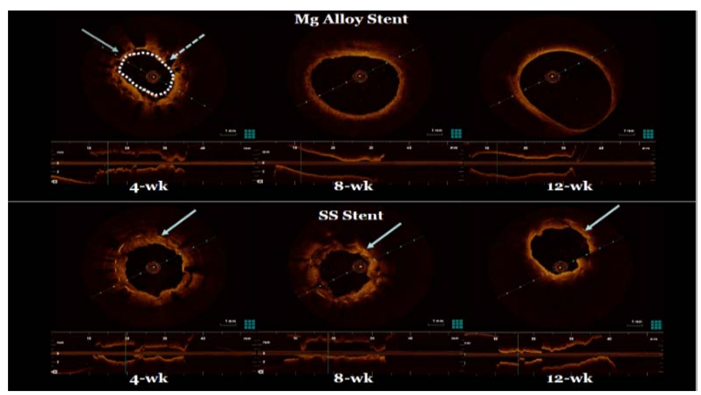


Figure 2. OCT images showing rabbit stented airway for UHD Mg alloy and SS stents.

Project Title: Non-thrombogenic/Biocompatible Coatings on Magnesium Alloy Stents
Thrust Area: ES2: Cardiovascular and Thoracic Devices (II.10-04)
Principal investigator(s): William R. Wagner
Participants: Sang-Ho Ye, Xinzhu Gu, Yingqi Chen,
Affiliations: McGowan Institute for Regenerative Medicine *(*UPitt)

Abstract: The project goal of this study is the development of an effective blood/biocompatible coating technology on a bioabsorbable Mg alloy cardiovascular stent device. The ideal coatings should firstly improve the blood compatibility on a Mg alloy device in acute blood contacting circumstance (I. Prevent acute thrombogenicity), provide bioproactive functionalities to help the appropriate tissue regeneration (II. Promote endothelialization as well as control of smooth muscle cell growth) by delivering bioactive molecules, and both biodegradation rates of the coating layer and Mg alloy substrate should be harmonized with the tissue restoration (III. Degradation control of the coating and Mg alloy substrate) (**Fig. 1**).

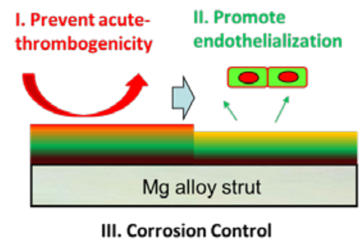


Fig. 1. Design of an multi-functional coating on a Mg alloy cardiovascular device.

To achieve a multi-functional coating layer on Mg alloy surface, a multi-layered functional coating was achieved via step by step process. Firstly, a thin film coating using a 6-phosphonohexanoic acid (PHA) was applied on a cleaned/polished and oxidized Mg alloy surface ($Mg(OH)_2$) to stabilize the substrate and provide a functionality. Then, the samples surface was coated with surface eroding polymers, poly (amino-1,3 trimethylene carbonate) (PTMC) as well as the polymer with amine groups (PTMC-NH_2). Subsequently, a non-thrombogenic SB bearing polymer with carboxyl groups (PSB-COOH) was also conjugated onto the PTMC-NH_2 coated layer by using EDC/NHS chemistry. The surface morphology and surface eroding property were investigated after the samples immersed into ovine poor platelet plasma (PPP) at 37°C for 1, 2 and 4 weeks. In vitro blood test was performed for each layer of the coated sample with a fresh whole ovine blood and the thrombotic deposition will be evaluated by a scanning electron microscope as well as a lactate dehydrogenase assay. In vitro cell culture study was performed on the coated Mg alloy surfaces. In vitro corrosion performance was also investigated by an electrochemical analysis method.

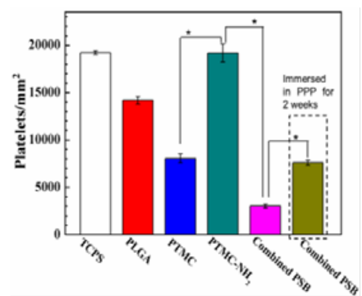


Fig. 2. Platelet deposition quantified after contact with a fresh ovine blood.

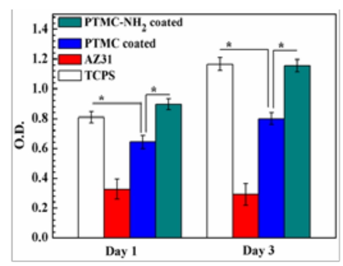


Fig. 3. The metabolc index measured after cultured a mouse endothelial cell for 1 and 3 days on the samples.

The designed surface combined with the PSB polymer showed a significant reduction of platelet deposition firstly compared with other controls which have not a PSB polymer layer (**Fig. 2**). Then, the platelet deposition was also gradually increased with the erosion of the PSB polymer as well as exposure of PTMC/PTMC-NH_2 layer from the sample immersed in PPP solution for 2 weeks. **Fig. 3** showed a mouse endothelial cell growth on a Mg alloy (AZ31) and the PTMC and PTMC-NH_2 polymer coated surfaces. The PTMC-NH_2 polymer coated surface showed a better attachment and growth of the endothelial cells than the other surfaces which showed the consistency of increased platelet deposition compared with PTMC coated surface. From the surface morphology observation as well as electrochemical analysis, the combination of surface eroding polymer coating with PTMC/PTMC-NH_2 showed the improvement of coating stability compared to a simply physical polymer coating and a significant corrosion protection on AZ31 surface (**Fig. 4**). The corrosion protection could be further improved when the PSB polymer was added on the top layer via a chemical conjugation with the PTMC-NH_2 polymer.

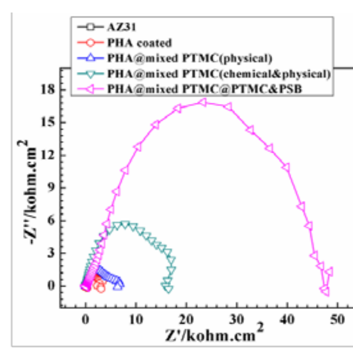


Fig. 4. Nyquist plot of electrochemical impedance spectroscopy for the samples.

In conclusion, this developed multi-layered coating technique on a Mg alloy could provide some dynamic functionalities to an advanced Mg alloy cardiovascular device which could prevent an acute thrombotic deposition firstly, then promote tissue restoration with the control of bioadsorption rate.

Project Title: ES-III-01a: Neural Repair and Biosensors.
Principal investigator: Sarah Pixley (UC)
Thrust Area: ES3: Responsive Biosensors and Neural Applications
Faculty Participants: (UC): V Shanov, W Heineman, Z Dong, M Schulz, John Yin. (NCAT): N. Bhattarai, Z Xu. (Pitt): P. Kumta, K. Marra, T. Cui. **Clinical Advisory Board:** Tejas Sankar, (UC/Cincinnati Children's Hospital); Kevin Little, David Hom; **Student and Post-doc Participants:** (UC): Xiaoxian An, Dr. Shanov's lab. (NCAT): U Adhikari, S Khanal, Nava Rijal.
Technical Support: Tracy Hopkins (UC), Dr. Donita Bylski-Austrow & David Glos (Cincinnati Children's Hospital Biomechanics Lab).
Industrial Partners: Ft. Wayne Metals (Jeremy Schaffer, Tom Hamilton and Adam Griebel)
Project Overview: The project goals are to characterize biomedical uses of metallic magnesium (Mg) in neural and soft tissue repair and to understand the mechanisms of Mg corrosion using sensors.
Achievements in year 10:

1. **Mg metal wire to support axonal growth across injury gaps:**

- **Paper Published:** Hopkins TM, Little KJ, Vennemeyer JJ, Triozzi JL, Turgeon MK, Heilman AM, Minteer D, Marra K, Hom DB, Pixley SK (2017) Short and long gap peripheral nerve repair with magnesium metal filaments. J Biomed Mater Res Part A 105:3148-3158.
- Manuscripts in preparation on rat nerve repair experiments:
  - Paper 1: Cleaning and anodizing commercial 99.9% Mg wire does not alter in vivo degradation of wire when used in nerve repair, or aid functional recovery. PCL nerve conduits collapsed, and wire broke.
  - Paper 2: Commercial 99.9% Mg wire placed inside silicone guides shows better cell attachment and growth along the wire with concentrated $MgSO_4$ solution added to guides at surgery. Function was not improved.
- New rat nerve repair experiment: Nerve guides made of electrospun polycaprolactone (PCL) nanofiber fabric, with and without embedded Mg metal micro-particles, were produced by Dr. Bhattarai's students at NCAT and made into nerve guides. Ft. Wayne Metals group provided stranded (6 around 1) wire made of the Mg alloy, Resoloy™. The wire was placed in conduits to guide cells. Histological analysis is still underway. Preliminary Results:
  - In vitro: stranded Mg wires degraded more rapidly than commercial Mg wire in cell culture medium.
  - In vivo: stranded Mg wire degradation was slower in vivo than commercial pure Mg wire (in previous exps).
  - PCL fabric guides showed swelling and delamination. Function was not improved.
  - Stranded wire corrosion left a solid core. SEM/EDX analysis showed this has a significant amount of the rare earth element Dy (Dysprosium) (average of ~15% by weight).
  - Tissues attached to the wires and nerves were able to grow through to distal end in all cases.

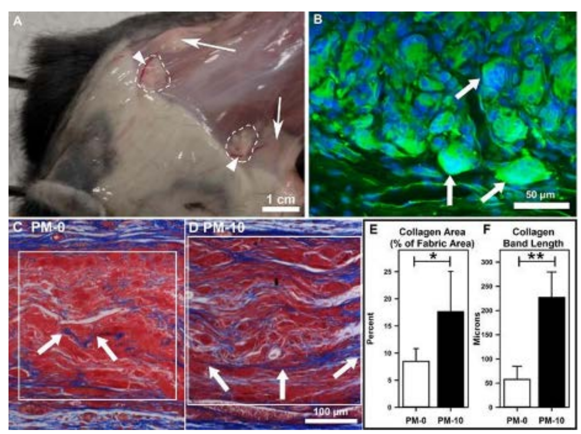


Fig. 1. Pixley lab placed PCL nanofiber fabrics (made at NCAT) under skin of mice (A, circled). (B) After 28 days in vitro (DIV), fabrics were filled with macrophages and foreign body giant cells (arrows). C-F) Mg fabrics had more collagen and more organized collagen (C,D, arrows), signs of more advanced tissue healing.

2. **Optimizing Mg wire properties with Ft. Wayne Metals: Paper published**: Griebel, AJ, Schaffer, JE, Hopkins, TM, Alghalayini, A, Mkorombindo, T, Ojo, KO, Xu, Z, Little, KJ, Pixley, SK 2017. An in vitro and in vivo characterization of fine WE43B magnesium wire with varied thermomechanical processing conditions. J Biomed Mater Res Part B 2015:00B:000–000.

3. **Mg-containing PCL electrospun nanofiber fabrics made and characterized. 2 manuscripts in preparation.**
    - Paper 1: Fabric manufacture, physical qualities and cell cytotoxicity were analyzed at NCAT and UC.
    - Paper 2: Fabrics with and without embedded Mg metal particles were placed in vivo, under skin in mice. Tissues analyzed at 3, 8, and 28 days in vivo (DIV) showed significant effects of Mg on reducing inflammation and activating tissue-reparative macrophages, speeding resolution of the foreign body response to PCL. Suggests that Mg metal could reduce the negative tissue reactions to a foreign body.
4. **C2C project:** Mg "bone nails" were made by 3 ERC engineers (using ERC Mg: Kumta, Xu, Shanov) and by Ft. Wayne Metals. At UC, Mg nails were tested for ease of insertion into pig cadaver bones (Dr. Little) and the biomechanical properties of bone with nails were analyzed by Cincinnati Children's Biomechanics Lab, with Dr. Bylski-Austrow, David Glos & students. Data were provided to Irish C2C members for use in modeling of stresses and coatings of nails, to perfect Mg bone nail use.

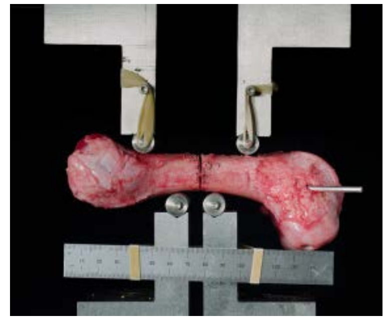


Fig. 2. C2C work. Mg bone nails provided by ERC and industrial members were inserted into cut pig cadaver bones and tested for mechanical properties on a 4-point bending instrument. Comparisons with Ti and stainless steel nails are providing data for Irish groups doing stress modeling and Mg coating.

Project Title: Embedding magnesium particulates into PCL nanofibers

Principal Investigators: Narayan Bhattarai, North Carolina A&T State University

Thrust Area: ES3: Responsive Biosensors and Neural Applications

Faculty Participants: Sarah Pixley (UC)

Student and Post-doc Fellow Participants: Udhab Adhikari (PhD student, NCAT), Shalil Khanal (PhD student, NCAT),Sunghyun Jun (MS student-NCAT), Kalene Johnson (MS-Student NCAT), Xiaoxian (PhD, UC), Svitlana Fialkova (NCAT)

Project Overview:

Magnesium metal has great potential for use as medical implants in bone as well as vascular stents because of its unique physical properties (strength but lighter in weight and greater similarity to bone in tensile properties) and biological properties such as biodegradability and biocompatibility. During previous years we could show that nerve repair was improved by placing a magnesium (Mg) metal filament inside a hollow nerve guide with reduced tissue inflammation. Mg^{2+} are also being explored as therapeutic agents for several conditions related to central nervous, cardiovascular, respiratory and gastrointestinal systems and has been proven to reduce acute as well as chronic pain. To study the effects of degradation products of Mg metal during the acute phase of recovery after injury, we embedded Mg metal particles within the nanofibrous meshes by electrospinning. Embedding Mg particles into nanofibers through electrospinning allows homogeneous distribution of Mg within the polymer matrix, as well as reduce the agglomerations for control over the timing and placement of local delivery of the products of Mg metal breakdown, which include Mg^{2+} and hydrogen gas. We fabricated nanofiber meshes with different concentration of Mg and characterized their physical properties, release of degradation products, cytotoxicity in culture and tissue responses in vivo.

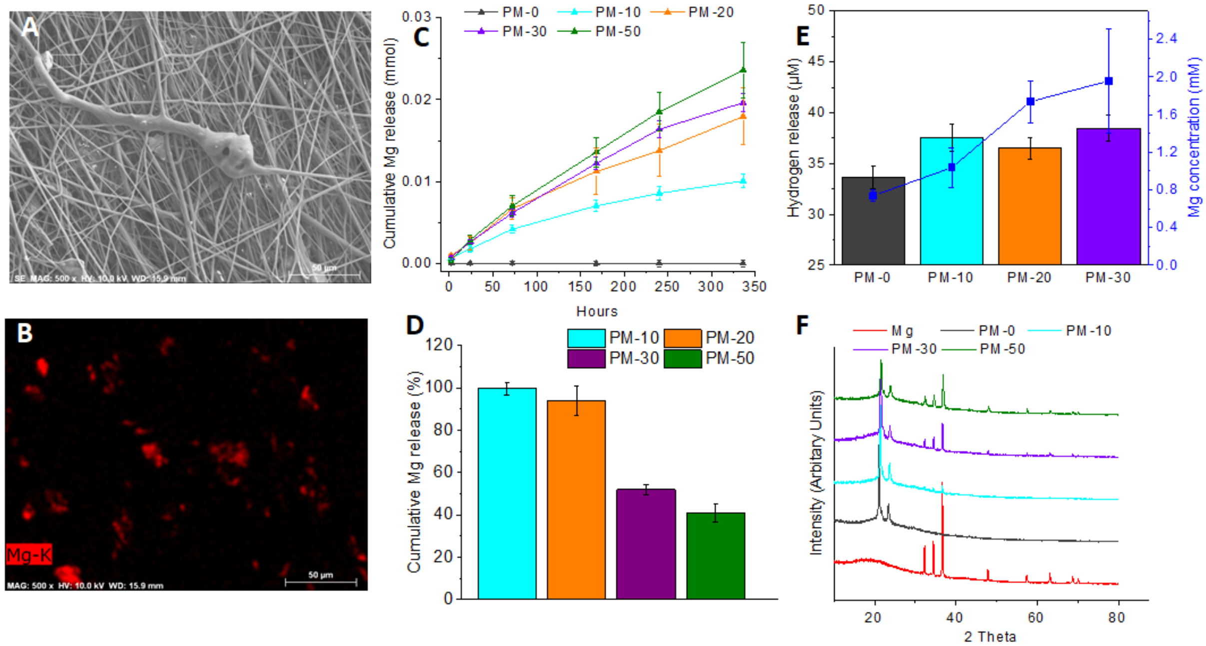


Figure: Metal particles were coated by PCL and embedded in nanofiber meshes (Fig. A). PM-10 to PM-50 represent the nanofiber meshes with PCL+10% to PCL+50% Mg respectively. Mg particle presence within electrospun nanofibers was confirmed by energy dispersive X-ray spectroscopy (EDS) analysis (Fig. B). After 14 days (336 h), the cumulative amount of Mg^{2+} released from PM-10, PM-20, PM-30 and PM-50 was estimated to be 0.0100, 0.0179, 0.0196 and 0.0236 millimoles, respectively (Fig C). It was estimated that these corresponds to 99.56 %, 94.058%, 51.87% and 40.78% of total Mg present each nanofiber mesh type (Fig D). Hydrogen release was significant and fabrics were not cytotoxic in vitro (not shown) (Fig E). The PCL nanofiber mesh showed two strong peaks at 21.5 and 23.6°, corresponding to the (110) and (200) crystallographic planes of PCL [31]. Similarly, major Mg peaks were observed at 32.2, 34.4, 36.6°, 47.8°, and 57.4° corresponding to (100), (002), (101), (102), and (110) crystallographic planes of Mg respectively in PCL nanofiber samples containing Mg (Fig F).

Peer Reviewed Journal Publications (2)

- Thompson Z, Rahman S, Yarmolenko S, Sankar J, Kumar D, Bhattarai N. **Fabrication and Characterization of Magnesium Ferrite-Based PCL/Aloe Vera Nanofibers**. Materials 2017;10

- Rijal NP, Adhikari U, Khanal S, Pai D, Sankar J, Bhattarai N. **Magnesium oxide-poly(ε-caprolactone)-chitosan-based composite nanofiber for tissue engineering applications**. Materials Science and Engineering: B 2018;228:18-27

Project Title: Sensor Development and Miniaturization

Principal Investigators: William R. Heineman (University of Cincinnati)

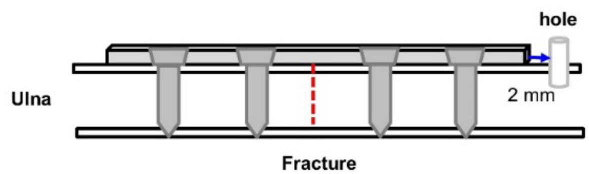
Thrust Area: ES3: Responsive Biosensors and Neural Applications

Faculty Participants: Z. Dong, S. Pixley, M. Schulz, V. Shanov, J. Yin (University of Cincinnati); C. Sfeir, P. Kumta (University of Pittsburgh); Z. Xu, Y. Yun (North Carolina A&T University); F. Witte (Charité University of Medicine, Berlin, Germany)

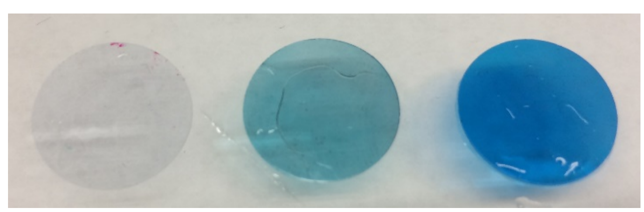
Student and Post-doc Fellow Participants: D. Rose, J. Lynch, K. Ojo (Postdocs)

Established feasibility of transdermally monitoring H_2 evolution from Mg implants in patients (Witte). A study is in progress with Dr. Witte to establish the feasibility of measuring the H_2 evolving from biodegrading Mg (MAGNEZIX®, Syntellix Co., Germany) fracture–compression screws implanted for the surgical correction of *hallux valgus* (bunions) in patients at up to three years after surgery. The very sensitive electrochemical H_2 sensor is being evaluated on a selection of patients in Europe at different times after surgery. The measurements are done noninvasively by touching the sensor tip to the skin of the patient above the implanted screw. Results confirm the ability of the sensor to detect H_2 emanating from the biodegrading Mg screw and permeating through the skin.

***Rabbit bone fracture study detects H_2 in bone marrow* (Sfeir).** H_2 in bone marrow from a biodegrading Mg implant has been measured for the first time in a rabbit bone fracture study performed with Dr. Sfeir. The electrochemical H_2 sensor was used for measuring H_2 transdermally to monitor biodegradation of an implanted plate and and invasively inside the bone marrow to monitor the screw thread biodegradation. The H_2 needle sensor inserted through the measurement hole in the bone was able to easily detect H_2 in bone marrow where the H_2 level was found to be very high (exceeding a saturated water solution), suggesting that H_2 generated in situ was trapped in bone marrow. A manuscript is under review at *Acta Biomaterialia*.



Develop visual H_2 sensors (Dong, Witte). A novel H_2 sensor based on color or luminescence change of a membrane when exposed to H_2 with substantially improved sensitivity and response time is being developed. The ideal sensor will be rugged, easy to use and will allow rapid visual assessment of H_2 levels *in vivo*. An H_2 sensitive colorimetric system has been incorporated into rugged polymer films. Protection of the IP is being aggressively pursued and a provisional patent has been applied for.



Calibration procedure for H_2 sensors. A novel method for calibrating H_2 sensors eliminates the need for a compressed gas cylinder for H_2. Commercially available Aqua H_2 tablets are used to prepare calibration solutions. This is a major safety consideration in the use of H_2 sensors that require routine calibration!

Project Title: In Vivo Biocompatibility and Toxicity Assessment of Mg/Mg Alloys and Coatings
Principal Investigators: Zhongyun Dong
Thrust Area: ESIII-3: Responsive Biosensors and Neural Applications
Faculty Participants: Yeoheung Yun (NCAT), Prashant Kumta (Pitt), Zhigang Xu (NCAT), Vesselin Shanov (UC), William Heineman (UC), Mark Schulz (UC)
Student and Post-doc Fellow Participants: Dr. Shan Lu and Dr. Daoli Zhao (UC)
Industry Participants (if applicable): N/A.

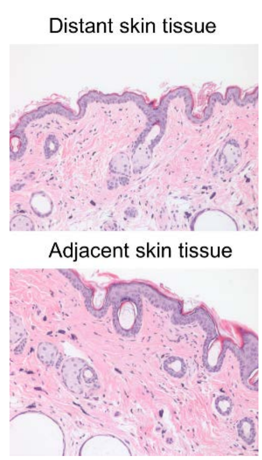
A. Project Overview

Our projects in ERC are to determine corrosion and biocompatibility of novel ERC Mg alloys and single crystal Mg in mice and to serve as an animal core for testing biosensors developed by investigators across ERC. Corrosion of Mg alloy and single crystal implants in the subcutis can be easily accessed, monitored, and measured. Hydrogen gas generated from a subcutaneously implanted pellet will form a bubble under the skin, which can be easily observed and measured with calipers. Degradation and toxicity are monitored and determined as described below. In combination with data from orthotopic models, information from our studies is invaluable for characterization of the novel materials and fundamental for the development and modification of novel revolutionary Mg alloys.

B. Achievements

We have established the subcutaneous implant model and evaluated samples of various Mg alloys and single crystal Mg in the model. The implants can be monitored in live animals over a long period time using an X-ray machine. The corrosion rates of alloy implants correlate directly with the volumes of gas bubble and densities of X-ray images. Upon sacrificing the mice, the extracted implants have been sent to other investigators of the ERC for in vitro analyses, including surface depositions and strength. Host responses to the materials are determined by examining in the hematoxylin and eosin (H/E)-stained sections of the tissue adjacent to the implants. Potential toxicity of the corroded materials to major organs (heart, lung, brain, liver, kidney, and spleen) is evaluated in H/E-stained tissue sections. Blood samples were collected for measurement of the blood urea nitrogen (BUN), the alanine aminotransferase (ALT), and aspartate aminotransferase (AST) of animals carrying the implants, which indicates potential toxicity of the corroded implants to liver and kidney. The expression of inflammatory cytokine genes, including interleukin-1 alpha (IL-1a), tumor necrosis factor-alpha (TNF□), and murine IL-8 homologous KC (mKC), as well as the inducible nitric oxide synthase (iNOS) were quantitatively determined by using reverse-transcription polymerase chain reaction (RT-PCR). deposit and accumulation of Mg in specific organs was measured using the inductively coupled plasma mass spectrometry (ICP-MS). Some data from these studies been published in multiple manuscripts.

The accomplishments in year 10:



B.1. Materials tested in year 10: Carbon nanowire, provided by Dr. Schultz, was tested in mice.

B.2. Methods: The immune competent SKH1-elite mice were anesthetized for surgical implantation. Two small incisions were created on two sides of mouse back and inserted with a carbon nanowire. The two ends of the wire were kept outside of the skin and stabilized with wound clips. The experiment was terminated two weeks later to recover the wire and the skin adjacent and distant site was sampled for histology staining.

B.3. Results: (a) The carbon nanowire did not cause significant discomfort or toxicity to the mice, based on body weight evaluation. (b) It appeared that the carbon nanowire did not cause significant pathological alterations to the adjacent tissue (Fig. 1). Moreover, there was no apparent degradation of the material during the experiment period.

B.3. Conclusion: Carbon nanowire could be a safe material for use as wire with a biosensor or other biomedical applications.

Project Title: Magnesium Based Materials for Implant Applications.
Principal Investigators: Vesselin Shanov
Thrust Area: ES3: Responsive Biosensors and Neural Applications.
Faculty Participants: Drs. Schulz (UC), Heineman (UC), Dong (UC), Pixley (UC), Kumta (U. Pitt), Wagner (U. Pitt), Yarmolenko (NCAT), Yun (NCAT), Xu (NCAT), and Roy-Chaudhury (UA)
Student and Post-doc Fellow Participants: Guangqi Zhang-PhD student, Pavan Kumar-MS student, two undergraduate student from the UC Biology Department-Sydney Hoffman and Jacob Sekinger and Dr. Pravahan Salunke
Industry: Inovasc LLC commercializing Mg stent for AVF
Project Overview: The project goals are to grow and characterize Mg alloy single crystals for device applications, based on alloys from NCAT and from U. Pitt, and to create and test multiple stents with different design & materials.

ACHIEVEMENTS IN YEAR 10:

1. Sustainability of the Mg single crystal research: During Year 10, the single crystal growth of Mg based alloys from both the ERC family's alloy systems (Dr. Xu and Dr. Kumta) and commercially available Mg alloys like WE43 has been a priority. A single crystal of 6.5 mm in diameter and 50 mm in length was grown from Alloy 59B, provided by Dr. Xu, as illustrated in Fig 1a. In addition, large Mg single crystals have been fully characterized. Thanks to the extraordinary ductility of pure Mg single crystal, fabricated from this material stent showed balloon expansion from 4 mm to 10 mm without any damage-Fig 1b. In-vitro study revealed better corrosion resistance of Mg single crystal stents compared to AZ31 stents.

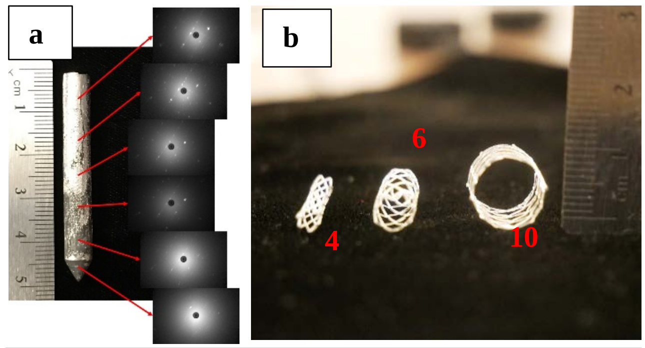


Fig 1. Laue patterns of the ERC's first Alloy 59B single crystal-a; Remarkable expansion of Mg single crystal stent from 4 mm to 6 mm and up to 10 mm-b.

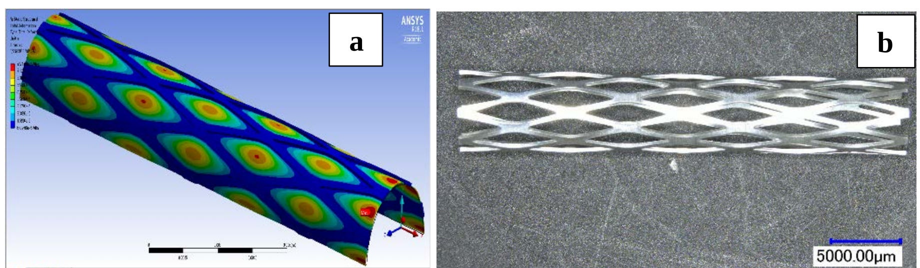


Fig 2. Simulation of rhombus design stent to replicated the expansion using balloon catheter-a; Fabricated rhombus design stent from AZ 31 foil-b.

Publications: 1. P. Salunke, S. Yarmolenko, V. Shanov, et al, "*Mechanical and Microstructural Characterization of Magnesium Single Crystals*", J. Materials Research, 32, 22, pp. 4196-4205, (2017).

2. Continuous improvement of the stent design through simulation and materials selection: A new robust design with rhombus patterns was created for AZ31 stents. Rhombus design stents has great crimpabilty and uniform balloon expansion when applying water pressure up to 26 atm. -Fig 2a and b. Mg stents with rhombus design are in a process of in vivo testing for AVF application at the U. Arizona by Dr. Roy-Chaudhury. Multiple heart valve stents and meshes have been also manufactured by photo-chemical etching and tested by Dr. Wagner`s group. Photo-chemical etching was employed for fabricating different designs of Zn stents and to test them in vitro-Fig

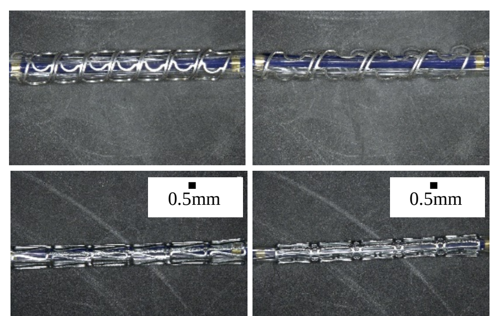


Fig. 3. Zn stents with different design fabricated by photo-chemical etching. (a) Helical stent made of 3mm wide ribbon; (b) Helical stent made of 5mm wide ribbon; (c) Cylindrical stent with Ω design-crimped; (d) Cylindrical stent with U design-crimped.

3. In some cases, conformal parylene coating was applied by vapor deposition, which enhanced greatly the corrosion resistance of both Mg and Zn stents.

Publications and IPs: 1. Y. Chen, V. Shanov, W. Wagner, et al, "*Hybrid Scaffolds of Mg Alloy Mesh Reinforced Polymer/Extracellular Matrix Composite for Critical-Sized Calvarial Defect Reconstruction*", Tissue Engineering and Regenerative Medicine, in review, 9/22, (2017); 2. W. Wagner, V. Shanov, et al, "*Mg Alloy Mesh Reinforced Polymer/ECM Hybrid Scaffolds for Critical-Sized Bone Defect Regeneration*", U.S. Provisional Patent 62/481,206, filed 4/4, (2017); 3. A. D'Amore, V. Shanov, W. Wagner, U. Pitt Invention Disclosure 04492, 01/23/2018, "*Biodegradable Metallic Stent for Heart Valve Tissue Engineering*".