#### 2018-2019 ERC-RMB Research Highlights

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), Christopher Hale (PhD)
Industrial participants: Luminal Solutions, Fort Wayne Metals

**Project Overview:** In last year, our research was focused on processing Mg alloys with differential speed rolling (DSR) - a newly established materials processing technology at NCA&T. Mg-6Al was selected as the model alloy. The emphasis was to compare the advantage of DSR over conventional rolling (CR). The alloy was first solutionized at 695 K for 18 h. Then, multiple plates were cut from the ingot and rolled with rolling speed ratio of 1 and 1.3 at temperature of 540 K with thickness reduction of 0.38 and 0.76 mm per pass and a total thickness deduction of  $\sim$ 50%.

Figure 1 shows electron backscattering diffraction (EBSD) inversed pole figure (IPF) maps observed in normal direction (ND) of the alloy plates processed with different conditions. The grain size in DSR processed sample is slightly smaller than that processed with CR. About 10° tilt of the (0001) pole towards the rolling direction (RD) in DSR processed sample can be seen, while there is almost no tilt in the CR processed sample.

Both DSR and CR processes increased the UTS and elongation by nearly two times as compared to the solution treated samples. From figure 2, it is observed that DSR process reduced in-plane ductility anisotropy, with increased ductility in RD and TD directions in comparison to CR.



Figure 1. a) and c) show the EBSD IPF map and the pole figure of Mg-6Al processed in a speed ratio of 1 (conventional rolling); b) and d) show the EBSD IPF map and the pole figure of the same alloy rolled at a speed ratio 1.3 (differential speed rolling).

#### Year 11 Accomplishments: *Differential speed rolling:*

The equipment is successfully used to process Mg alloys and shows promising results in grain refinement, improve the in-plane plastic isotropy, which is very important to improve the forming ability of Mg alloys. *Electron Backscattering Diffraction:* 

The application of this advanced microstructure characterization method greatly advanced the study of the rolling processed alloy samples. It enables is to obtain detailed information, including orientation of each individual grain, misorientation of grain boundaries, twins, internal strain, and so on, in the processed samples, which makes the adjustment and optimization of the processing conditions for the best performance of Mg alloys possible.

#### Other progresses:

More Mg-based sternotomy wires has been fabricated in collaboration with Fort Wayne Metals. The wires were annealed in two different temperatures (400 and 450 °C). Their tensile properties are shown in Figure 3. Wires in as-drawn condition showed very brittle feature, through their strength are high. After annealing, both their UTS and YS were reduced, but their ductility were significantly improved. Further studies of these wires were focused on corrosion property. Luminal Solutions is also seeking a specialized company to coat the wires so to make them closer to *in vivo* studies.



Figure 2. Mechanical properties of the rolled samples in tested in three different orientations. Thickness reduction for condition 1 and 2 are 0.38 and 0.76 mm per pass, respectively.



Figure 3. Tensile properties of the sternotomy wires in different processing conditions.

**Project Title:** Endothelial cell attachment on surfaces of biodegradable polymer-coated magnesium alloys in a microfluidic environment

Project leader: Yeoheung Yun, North Carolina A&T State University

Thrust Area: ES-1 Craniofacial and Orthopedic Applications (Materials Development and Processing)

Faculty Participants: Sangho Ye, Yeoheung Yun, William Wager,

Students: Lumei Lui, Teal Russell, Travor Surratt, D'Kaila Price

**Project Overview:** Polymeric coatings can provide temporary stability to bioresorbable metallic stents at the initial stage of deployment by alleviating rapid degradation and providing better interaction with surrounding vasculature. To understand this interfacing biocompatibility, this study explored the endothelial-cytocompatibility of polymer-coated magnesium (Mg) alloys under static and dynamic conditions compared to that of non-coated Mg alloy surfaces. Poly (carbonate urethane) urea (PCUU) and poly (lactic-co-glycolic acid) (PLGA) were coated on Mg alloys (WE43, AZ31, ZWEKL, ZWEKC) and 316L stainless steel (316L SS, control sample), which were embedded into a microfluidic device to simulate a vascular environment with dynamic flow. The results from attachment and viability tests showed that more cells were attached on the polymer-coated Mg alloys than on non-coated Mg alloys in both static and dynamic conditions. In particular, the attachment and viability on PCUU-coated surfaces were significantly higher than that of PLGA-coated surfaces of WE43 and ZWEKC in both static and dynamic conditions, and of AZ31 in dynamic conditions (P<0.05). The elementary distribution map showed that there were relatively higher Carbon weight percentages and lower Mg weight percentages on PCUU-coated alloys than PLGA-coated alloys. Various levels of pittings were observed underneath the polymer coatings, and the pittings were more severe on the surface of Mg alloys that corroded rapidly. Polymer coatings are recommended to be applied on Mg alloys with relatively low corrosion rates, or after pre-stabilizing the substrate. PCUU-coating has more selective potential to enhance the biocompatibility and mitigate the endothelium damage of Mg alloy stenting.

#### Year 11 Accomplishments:

Endothelial cell attachment: Here, endothelial cell attachment on Mg samples was tested to evaluate the compatibility of PCUU- and PLGA-coated alloys in both static and dynamic conditions. The viability of attached cells was assessed with live/dead cytotoxicity assays. The green color represents live cells, and the red color represents dead cells. Cells were more viable on the surface of both PCUU- and PLGA-coated alloys than non-coated alloys. The area of live and dead cells was calculated by ImageJ to analyze the viability (Figure 1). The live and dead cell areas (Figure 1) confirmed the observation that both PLGA and PCUU coatings can improve the viability of endothelial cells. In Figure 1, the viability represented by the live/dead area ratio in static condition is significantly higher than that in dynamic conditions. PCUU coating on the surfaces of 316 L SS, WE43, AZ31, and ZWEKC improved the endothelial cell live/dead ratio in both static and dynamic conditions compared with non-coated alloys (P<0.05). PLGA coating resulted in a higher endothelial cell live/dead ratio on the surfaces of WE43 and AZ31 in static condition, and on WE43 and ZWEKC in dynamic condition compared to non-coated alloys. The endothelial cell viability on both PCUU-coated and PLGA-coated surfaces of ZWEKL was not improved because the corrosion rate is the highest of all the tested alloys, which means a greater amount of hydrogen is released through the coating to repel the cells. The choice of base alloys to be coated is importance. Magnesium alloys with endocompatibility and re-endothelialization ability in vitro and in vivo such as Mg-Nd-Zn-Zr (JDBM) are potential choice. PCUU improved live/dead ratios significantly compared with the PLGA coated surfaces of WE43 and ZWEKC in both static and dynamic conditions, and AZ31 in dynamic condition (P<0.05). PCUU and PLGA improved initial endothelial cell adhesion and PCUU improved endothelial cell viability more than PLGA.



**Figure 1. Endothelial cell viability.** The green (living cells) and red (dead cells) areas were analyzed by ImageJ software. The quantified bar chart shows areas of living and dead endothelial cells on Non-coated, PCUU coated and PLGA coated surfaces of 316L SS, WE43, AZ31, ZWEKL and ZWEKC at static and dynamic conditions. \* represents the significant difference between groups of live/dead ratio.

Project Title: Embedding magnesium particulates into polymer biomaterials

Project leader: Narayan Bhattarai, North Carolina A&T State University

Thrust Area: ES3: Responsive Biosensors and Neural Applications

Faculty Participants: ES3: Sarah Pixley (UC), Jagannathan Sankar (NCAT)

**Students and Postdoc:** Udhab Adhikari (PhD-Student, NCAT), Shalil Khanal (PhD-Student, NCAT), Shekh Saudi (PhD-Student NCAT), Sara Tatum (PhD-Student NCAT), Kalene Johnson (MS-Student NCAT), Christian Chavis (MS-Student NCAT), Oreoluwa Alonge (MS-Student NCAT), Alessia Steward (BS-Student NCAT), Xiaoxian An (PhD-Student UC), Svitlana Fialkova (Postdoc-NCAT)

#### Industrial participants: None

#### **Project Overview and Highlights:**

The main objective of this project is to develop magnesium-polymer composite platform technology that has applications in regenerative medicine applications such as development of a tissue-engineered nerve conduit device to promote nerve regeneration and anti-inflammatory nanofiber membrane for wound healing. The developed composite platform consists of Mg contained biodegradable polymer microparticles, hydrogels, nanofiber membranes and nerve guides.



Figure 1: Left: SEM images of metallic magnesium particles used (a), schematic illustration showing the fabrication of PCL-Mg composite nanofibers (b), nerve guide conduits of b (c). Middle: SEM image of PCL nanofibers containing Mg in nanofiber meshes (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 EDS analysis (B). After 14 days, the cumulative amount of Mg<sup>2+</sup> 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 (C) corresponds to 99.56 %, 94.058%, 51.87% and 40.78% of total Mg present each nanofiber mesh type (D). Hydrogen release was significant, and fabrics were not cytotoxic in vitro (not shown). Education module developed by Bhattarai Lab presented in NC Science Festival activity at NC A&T State University with middle to high school students from Guildford county public schools (E).

In last year, we continued a project to develop Mg-based nanofiber mesh in collaboration with Dr. Pixley's lab at UC (Figure 1). We confirmed that the biodegradable metal, Mg, safely biodegrades in the body, releasing beneficial byproducts. To improve tissue delivery, magnesium metal particles were incorporated into electrospun nanofiber meshes composed of a biodegradable, biocompatible polymer, polycaprolactone (PCL). Magnesium addition, at several concentrations, did not alter PCL chemistry, but did alter physical properties. Under cell culture conditions, meshes released magnesium ions and hydrogen gas and were not cytotoxic for two cell types. After implantation in mice, the mesh with magnesium resulted in earlier appearance of M2-like, reparative macrophages and improved tissue healing versus mesh alone. This is in agreement with other studies showing beneficial effects of magnesium metal and provides a new type of scaffold material that will be useful in clinically relevant tissue engineering applications.

This year our focus of the research seeks to continue the development of electrospun nanofiber meshes of

biocompatible polymers containing embedded Mg metal particles of various sizes, dimensions and amounts, with the ultimate goal of using these for broad applications, from bone to nerve repair. Electrospun nanofiber meshes will be composed of polycaprolactone (PCL) or PCL mixed with other biodegradable polymer, containing embedded Mg metal particles. Uniform size of Mg partiles will be synthesized by using laser ablation technique (see our preliminary results in Fiure 2). As assays for



Figure 2: STEM image (A) and TEM image of as synthesized Mg particles by using laser ablation technique, and composite nanofiber of PCL/ Mg particles (C).

release of Mg degradation products, we will not only use standard analytical techniques, we will test cell exposure to meshes in culture and tissue responses to implantation in vivo. The latter is necessary because it is widely understood that Mg degradation in vivo differs drastically from that in vitro.

Project Title: Biofunctional coatings from WPC
Project leader: Svitlana Fialkova, North Carolina A&T State University
Thrust Area: ES-1 Craniofacial and Orthopedic Applications (Materials Development and Processing)
Faculty Participants: Jag Sankar (NCAT)
Students: NA
Industrial participants: DentsplySirona Endodontics

**Project Overview and Accomplishments:** We continue the collaboration with the DentsplySirona in development and improvement of WPC-gutta-percha obturators for the root canal treatments. We produce a several butches of WPC coated gutta-percha points for biological and animal trials. We also assist the company to troubleshoot several issues with the quality of stored gutta-percha points. We assist and train the research technician, Sheridan Rose at our facility. Sheridan was introduced to the PLD process, been trained and used SEM/EDS, Raman microscopy and FTIR techniques for several samples/products she brought with her. The future projects that are currently under discussion/proposal stage include: evaluation of different radiopacifiers on biological performance of WPC sealer, and benchmarking filling effectiveness of different endodontic sealers.

We also provided assistance for local start-up company "Shefabone Inc.", conducting the analysis of bioactive silica-calcium phosphate composite (SCPC\_ powders. The crystallographic phase content of SCPC powders was evaluated by X-ray diffractometry. The size of the powder particles and pore distribution inside the particles, and elemental composition were determined with the high-resolution SEM/EDS system. **Project Title:** Localized corrosion studies: Evaluation of interface properties and corrosion processes **Project leader:** Svitlana Fialkova, North Carolina A&T State University

**Thrust Area:** ES-1 Craniofacial and Orthopedic Applications (Materials Development and Processing) **Faculty Participants**: Zhigang Xu, 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 and Accomplishments: In a past few years, Dr. Xu group in collaboration with Luminal Solutions started the development of Mg-based sternotomy wires. The several wires prototypes from pure magnesium and newly developed Mg-alloys (ZXK, ZXKWE, etc.) were produced through cold drawing process. The process also includes the post-draw heat treatment of wire to achieve the enhanced ductility, lower vield strength and higher strain-hardening rate. This feature is particularly valuable, which provides wires with ease in twisting and strength for tightening during median sternotomy. The part of the project is the establishing the method for evaluation of corrosion resistance of the produced and subjected to optimal heat treatment sternotomy wires. The research achievements are summarized as follows: we establish the procedure of corrosion evaluation by two methods: hydrogen evolution, weight and optical methods. We use dynamic conditions, Hank's BSS at 37°C were used as immersion media, and the immersion time was 28 days. Using eudiometer data (hydrogen evolution method) we observed and determined several stages of the complex corrosion process: a) the formation of protective oxide layer in a first 15-30 minutes, b) that slows down the degradation process in a first 4-8 hours, c) then formation of pits and degradation acceleration that last up to 5 days and finally d) slowing down of degradation process when the balance between Mg dissolution and corrosion product precipitation was reached. Both prototypes (wires from ZXK and ZXKWE) shown superior corrosion resistance compared to the pure Mg wire, or wire drawn from AZ31. The corrosion rate of ZXK wires was in range 0.3- 0.8 mm/year comparing to 3.4 mm/year for pure magnesium wire. We also determined that heat treatment reduced the corrosion resistance, so the future efforts will be focused on finding the optimal heat treatment conditions to satisfy both mechanical performance and chemical stability of the sternotomy wires.

Project Title: Corrosion characterization for biodegradable magnesium medical devices
Project leader: Yeoheung Yun, North Carolina A&T State University
Thrust Area: ES-3 Responsive Biosensors and Neural Applications
Faculty Participants: Yeoheung Yun (NCAT), Boyce Collins (NCAT)
Students: Dustin Trujillo
Industrial participants:

**Project Overview:** In last year, Dr. Collins and his student continued to support Mg alloy innovation associates with many of the projects by providing X-ray CT images and analysis for new materials and processes. This has included characterization of Casting quality leading to a refined mold desing and the quantitative characterization of precipitates and voids in materials.

**Project Title:** US-Ireland R&D Partnership Program: Centre to Centre Proposal - Bioresorbable Magnesium Alloy Systems for the Promotion of Regenerative Biological Function in Orthopedic Implant Devices

Project leader: Jagannathan Sankar, North Carolina A&T State University

Thrust Area: ES-1 Craniofacial and Orthopedic Applications

Faculty Participants: S. Pixley (UC), V. Shanov (UC); P. Kumta (U Pitt), P. Lemoine, P.(NIBEC);

McGarry, P. (CURAM) Z. Xu (NCAT), S. Yarmolenko (NCAT); B. Meenan (Ulster)

Industrial participants: OrthoKinetics, Fort Wayne Metals

**Project Overview:** Our team shares the goal of developing bioresorbable magnesium alloy systems for clinically-capable orthopedic implant devices. The regulatory landscape, especially for biomedical materials and devices, is highly dependent on intercountry efforts. This tripartite partnership via the C2C program has created a unique convergence of world-leading expertise from academia and industry in the fields of materials processing (ERC-RMB, Fort Wayne Metals), surface modification (ERC-RMB, NIBEC), characterization (ERC-RMB, NIBEC), computational modeling (NUI Galway), and regulatory issues (Ft Wayne Metals, Orthokinetics, C2C universities). Our groups have, with the input of clinician team members, chosen applications ranging from thin wires (for clinical use as "k wires") to thicker pins, rods and elastic stable intramedullary nails (IMs or ESINs) and meshes for the treatment of complex bone fractures.

**Materials and Data Exchange**: One of the many outcomes of the matured ERC-RMB program is the ability to tailor magnesium (Mg) materials and characterize the resulting materials at the atomic and microscale and relate these characteristics to bulk mechanical properties. Unique Mg materials including polycrystalline Mg alloys (Dr Xu, NCAT; Dr Kumta, Pittsburgh), and monocrystalline pure Mg (Dr Shanov, Cincinnati) have been shared with NIBEC for coating studies and analysis. Related microstructural, fatigue, and corrosion data have been sent to NIU Galway for Finite Element Analysis of these materials in application relevant scenarios, developed in conjunction with our clinical partners. Inputs from our C2C partners have helped modify the subsequent rounds of materials produced. NCAT (Dr Yarmolenko) has prepared a living document materials data base distributed to both academic and industrial members. This C2C partnership has demonstrated the efficacy of Mg materials as a K-wire material and resulted in the development of a monocrystalline Mg alloy driven by the demands and needs of the EISN application (see below).

Center Visits and Meetings: Modern industry is multi-national and so should be the scientific teams and the training of our current and future researchers. An essential component of the collaboration is the efficient exchange of ideas that allows student researchers to operate and succeed in an intercontinental arena. We can communicate person-to-person on the different challenges associated with each country's academic, industrial, and regulatory challenges. We have leveraged this opportunity via student and faculty transatlantic visits and cross-institution webinars and person-to-person communication. A highlight and catalyst of this program has been the physical presence of researchers at one another's' universities: Ireland to US, June 17-27, 2018: One graduate student and 3 postdocs from CÚRAM and NIBEC visited all three campuses of the ERC-RMB. They gave and heard presentations, toured the facilities and spent extensive time in collaborative talks. US to Ireland, August 31-September 2, 2018:. Drs. Pixley and Little (Cincinnati) visited NIBEC at Ulster University, for collaborative talks and tours of the facilities. Our C2C experience has allowed us to contribute to the international community through participation in the NSF sponsored C2C Collaboration Workshop, August 14-15, UCLA, Los Angeles. (Drs. Pixley (Cincinnati) and Collins (NCAT)) and the 10<sup>th</sup> Annual Biodegradable Metals for Biomedical Implants, Oxford University, Oxford, England, August 26-31, Several C2C members presented and met for discussions: Keynote talks: Dr. Kumta (Pittsburgh), Adam Griebel (Ft. Wayne Metals), Dr Pixley (Cincinnati); Presentations: Dr Shanov (Cincinnati), Dr Little (clinician, Cincinnati), Dr Acheson (NIBEC)

**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-06.3 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 11 accomplishments: The broad goal of this project is to design and develop patient specific, customizable and complex 3D anatomical structures from biodegradable metals and biodegradable metalpolymer composites. These structures by design contain hierarchical pores mimicking the macroscopic and internal microstructure of various and specific organs and tissues while also providing temporary mechanical structural functions and mass transport properties using various 3D printing techniques. The flexibility in printing these parts from CAD/CAM data also addresses the overarching goal of the ERC-RMB in developing prototyping devices without the need for developing specific tooling as typically 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 constructs fabricated by binder jetting and selective laser melting additive manufacturing techniques 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 including the generation of a goat mandible using computerized tomography images. 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 also 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 and layer height strongly influence the various geometrical dimensions of the printed scaffolds. The results demonstrated that when printing at higher temperatures, 160 °C to 180 °C, the smallest achievable layer height was 0.25 mm, whereas printing at 130 °C allows achievable layer heights of 0.10 mm. Additionally, smaller layer heights counteracted the decrease in mechanical properties associated with decreased printing temperatures, resulting in no significant change in mechanical properties. In-vitro degradation testing of the 3D printed Mg-PCL constructs proved that smaller layer heights resulted in a flatter morphology allowing for a more homogenous distribution of precipitates at various time points. We are also developing diffusion bonding (DB) 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 having minimal compromise of the desired material and structural attributes. In this process, micron to millimeter sized thick Mg alloy plates are pressed inside a hot press applying a precise pairing of pressures and temperatures for a specified amount of time. This process allows diffusion to bond the sheets into a singular structure retaining any initially created sheet geometries. Our initial feasibility studies provided evidence that complete joining of two or more Mg plates is not only possible, but the nature and strength of the bonded layers depends on the intrinsic physicochemical nature and surface finish of the alloy sheets, including the time, temperature, pressure, and surrounding atmosphere of the DB process. Results of these studies offer insights into the mechanisms of the bonding process providing a path to remove the oxide layer during the process, thus increasing the shared contact area of the structure correlating to the strength of bond. Research is currently ongoing to understand the processing-property-performance relationships of DB systems of Mg alloys.

**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: 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 – Matthew Criado (PhD candidate)
Industry Participants: Dr. Howard Kuhn, ExOne Company, LLC.

Year 11 accomplishments: The goal of this project was to process and manufacture near net shaped biocompatible and biodegradable novel patent pending and proprietary magnesium (Mg) 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<sub>6</sub> + 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 alloys. 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. The in-vivo corrosion rates of these alloys were tested in rat gluteal muscle over a period of 4, 8, 16 and 26 weeks and corrosion rates of both these alloys were observed to be higher than that of pure Mg which may be attributed to the presence of secondary phases in these alloys. The local and systemic toxicity of various alloying elements present in the alloys were also tested in the rat gluteal muscle and the results demonstrated absence of any detectable toxicity in various organs analyzed. Based on the above invitro and in-vivo results, some of these Mg-Y-Sr (WJ) and Mg-Zn-Sr (ZJ) containing alloys were processed in large scale (> 10 kg/batch) from a commercial vendor in China. The in-vitro degradation results on these commercially made alloys indicate improved corrosion resistance compared to laboratory scale manufactured alloys. In-vitro corrosion rate of the WJ11 alloy was found to be comparable or even better than common bio-medically used alloy WE43. The in-vivo local and systemic bio-toxicity, degradation, and bone regeneration efficacy of this alloy, WJ11, were tested in rat critical sized calvarial bone defect. The results demonstrated absence any hydrogen pocket formation as well as absence of any apparent local and systemic toxicities. In addition, the alloys appeared to be safe and comparable to clinically used nondegradable PEEK scaffolds. The fluoride coated WJ11 scaffolds begin to degrade with time and the amount of new bone formation appears to be comparable to PEEK. Iron based alloy films also showed good cytocompatibility. Furthermore, using the density functional theory (DFT) computational approaches several Zn-based alloys with cubic symmetry demonstrating improved ductility and ultimate tensile strengths have been identified theoretically. The roles of many biocompatible elements added to Zn-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: Evaluation of Magnesium-based alloys for airway stenting

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

Thrust Area: ES2: Cardiovascular and Thoracic Devices

Faculty Participants: Abhijit Roy, PhD, Oleg I. Velikokhatnyi, PhD.

Student and Postdoc: Matthew Criado (PhD candidate), Mubin Ali Aral (Postdoc).

Industry Participants (if applicable): Acell, Inc.

Year 11 accomplishments: In the past years, we have successfully manufactured Mg stent using our UHD (ultra-high ductility) Mg alloys. Prototype Mg stents were manufactured from extruded magnesium alloys rods. The design of the stents was determined prior to the fabrications based on the mechanical properties of the alloy (Young's modulus and Poisson's ratio). Stents of outer diameter, 4.2 mm, 300 um wall thickness and 10.0 mm length were manufactured using wire-EDM (EDM: Electrical discharge machining) followed by laser cutting. To achieve a better surface condition, all the stents were electrochemical polished. Initially, we implanted the balloon expandable stents made of our UHD Mg alloys into rabbit tracheal model. 316L stainless steel stent with the same dimension and design acted as the control group in this study. The stents were implanted for 4 weeks, 8 weeks and 12 weeks respectively, and 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, and totally degraded in the trachea at 4 weeks and 12 weeks' time point. Tracheobronchial endoscopy showed that the airway patency was maintained throughout the study for the Mg stent group. However, for the 316L stainless steel stent group, there was thick stenosis tissue formed around the stent after 4 weeks of implantation. At 8 weeks and 12 weeks, the airway stenosis continued to grow and narrowed the airway in 316L stainless steel stent group. On the other hand, in the Mg stent group, normal healthy trachea was observed after the stent degraded. Optical coherence tomography (OCT) imaging of the stented airway verified this observation. The lumen



size of the rabbit airway continued to grow for the Mg stent group, while the lumen size either remained the same for the 316L stainless steel stent group or stopped growing and even decreased due to the formation of airway stenosis (**Fig. 1**). Micro-CT analysis showed that about 35% of the Mg tracheal stents degraded after 4 weeks of implantation, and completely degraded at 4 weeks and 12 weeks. The stented airway session was harvested for histology analysis. The H&E staining showed encapsulation of stents at 4 weeks, and the CD68 staining showed less inflammatory response for the Mg stents compared to 316L stainless steel stents. In the past one year, we also developed a reliable and

reproducible rabbit laryngotracheal stenosis model. We designed a micro-suspension laryngoscopy device to augment subglottic injury with a nylon brush as well as endoscopic application of the balloon expandable Mg stent in the rabbit airway. Our initial results on one-week post-injury trachea indicated creation of grade 2-3 laryngotracheal stenosis. Studies are currently ongoing to understand the advantages of this novel biodegradable Mg stent as a treatment approach over normal balloon dilation for the treatment of laryngotracheal stenosis. Additionally, density functional theory (DFT) calculations of the mechanical properties of various Mg-based alloy compositions have been conducted in conjunction with corresponding stent manufacturing validating the influence of the alloying elements on the observed improved ductility. Project Title: C2C: Degradable Magnesium Based Kirschner Wires (K-wires) for Bone Fixation

**Principle Investigators**: Prashant N. Kumta, PhD (University of Pittsburgh)

**Faculty Participants:** Dr. Abhijit Roy, PhD, University of Pittsburgh; Dr. Oleg I. Velikokhatnyi, PhD, University of Pittsburgh; and Dr. Vijay Gorantla, MD, PhD, Wake Forest University.

**Student and Postdoctoral Fellow Participants:** John Ohodnicki (MS student), Mubin Ali Aral, MD (Post-doctoral associate)

**1. Goals**: Kirschner wires (K-wires) and other pins, wires, rods, and nails derived typically from metallic systems are commonly used as fixation systems for supporting fractures in orthopedic, reconstructive plastic, and pediatric surgery, because of their simplicity in handling and the application needs as desired based on the complexity and nature of the fracture. The overall goal of this project is to design and implement new and novel degradable magnesium (Mg) alloy K-wires and Elastic stable intramedullary nails (ESINs) for bone fracture fixation that exhibit the unique property to degrade after bone healing. The aims of this project are:

(i). Optimize the alloy compositions based on the mechanical and corrosion properties predicted by *ab-initio* calculations based on the density functional theory (DFT) approaches.

(ii). Improve the physicochemical properties of these alloy by further optimizing the alloy processing and post processing steps.

(iii). Assess the *in-vitro* biomechanical and degradation characteristics of the wire based devices (e.g. K-wires and pins).

(iv). Assess the *in-vitro* degradation and electrochemical corrosion characteristics of the pure and substituted hydroxyapatite (HA) coated substrates and fixation devices.

(v). Utilize the *in-vitro* results to further refine the DFT computational modeling as well as the Finite Element Analysis (FEA) modeling studies further for improving the alloy design and device optimization to identify the desired physicochemical and design parameters.

Aims	Achievements	% Task completed
Manufacture of biodegradable Mg K-wires and ESIN implants.	K-wires of various diameters and tip designs were fabricated and tested in cadaver porcine and rabbit bones. These tests showed that the Mg alloys based diamond tip K-wires can be used to drill both the compact and the spongy bones of the porcine and rabbit cadaver bones. These results were shared with the Ireland groups.	50
Pre-clinical testing of Mg K- wires and ESIN implants.	The in-vivo biocompatibility of the two ERC alloys were tested in rat gluteal muscle. No observable toxicity was observed. Pilot study on K-wires was also performed in rabbit ulna fracture model. K-wires showed improved bone growth and complete union of the bone on fracture sites.	50
Identification of optimal alloy processing parameters for orthopedic applications.	Two optimally processed and patented ERC alloys were tested in-vitro and in-vivo. For the K-wire design, we have particularly focused on melting	70

### 2. Achievements in Previous Years:

	casting of various Y and Sr containing novel patent pending Mg alloys. Based on the encouraging in-vivo outcomes, we planned to make large scale melting and casting of two of these alloys. Large scale (>10 kg/batch) manufacturing of two patented alloys were performed using a commercial vendor in China. The large-scale processed alloys showed better in-vitro corrosion resistance compared to small lab scale made alloys.	
Analysis of elastic properties and biodegradation rates for a range of alloy compositions.	In-vivo and in-vitro biodegradations were tested for small-scale lab processed alloys. In-vitro and electrochemical corrosion measurements were performed on the large-scale manufactured alloys. Moreover, corrosions of the fluoride coated devices were also tested. In-vivo corrosion rate and biocompatibility of one of the large-scale commercially processed alloy was tested in a rat calvarial critical sized bone defect model.	40
Characterization of Mg alloy mechanical and corrosion behavior	In-vivo and in-vitro corrosion characteristics were tested. Tensile properties of the small scale lab- processed alloys were measured. Evaluation of the mechanical properties of the commercially processed alloys are currently ongoing.	60
Characterization of Mg implant degradation in a bioreactor.	The bioreactor has been procured and installed. The degradation of the alloys will be tested in future.	20
Optimized pure and substituted hydroxyapatite (HA) coating for orthopedic applications.	ure and substituted te (HA) coating for pplications. Test samples of a proprietary alloy were supplied the coating team at Ireland for pilot study. Pure H and strontium substituted HA coatings of vario thickness were deposited on these substrates usin sputtering deposition techniques. The static in-vit corrosion and electrochemical corrosion studies of these alloys are currently ongoing. Cytotoxicity these coated samples will be also tested.	
Ab-initio and semi-empirical computational study.	Using DFT computational methods several Zn-based alloys with cubic symmetry have been identified for improving the ductility and ultimate tensile strength. Also, roles of different biocompatible elements in improving of the mechanical properties have been established.	30

**Project Title:** Functionalized Organic-Inorganic Coatings on 3-D Magnesium Based Scaffolds-Biocompatible Ceramic Coatings

**Principle Investigators**: Professor Prashant N. Kumta, PhD, Bioengineering, Mechanical Engineering and Materials Science, Chemical and Petroleum Engineering, University of Pittsburgh

Thrust Area: ES-I: Craniofacial and Orthopedic Applications

**Faculty Participants:** Dr. Abhijit Roy (Pitt), Dr. Oleg I. Velikokhatnyi (Pitt) and Dr. Zhongyun Dong,(UC) **Student and Postdoc Fellow Participants:** Dr. Sangeetha Kunjukunju, PhD and John Ohodnicki, BS **Industry Participants:** Dr. Howard Kuhn, Ex-One Industries, PA.

Year 11 accomplishments: The kinetics and quality of bone tissue response are dictated not only by the intrinsic properties of the implanted biomaterials but also largely by the implant surface chemistry. Therefore, tailoring of the biomaterial surfaces with a suitable inorganic (e.g. pure and doped hydroxyapatite, calcium or magnesium silicates and fluorides) and organic (e.g. proteins, enzymes, peptides) coatings are of immense importance mainly to increase the biocompatibility of novel degradable implants and existing degradable polymeric as well as non-degradable metallic implant materials. This is mainly possible by achieving a complex combination of controlled degradation, mechanical, antibacterial, and osteo-conductive properties. The broad purpose of this project is the tailoring of degradable biomaterials surfaces with suitable pre-treatment methods (e.g. Micro-arc-oxidation (MAO), fluoride treatment, etc.), inorganic (e.g. pure and doped calcium phosphates, calcium silicates, and bioactive glasses) and organic coatings to increase the corrosion resistance and biocompatibility of novel degradable magnesium and magnesium alloy implants. The uncontrolled corrosion of magnesium and magnesium alloys and the limited osteo-conductive as well as absence of osteo-inductive characteristics of these materials demand the need to develop functional coatings. Therefore, the goal of this project is to develop biocompatible and bio-functionalized inorganic coatings that can control the corrosion rates of novel patent pending magnesium and magnesium alloy based scaffolds while also promoting rapid bone regeneration. Various coating approaches have been studied to form calcium phosphate (CaP) coatings on magnesium alloys, however, only a few of these techniques can be used to coat complex geometries for various medical devices such as orthopedic and dental fixation devices (screws and plates) and porous scaffolds. In addition, the relatively low melting point of Mg based alloys and its reactive nature further limits the application of coating processes requiring subsequent high temperature treatments, which are routinely used to deposit CaP coatings on non-degradable alloys such as titanium and cobalt-chrome based alloys. As a result, we have chosen to study an aqueous biomimetic approach and non-aqueous sol-gel based approaches to deposit CaP coatings on Mg alloys. These techniques all require the immersion of substrates into solutions, enabling the coating of complex structures and porous scaffolds. Also of interest is to use these techniques to study the influence of doping with elements such as strontium and silicon, which are critical in bone regeneration and are yet to be studied in detail for Mg based alloy substrates. We have also studied the influence of micro-arc oxidation (MAO) and fluoride pre-treatments on the corrosion characteristics of the various ERC developed alloys. Furthermore, we have explored the possibility of release of antimicrobial drugs such as vancomycin and model protein bovine serum albumin (BSA) from the inorganic-organic composite coated surfaces of magnesium substrates. One of the ERC developed Mg alloy based K-wires was coated with MgF<sub>2</sub> and was tested in a rabbit ulna fracture model. The results show that the fluoride coated pins promotes fracture healing providing complete union of the bone. Over the past 12 months, we have coated a proprietary ERC alloys using fluoride treatment and the coated scaffolds were tested in rat critical sized calvarial bone defect model. The results show that the fluoride coated calvarial scaffold promotes bone formation without the formation of any hydrogen bubble. Moreover, the scaffolds appear to degrade slowly over time without causing any local and systemic toxicities. We are also performing in-vitro corrosion and cytotoxicity testing on the sputter coated pure and doped hydroxyapatite coatings of the ERC developed alloys. In addition, as a continuation of the last years research, an *ab-initio* computational study has been conducted for identification of the most stable coatings on various novel patent pending Mg-alloys studied and developed within the ERC.

**Project Title:** Sensor development and miniaturization: Developing of novel platforms for rapid biomarkers detection

**Principle Investigators**: Professor Prashant N. Kumta, PhD, Bioengineering, Mechanical Engineering and Materials Science, Chemical and Petroleum Engineering, University of Pittsburgh

Thrust Area: ES-3: Responsive Biosensors and Neural Devices

Faculty Participants: Dr. Abhijit Roy (Pitt) and Moni Kanchan Datta (Pitt)

Student and Postdoctoral Fellow Participants: Dr. Sangeetha Kunjukunju, PhD

Accomplishments: Cardiovascular diseases (CVDs) are the major cause of death worldwide, resulting in ~ 17.5 million deaths in 2012 corresponding to 31% of all global deaths. Neurodegenerative diseases (NDs) affect millions of people worldwide. Alzheimer's disease and Parkinson's disease are the most common types, with more than five million Americans living with Alzheimer's disease, and at least 500,000 Americans living with Parkinson's disease, although some estimates are much higher. Additionally, traumatic brain injury (TBI) is often a diagnostic and therapeutic challenge, particularly at the mild end of the injury spectrum. The goal of this project is to simplify biochemical marker testing for CVDs, NDs and TBI diagnosis by developing a portable biosensor specific to rapidly detecting cardiac, neurodegenerative diseases or TBI markers utilizing few drops of blood to provide readings of the biomarkers levels in blood within minutes. Current procedures for clinical evaluation of these diseases rely on testing of the biomarkers present in the whole blood or serum sample obtained from the patient. These methods are generally expensive, time-consuming requiring specialized instrumentation and trained personnel, or necessitate extensive processing and turnover time. Therefore, there is a need for an efficient and robust 'point-of-care' (POC) device enabling rapid screening, early detection and monitoring of the disease risks to decrease the deaths, and healthcare costs. In this project, we have developed vertically aligned platinum wire aptamer or antibody-based multi-array biosensors to accurately measure the specific levels of relevant CVD, NDs or TBI biomarkers in the blood/serum using electrochemical impedance spectroscopy (EIS). For detection of CVDs and NDs/TBI, we have chosen Brain Natriuretic Peptide (BNP) and Tau protein (Tau), respectively, as the disease specific biomarkers. Over the past 10 years, we have achieved the following goals: (a) Fabricated and optimized the parameters of the platinum wire electrodes. (b) Optimized the concentrations, incubation times, and self-assembled monolayer (SAM) parameter combinations including antigen incubation times to improve and enhance the biosensor precision, accuracy, selectivity and sensitivity. (c) Determined a single-frequency for biomarker detection, and tested the biosensor against clinically relevant samples using only few drops of whole blood/plasma/serum for CVDs biomarker detection. (d) Developed and optimize the two-electrode approach to eliminate any interference due to nonspecific adsorptions (or bio fouling) of signaling molecules and proteins on the sensor surface, especially in whole blood and plasma. In past one year the BNP biosensor response was recorded using known concentration of BNP added to a very low BNP containing human whole blood and serum to generate linear calibration curves. The range of BNP concentration used for this calibration curves was between 100 to 1200 pg/mL. Clinically derived unknown blinded serum and whole blood samples (WB) from patients were obtained to assess the sensor response utilizing the developed biosensor and the various concentrations of the BNP was determined using the calibration curve. The amount of BNP present in these twelve unknown serum samples were measured using the ELISA assay (clinical gold standard for the determination of BNP concentration). The results clearly demonstrated that BNP values obtained using the developed novel aptamer biosensor are very similar to the gold standard ELISA values. We also tested antibody based Tau biosensor and the results confirmed that the biosensor is highly sensitive at detecting even extremely low concentrations of the Tau protein, thus ensuring that even minimal fluctuations in Tau could be detected by the novel biosensor, which could be useful for monitoring the progression of brain injuries (especially in sports) or neurodegenerative diseases (especially Alzheimer's).

Project Title: Sensor Development and Miniaturization

Project leader: William R. Heineman (University of Cincinnati)

Thrust Area: ES-3: 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: J. Lynch (postdoc), M. Smith (PhD student)

**Project Overview:** The overall goal has been to develop sensors for measuring alloy biodegradation products *in vitro* and *in vivo* to provide an in-depth understanding of the biodegradation process for alloys developed by the ERC and the biological effects these products have on tissues and organs. Sensors and associated instrumentation were developed that allow real-time monitoring of the soluble corrosion products (OH<sup>-</sup>, Mg<sup>2+</sup>, H<sub>2</sub>). The electrochemical H<sub>2</sub> sensor has been especially useful in multiple *in vivo* studies in which H<sub>2</sub> was measured for the first time with a sensor: measuring H<sub>2</sub> levels in bubbles forming adjacent to Mg implanted subcutaneously in mice; monitoring biodegradation of a Mg implant in real time by non-invasive H<sub>2</sub> sensing; measuring H<sub>2</sub> in bone marrow from a biodegrading Mg implant in a rabbit bone fracture; and demonstrating the ability to measure H<sub>2</sub> through the skin of patients with Mg implanted for bunion surgery. With Element One we showed that a material changing color when exposed to H<sub>2</sub> can be used for non-invasive H<sub>2</sub> sensing. In a comprehensive study of Mg alloys from NCAT, UPitt and UC using the subcutaneous implant mouse model, biodegradation was monitored by measuring H<sub>2</sub> transdermally with the electrochemical H<sub>2</sub> microsensor, surface corrosion products by XPS, and alloy metal ion (Mg, Zn, Zr, Sr, Ca and Y) distribution in organs by ICP-MS

**Year 11 Accomplishments:** In year 11 our focus has been the development of a sensitive, fast visual  $H_2$  sensor "band-aid" that could easily monitor biodegradation rate in *in vivo* animal studies used to develop new alloys and devices and in routine post-surgery doctors' office checkups for biodegradation progress of implants in patients.

## 1. Discovery of various indicator dyes that undergo irreversible color changes in response to $H_2$ in the presence of metal nanoparticle catalysts.

Bubbling H<sub>2</sub> gas into buffered solutions (pH~7) containing certain indicator dyes and bimetallic gold-palladium nanoparticles (Au-Pd NPs) resulted in color changes that can be detected spectroscopically and visually (Fig.1).



Figure 1. UV-Vis spectra and photographs showing the color of solutions before and after exposure to  $H_2$ . All spectra and photographs correspond to buffered solutions (pH~7) containing Au-Pd NPs and an indicator dye. Dyes include resazurin (A), bromothymol blue (B), and methyl red (C).

Resazurin dye underwent an irreversible blue to pink transition when exposed to  $H_2$  followed by a reversible pink to a colorless transition (Fig.1A); bromothymol blue dye (Fig1B) underwent an irreversible blue to yellow transition; and methyl red dye (Fig.1C) underwent an irreversible yellow to colorless transition with a subsequent reversible colorless to red transition.

## 2. Incorporation of dye and nanoparticles into thin polymer matrices exhibiting color changes in response to $H_2$ gas, and the construction of a wearable $H_2$ sensing prototype

Visual color changes of resazurin dye molecules and Au-Pd NPs incorporated into thin Nafion polymer and Alginate/Agarose hydrogel matrices with subsequent exposure to H<sub>2</sub> were observed. A linear response to H<sub>2</sub> yielded a limit of detection and quantification for the film of 8  $\mu$ M and 27  $\mu$ M; respectively. When resazurin and Au-Pd NPs were loaded into a thin alginate/agarose hydrogel in a prototype band aid-like sensor, visual color changes in the hydrogel were observed upon exposure to H<sub>2</sub>, This achieved our goal for this year to demonstrate the concept of a band aid-like (wearable) H<sub>2</sub> sensor.

Project Title: Soft Tissue Injury Repair and Biosensors

Principal Investigator: Sarah Pixley (UC)

Thrust Area: ES3: Responsive Biosensors and Neural Applications.

**Faculty Participants:** V Shanov (UC), W Heineman (UC), Y Yun (NCAT), N Bhattarai (NCAT),

Z Xu (NCAT), P Kumta (Pitt), K Marra (Pitt), T Cui (Pitt).

**Clinical Advisory Board:** Tejas Sankar, Kevin Little (UC/Cincinnati Children's Hospital), David Hom (UCSD), Ryan Collar (UC)

**Technical Support:** T Hopkins (UC)

Industrial Partners: Ft. Wayne Metals (J Schaffer, T Hamilton & A Griebel)

## **Project Overview:**

Our ongoing efforts are focused at understanding the biological responses to biodegradable metals and advancing their use as biomedical implants. Projects include:

- 1) Nerve repair with Mg filaments placed inside hollow nerve guides (conduits), where the Mg filaments are designed to provide physical contact guidance and improve nerve regeneration.
- 2) Determine the cellular reactions to and anti-inflammatory effects of embedding Mg metal in another biomaterial for use in biomedical implants. We have observed that the presence of Mg metal improves tissue acceptance of the other biomaterial.
- 3) Determine the cellular reactions to zinc as an in vivo implant.
- 4) Associated project: Mg for pediatric bone nails, as part of the C2C collaborative project.

## Year 11 Accomplishments:

*Mg in electrospun meshes:* We published work with Dr. Narayan Bhattarai (NCAT) on embedding Mg metal particles in electrospun meshes and characterizing cellular reactions to the materials, in vitro and in vivo, using mice. This publication is part of a Special Issue of Acta Biomaterialia.

## Mg in Nerve Repair:

*Continued Projects:* In this year we have continued writing up experiments on nerve repair with Mg metal. Mg plus Growth Factors for Nerve Repair: We started a new project to improve nerve repair by combining a) a new type of hollow nerve conduit material, b) pure Mg filaments to provide physical contact guidance support and c) a nerve growth factor to enhance nerve outgrowth. The conduits were polysulfone from Koch Membrane Systems, Inc., the Mg filaments were pure Mg provided by Ft. Wayne Metals and the growth factor was ciliary neurotrophic factor (CNTF). The project received additional funding support via a UC grant from the UC Gardner Neuroscience Institute. We had five experimental groups (adult Lewis male rats, 5-7 per group, surgical model: repair a sciatic nerve gap of 10 mm): 1) empty conduits filled with phosphate buffered saline (PBS) at surgery, 2) empty conduits with CNTF in PBS injected into the conduits at surgery, 3) conduits with one pure Mg filament (250 micron d) and PBS, 4) conduits with Mg wire and CNTF and 5) conduits with Mg wire, CNTF and MgSO<sub>4</sub> in PBS. The animals were allowed to survive for 18 weeks, during which behavioral measures were collected. The animals have been sacrificed. Their regenerated nerves were removed and imaged by microCT with iodine as a soft tissue contrast agent. Regeneration was variable and groups were not statistically different. However, there was a trend for the wire + PBS and wire + CNTF to have higher behavioral return and better tissue growth. Samples are being processed for histological analysis.

**End-to-End Nerve Repair:** We started a collaboration with a new MD partner, Dr. Ryan Collar, a facial plastic surgeon in the UC Otolaryngology Dept. With him, we are exploring the effects of using a Mg filament within a direct end-to-end repair of a nerve, which is called a neurorrhaphy repair. This type of repair is one that Dr. Collar does on a routine basis in facial surgery. He re-routes an intact nerve to provide nerve fibers to a cut facial nerve, to enable a person to regain the ability to smile. We did a pilot study with rats where we put Mg filaments inside a direct cut and repair of the rats' sciatic nerves. In the regenerating tissues, we saw a phenomenon that we had previously observed when doing repairs of gaps in nerves. That is that the regenerating nerves appear to cluster around the Mg filament within the nerve tissues. This suggests that the Mg filament provide an "attractive force". This phenomenon is likel to lead to better nerve regeneration through the scar tissue that forms at the reconnection site. Dr. Pixley is currently using these

data and her previous data to write an NIH R01 to investigate the mechanisms underlying this attractive force.

*Surgical improvements to Nerve Repair:* In collaboration with Dr. Collar and a chemical engineer at the UC College of Engineering, the Pixley lab is developing ways to improve surgical repair of nerves, based on ideas from Dr. Collar. These ideas have been written up as Invention Disclosures.

*Zinc as a biomedical implant material:* In collaboration with Dr. Shanov, Dr. Pixley's lab is investigating the cellular reactions to Zn as an implant material and comparing them to cellular reactions to Mg metals.

## Year 11 Industrial Impact:

- We are continuing our active collaboration with Ft. Wayne metals. No major changes have occurred this year, except for receipt of materials from Ft. Wayne for testing.
- We are establishing a relationship, with Dr. Ryan Collar, with the company, Axogen, which makes nerve guides.

**Project Title:** Biodegradable Metallic Stent for the Arteriovenous Fistula and Smart Degradation Control **Project leader:** Mark Schulz, University of Cincinnati

# Thrust Area: ES-2 Cardiovascular and Thoracic Devices, and ES-3 Responsive Biosensors and Neural Applications

**Faculty Participants**: Mark Schulz, Zhongyun Dong, V. Shanov, Begona Campos (UC); W. Wagner (Pitt); Prabir Roy-Chaudhary (UNC)

Students: Chenhao Xu (PhD), Priya Jaswal (MS), Yonghai Zhang (MS)

Industrial participants: Inovasc LLC (spin-off company from the ERC)

**Project Overview:** In the United States, almost 750,000 people live with end stage renal disease (ESRD). These patients are 1% of the U.S. Medicare population but account for roughly 7% of the Medicare budget. About 350,000 ESRD patients are on hemodialysis (a procedure to purify the blood), all of whom require some sort of vascular access. Hemodialysis treatment costs an average of \$89,000 per patient annually in the United States. A fully mature AV fistula is by far the best form of dialysis vascular access, resulting in reduced costs, improved patient survival, and decreased morbidity. However, AV fistula non-maturation remains a huge problem. Recent data from a large multi-center NIH funded clinical trial suggests that over 50% of AV fistula are **not** suitable for dialysis between 4-5 months following surgery. The combination of an increased dependence on catheters (while AV fistula are maturing) together with repeated endovascular and surgical interventions for non-maturing fistula have all resulted in hemodialysis vascular access dysfunction being perhaps the single most important cause of morbidity and hospitalization in the hemodialysis population at an economic cost of over 1 billion dollars per annum.

(a). Solution to hemodialysis vascular access dysfunction. The single most important intervention that could reduce both the morbidity and economic cost associated with hemodialysis vascular access dysfunction at the present time is thought to be a clinical care process or therapeutic intervention which would enhance AV fistula maturation, which is the focus of this project. The placement of a biodegradable maturation enhancing stent (bMES) at the time of surgery will ensure the presence of a good venous outflow during the critical 6-12 weeks post-surgery when maturation occurs even in small and/or diseased veins. The availability of good blood flow during this period should enhance vasodilation through normal or enhanced biological pathways. The placement of a bMES coated with agents (drugs, cells, genes or chemicals) to both enhance outward remodeling and also inhibit neointimal hyperplasia could play an important role in neutralizing the local endothelial dysfunction which characterizes patients with ESRD who receive AV fistula.

(b). Advantages of using metallic biodegradable stents. Conventional stents stay in the body permanently and are prone to develop an aggressive in-stent restenosis and create flow-limiting situations. A *b*MES on the other hand could provide the initial scaffolding to allow for increased flow and diameter in the initial critical 4-8 week maturation period. In addition, the *b*MES could also allow for optimization of anatomical configuration and hemodynamic shear stress profiles and be a conduit for the short term delivery of agents that enhance outward remodeling and inhibit neointimal hyperplasia. Most importantly, biodegradable drug-eluting stents with the degradation rate properly controlled (e.g. by a smart stent) can degrade and eventually disappear after the fistula matures.

#### Year 11 Accomplishments:

*New Self-Expanding Biodegradable Metal Stent.* A self-expanding biodegradable metal stent (SEBMS) was designed using computer simulation. The SEBMS expands totally elastically upon insertion into a blood vessel and is easier to install than balloon expandable stents and will maintain a small force on the vessel wall to keep the vessel open. SEBMS do not have plastic regions with cracks and thus will corrode more evenly and possibly have less risk of pieces of the stent being released into the blood vessel. Many surgeons prefer self-expanding stents. Dr. Tarek Helmy, MD, a vascular surgeon, has asked us to design a self-expanding biodegradable stent. The concept for the self-expanding stent is that the stent struts are designed to provide large elastic displacement of the stent size (diameter) without going into the plastic range. Biodegradable stents used now or that are currently in development are all balloon expandable and rely on large plastic deformation to expand and to remain expanded. These stents have recoil (do not hold

the expanded size and decrease in size when the balloon is removed). Balloon expandable Mg deformable stents also produce a force on the vessel wall based on the expansion size and recoil of the stent. But the deformable stents do not provide much elastic force on the vessel wall once expanded because small deformation will cause the stent to go into the plastic range. Thus a plastic Mg stent is not flexible or compliant to reduce stresses on the vessel due to pulsations in blood pressure. Also, a plastically deformable Mg stent, if loaded by an impact may deform and close, whereas the SEBMS may deform and restore its shape due to its elastic nature.

It is difficult to design a self-expanding biodegradable Mg stent that can match the characteristics of a Nitinol shape memory alloy stent. Thus the elastic self-expanding stent proposed has a different strut design that allows high flexibility while maintaining elastic stresses. The Mg material also may have yittrium to provide high strength. AZ31 is the first material considered and used in the initial design of the SEBMS, Fig-1. However, other alloys like WE43 (a commercial Mg alloy with yittrium) or alloys from Pitt (Kumta Lab), or NCAT, (Zhigang Xu lab) will also be considered for this application. The SEBMS stent may be Mg, Fe, Zn, another metal, an alloy, or a polymer/metal.

Current Mg stents are balloon expandable which causes the stent to go into plastic deformation in many sections of the stent, Fig-1. Plastic deformation produces cracks in the Mg which are sites for stress corrosion and also the cracks may breach any coating on the stent. Plastic deformation and cracking cause faster corrosion in the plastic region and the stent may not corrode uniformly producing uneven support of the stent in the vessel and particles of the stent that corrode faster may be released into the blood stream.



Figure 1. Stent development in the ERC, Gen III plastic stent design.

The self-expanding elastic stent has no significant plastic deformation, Fig-2. Thus the above problems are avoided. The self-expandable stent has 3 states:

- 1. Original State: the stent as made, the state before crimping
- 2. Crimped State: This is the delivery state, the state before release in the blood vessel
- 3. Spring-back State or self-expanded State: It's also the released state in the blood vessel.

Fig-2 shows the original state, crimped state, and the spring back state outside of the vessel (no constraint on expansion). The Mg biodegradable stent field and market are still in development and emerging. No Mg stents are on the market yet but they are in clinical trials in Germany by Biotronik Company. The present research could be a product with a competitive advantage to the Biotronik stent. The SEBMS is particularly useful in veins in which the restoring force needed is not as large as in arteries that are smaller and where arteriosclerosis may require a large outward force that is typically provided by self-expanding nitinol permanent metal stents. Also, the SEBMS does not need to expand beyond its final size whereas plastic range stents must be expanded beyond their final size over-straining the vessel wall in order to recoil to their final dimension.



Figure 2. Radial displacement comparisons of: (a) the original state; (b) the crimped state; and (c) the spring back state of one segment of the self-expanding stent outside the vessel. Color bar scale is different on each figure a-c.

Table 1 shows the design parameters used for the simulation study of the self-expandable stent. Spring-back can vary from 98% to 100% (completely elastic) based on the designs considered.

	Type 1 Self-expanding	Type 2 self-expanding	Type 3 self-expanding
	with 10 units.	with 10 unit and 10%	with 12 units and
		reduced thickness	original thickness
		strut.	strut.
# strut units	10	10	12
Cross section	0.2 x 0.3	0.18 x 0.3	0.2 x 0.3
Maximum Von Mises	227 MPa	202 MPa	223 MPa
Maximum Principle	233 MPa	208 MPa	227 MPa
Stress			
Maximum Principle	7.2 E-3	2.6 E-4	3.3 E-3
Plastic Strain			
Maximum Equivalent	8.3 E-3	4.3 E-4	3.4 E-3
Plastic Strain			
Spring Back Diameter	9.79 mm	9.99 mm	9.98 mm
Recovery Ratio or 1 –	98%	100%	99%
Recoiling Ratio			

Table 1. Stresses and spring back of the Self-Expandable Stent.

Two SEBMS are being fabricated with different bending stiffness to compare with our plastic Gen III stent. A proposal will be written to request funding to test the SEBMS in a pig model to directly compare its efficacy with the Gen III plastic stent.

*Subcontract to University of Arizona*. Dr. Prabir Roy-Chaudhary of Univ. of Arizona with Dr. Diego Celdran worked with UC on stent design in a subcontract as part of the ERC. The subcontract was ended March 2019 since Dr. Roy-Chaudhary moved from Arizona to UNC. UC continued the work by fabricating stents and will send the stents to Dr. Roy-Chaudhary for evaluation on the bench. Two proposals are being written with Dr. Roy-Chaudhary (Dr. William Wagner also is participating by coating the stents) to continue development of the stents. Dr. Prabir Roy-Chaudhary and Dr. Diego Celdran provided guidance on development of the stent is a challenge and testing is continuing at UC based on feedback from Dr. Roy-Chaudhary and Dr. Celdran. We plan to test a smart stent that degrades on command electrically once the connection is improved.

## <u>C2C ERC-RMB Year 11 (C2C Year 2 Update):</u> US-Ireland- Northern Ireland R&D Partnership Between the NSF-ERC for Revolutionizing Metallic Biomaterials (ERC-RMB) in the US and CÚRAM at National University of Ireland Galway (NUIG), Ireland, Nanotechnology and Integrated BioEngineering Centre at Ulster University, Northern Ireland (NIBEC): NSF-SFI-C2C

Our NSF-SFI-C2C team is advancing the production of modifications to magnesium metals (new alloys, new coating methods) and testing paradigms (modeling backed up with experimental data) with the purpose of transforming the application of magnesium biomaterials to clinically relevant problems. This C2C experience and our efforts to overcome international logistical barriers enhances our researchers' experiences, generates a globally-thinking future work force, and demonstrates that global collaborations can be efficient and productive.

**Overview:** Our team shares the goal of developing bioresorbable magnesium alloy systems for clinically-capable orthopedic implant devices. The regulatory landscape, especially for biomedical materials and devices, is highly dependent on intercountry efforts. This tripartite partnership via the C2C program has created a unique convergence of world-leading expertise from academia and industry in the fields of materials processing (ERC-RMB, Fort Wayne Metals), surface modification (ERC-RMB, NIBEC), characterization (ERC-RMB, NIBEC), computational modeling (NUIG), and regulatory issues (Ft Wayne Metals, Orthokinetics, C2C universities). Our groups have, with the input of clinician team members, chosen applications ranging from thin wires (for clinical use as "k wires") to thicker pins, rods and elastic stable intramedullary nails (IMs or ESINs) and meshes for the treatment of complex bone fractures.

**Materials and Data Exchange**: One of the many outcomes of the matured ERC-RMB program is the ability to tailor magnesium (Mg) materials and characterize the resulting materials at the atomic and micro-scale and relate these characteristics to bulk mechanical properties. Unique Mg materials including polycrystalline Mg alloys (Dr Xu, NCAT; Dr Kumta, Pittsburgh), and monocrystalline pure Mg (Dr Shanov, Cincinnati) have been shared with NIBEC for coating studies and analysis. Related microstructural, fatigue, and corrosion data have been sent to NIU Galway for Finite Element Analysis of these materials in application relevant scenarios, developed in conjunction with our clinical partners. Inputs from our C2C partners have helped modify the subsequent rounds of materials produced. NCAT (Dr Yarmolenko) has prepared a living document materials data base distributed to both academic and industrial members. This C2C partnership has demonstrated the efficacy of Mg materials as a K-wire material and resulted in the development of a monocrystalline Mg alloy driven by the demands and needs of the EISN application.

The NIBEC team has been focusing on the chemical and physical characterization of various hydroxyapatite coatings on commercially sourced and custom designed magnesium alloys by the University of Pittsburgh partners.

A large batch of WJ11 magnesium alloy samples were prepared by the University of Pittsburgh, these samples were sent to NIBEC in Northern Ireland to be coated with hydroxyapatite coatings at a range of coating thicknesses. Five coating thicknesses were explored, with 50 samples coated at each thickness, these 250 samples were then returned to the University of Pittsburgh for in depth characterization and analysis. This study was conducted with the aim of publishing a joint article discussing the work.

Invigorating group calls between all the institutions lead to an interesting discussion about the ability for a barrier type coating to withstand the shear forces that would be induced upon it during

the insertion of a bone nail or k-wire. The NIBEC team then worked with the NUIG team to devise a series of experiments and models to examine the mechanical, adhesive and shear properties of the coating and model these parameters on a device scale to determine and optimize coating effectiveness. Lap shear, nanoindentation and nanoscratch testing is being conducted by the NIBEC team, with data passing through to models developed by the NUIG team.

The NIBEC team is examining the coating physical and chemical properties in detail and performing degradation studies to examine the effect of the coating to change the corrosion rate of magnesium alloys. So far chemically pure hydroxyapatite coatings and strontium substituted hydroxyapatite coatings have been investigated by the team, with current experiments studying the effects of annealing the coating and future planned work to examine multi-doped hydroxyapatite coatings.

Center Visits and Meetings: Modern industry is multi-national and so should be the scientific teams and the training of our current and future researchers. An essential component of the collaboration is the efficient exchange of ideas that allows student researchers to operate and succeed in an intercontinental arena. We can communicate person-to-person on the different challenges associated with each country's academic, industrial, and regulatory challenges. We have leveraged this opportunity via student and faculty transatlantic visits and cross-institution webinars and person-to-person communication. US to Ireland, August 31-September 2, 2018:. Drs. Pixley and Little (Cincinnati) visited NIBEC at Ulster University, for collaborative talks and tours of the facilities. Our C2C experience has allowed us to contribute to the international community through participation in the NSF sponsored C2C Collaboration Workshop, August 14-15.2018 UCLA, Los Angeles. (Drs. Pixley (Cincinnati) and Collins (NCAT)); the 10<sup>th</sup> Annual Biodegradable Metals for Biomedical Implants, Oxford University, Oxford, England, August 26-31, Several C2C members presented and met for discussions: Keynote talks: Dr. Kumta (Pittsburgh), Adam Griebel (Ft. Wayne Metals), Dr Pixley (Cincinnati); Presentations: Dr Shanov (Cincinnati), Dr Little (clinician, Cincinnati), Dr Acheson (NIBEC); 03/27-28/2019 - Dr Kumta (Pittsburgh) on behalf of the ERC-RMB attended the Science Foundation Ireland Site Review for CURAM-2, Galway, Ireland, March 27-28, 2019; and Drs. Sankar (NCAT) and Dr. Pixley attended the ERC Workshop on International Center-Level Collaborations to Enhance Research and Workforce Development, NSF, Alexandria, VA, April 11-12, 2019.

Several center-to-center webinar meetings have occurred in year 11 (year 2, C2C) and regular face-to-face and skype meetings between the NIBEC team and NUIG team have occurred to share data and plan further coating adherence experimentation.

## 3D ESIN parameter study ongoing



**Figure 1**. Representative slides presented during a webinar meeting illustrating experimental (ERC-RMB, NIBEC,) and computational (NUIG) approaches to improved application of Mg Biomedical devices.