Robert Morris University

Experiences as a Research Assistant at the University of Pittsburgh

All documents are of good quality!

Introduction

Cranio-maxilliofacial scaffolds are used to treat trauma and cancer patients with severe bone damage in the face, jaw, skull, and neck region. The current techniques used to administer these scaffolding involve bone grafting combined with external fixation devices. This results in a long procedure and often requires a secondary surgery to remove the fixation device. As part of my internship as a research assistant at the University of Pittsburgh's bioengineering department, I worked on a project which aimed to improve upon these treatments. In order to accomplish this task we concentrated specifically on three variables; the ability of the device to be imaged, the biodegradability of the materials, and the customization of the device's design. Focusing on these three areas of design allowed us to reach our ideal device characteristics. First we wanted to make sure the implant could be imaged with modern technologies such as MRI and CT scans, this will allow for proper monitoring of the recovery process. By improving the biodegradable we can create bioresorbable alloys. These alloys can degrade in the human body without harmful latent effects, therefore eliminating the need for a secondary removal. Customizability offers the ability to create patient specific devices, designed and manufactured based on the patients associated geometries. This simplifies the surgery and in turn reduces its duration. It also has the ability to improve the success of the fixation.

To begin the study we focused on material selection. Just as I learned in engineering materials, the first stage in every design process is material selection. We began with our first material criteria, the ability to be imaged. This led us to choose iron manganese, Fe-Mn, as our base alloy because it is known to be susceptible to imaging while also possessing favorable degradation characteristics. The only problem with this material is that it degrades too slowly

within the human body, resulting in unwanted latent effects. We know that other metals, such as Magnesium, which are known to be biocompatible and have a faster degradation rate than the Fe-Mn alloys. Using one of these metals as a third alloying element along without Fe-Mn base alloy could therefore result in an alloy both susceptible to imaging and bioresorbable. Thus we had a solution to our first two design criteria.

To obtain the desired customization of the devices we decided upon the integration of computer-aided design and modern imaging technology. Computer-aided design allows the patient's MRI or CT scans to be the base of their medical implant's design. First, the image file is converted into a CAD modeled allowing the clinician to make modifications to the design if necessary. The model is then converted into a STL file for use with 3D printing technology. Binder-jetting 3D printing is one technique used specifically for production of metal parts. This technology combines a glue-like binder with metal powders to create 3-dimensional objects layer-by-layer.

Now that the background research of the study had been done it was time to design the experimental study. The first objective of this study was to prove that the addition of a third alloying element with desirable biodegradation characteristics to iron-manganese will result in a bioreabsorbable alloy which can be imaged with modern technology. The secondary objective was to prove that this new alloy can be used to create a patient specific device through binder-jet 3D printing. To complete these objectives a theoretical study would be done to determine the top five alloy compositions. These alloys would then be synthesized and tested using both in the laboratory to determine their corrosion rate and biocompatibility. Of these original five alloys the two with the most desirable characteristics would be chosen for an in vivo study done using lab rats. Following these studies a sample device would be printed using a binder-jetting 3D printer

to prove the feasibility of the manufacturing process. My contribution towards this study came mostly in the laboratory. I was responsible for the manufacturing of the alloys, performing the electrochemical analysis of the alloys, and performing tests to determine cytocompatibility.

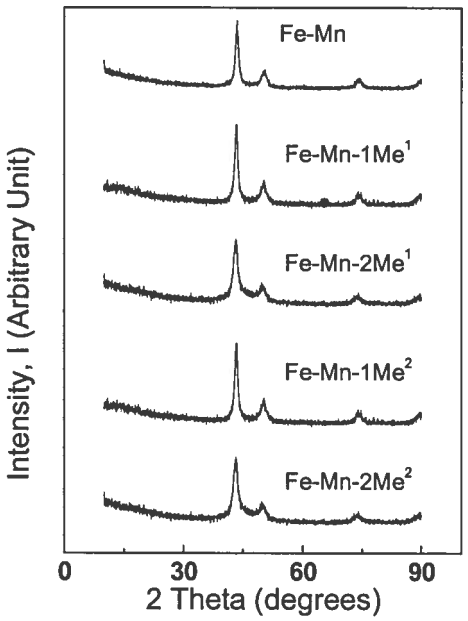
Manufacturing of the desired alloy compositions

High energy mechanical alloying was the process selected for the synthesis of the new alloys. This process involves combing pure elemental powders in a stainless steel vial while under a controlled Argon environment. This environment must be devoid of both oxygen gas and water molecules to avoid the possible reactions. Stainless steel pellets are also added to the vial which is sealed while in the controlled environment. These vials are then placed in the mill and spun at high speed for an extended period of time. High energy mechanical alloying allows for complete control over the final composition. By adding the elemental powders in exact quantities the percent composition can be precisely controlled. By controlling the total energy involved in the milling process the desired material phase can be controlled. The mass of the balls within the stainless steel vials and the duration and speed of the milling process are the only factors introducing energy to the system. Both of these factors can be varied by the operator of the machine. Different material phases can be associated with drastically different material characteristics.

Our desired alloys as included Fe65-Mn35, Fe65-Mn34-1Me[1], Fe65-Mn33-2Me[1], Fe65-Mn34-1Me[2], Fe65-Mn33-2Me[2]. In this case Me represents an undisclosed material. These compositions were selected due to their theoretical biodegradation characteristics which were determined using a calculation of phase diagram model. Furthermore, through the desired

material phase of the Fe-Mn alloys is the gamma-phase due to its susceptibility. In order to reach this phase the powders must undergo twenty hours of dry and wet milling.

After synthesizing the materials the success of the process had to be checked. This was done through the process of x-ray diffraction which uses X-rays generated by a cathode ray tube to determine sample purity as well as the characterization of crystalline materials. In order to complete the X-ray diffraction a small amount of the alloy powder is placed in a machine where a goniometer slowly rotates the sample. As this rotation occurs the cathode ray is emitting x-rays of a known angle towards the sample. When these rays collide with the powder they are diffracted according to the materials specific crystalline structure. By collecting these diffractions and converting them into a graph of diffraction angle versus counts per second the phase of the material can be determined [2]. The following are the results of the x-ray diffraction of our five materials:



The peak seen on this graph represents the gamma phase of our Fe-Mn based alloys, proving the alloy synthesis to be successful.

Now that we had our desired alloys in powder form we had to processes them so they may be tested. The first step in this process is to mold the elemental powder into a solid form. A cold-isostatic press, CIP, is used to complete this task. This machine uses intense pressures, up to 60,000 pounds per square inch, to compact the material. For the purposes of this experiment the desired shape was simply a cylinder which can be easily machined. Due to the fact that the University of Pittsburgh does not have a CIP, this part of the experiment was done at Robert Morris University. The process of using a CIP involves adding the alloy powders to a silicon mold, sealing the mold, and then submerging it in the CIP chamber. Proper sealing is essential to prevent the dirty water from within the CIP's chamber from coming in contact with the material in the mold. Such contamination could result in a faulty mold or skew the experimental results.

Even though the elemental powders hold the desired shape after compaction with the CIP they are still brittle. For this reason, the samples are must be heat treated. This involves carefully placing the samples within a furnace and heating them to 1,200°C for an extended period of time. Correct placement of the cylindrical samples is important to avoid deformation of the desired geometry. If the sample happens to fall onto its longer side rather than its circular face it will assume a flat tire shape which cannot be used for the ensuing experiments. Heat treatment also helps to burn away any foreign particles that may remain in the sample. These foreign particles may include water and other foreign substances from the CIP process.

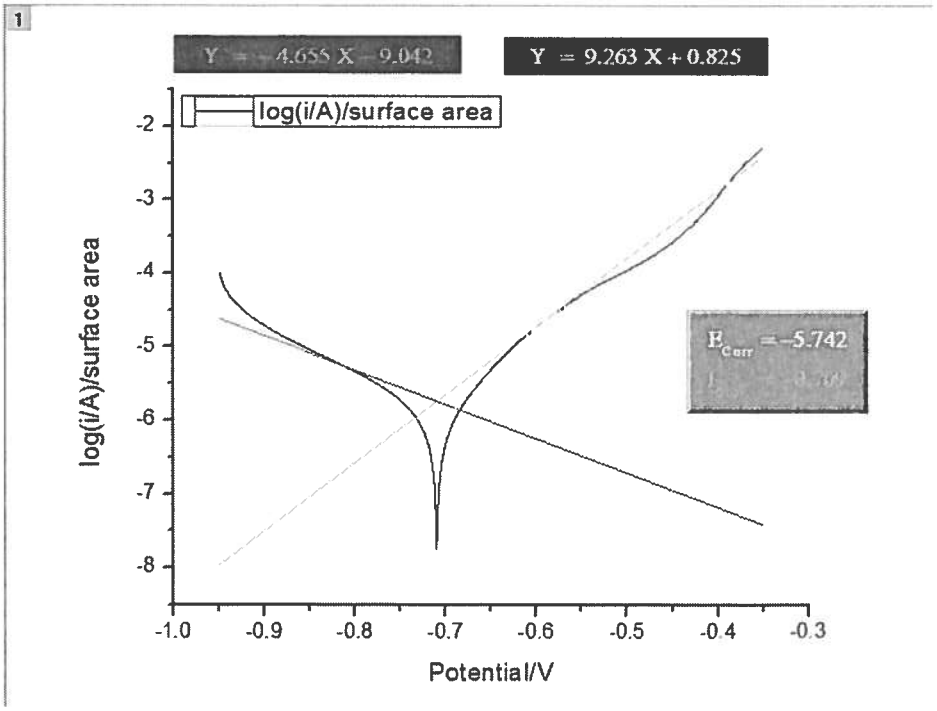
The final steps in alloy synthesis include simple machining tasks. The first of these being a lathe, used to remove any defects in geometry and clean up the outer edge of the sample. Creating individual samples from the collective rod of each material is the next task. Depending

on the composition of the material this can be done using either a lathe or a diamond saw. While the diamond saw requires much less effort, the wear on the blade and time required for each cut render the technique impractical. Once the samples have been formed to the correct shape – a small disc for the purposes of our experiment – they must be polished. By polishing the samples with sandpaper of increasingly fine grit we are able to refine the surface of the discs. The end result of this process should be a perfect surface, free of imperfections and completely flat. In doing so we are able to obtain more accurate measurements of surface area which are necessary for the majority of laboratory testing. To obtain such a refined state in the samples of this experiment were polished with 320 grit, 600 grit, 1200 grit, and finally a cloth polisher with the addition of a diamond based liquid polishing solution. Polishing was done using an automatic polisher which allowed for complete control of the speed of the sanding while also making the process less taxing for the individual performing the task. Alcohols such as isopropanol and ethanol were used as a polishing medium to reduce wear of the discs used for polishing and increase the quality of the final product. Between each of these different levels of grit the samples must be cleaned using a sonicating machine to remove all debris particles. If this is not done then the success of the following grit will be significantly reduced due to these particles which are larger than the grit of the paper, thus causing surface imperfections.

Electrochemical analysis

In order for a material to be bioresorbable it must corrode at a certain rate within the human body. To determine if our experimental alloys were within the range of bioresorbable corrosion characteristics we conducted an electrochemical analysis was conducted. Electrochemical corrosion experiments are conducted by immersing a metal sample within a solution typical of the environment in which the metal will be corroding. For our study Hanks Balanced Salts were

used to mimic the conditions within the human body. Electrodes are also immersed in the solution and are connected to a potentiostat. Our experimental setup involved three electrodes; one reference electrode and two counter electrodes. The potentiostat is used to apply a potential to the system through the counter electrodes. Application of a potential causes the oxidation and reduction reactions of an electrochemical to occur at different rates. This application must begin at the value of the corrosion current, E_{Corr}, and continue through the range of potentials associated with both cathodic and anodic reactions [5]. As a result of this varying potential the rate at which the cathodic and anodic reactions take place. Collecting this data regarding the electrical potential of the system while a corrosion reaction is occurring allows the construction of a tafel plot of the solutions potential voltage versus a function. Further analysis of such a tafel plot allows the corrosion current, i_{corr} to be determined from the anodic and cathodic tafel constants [3]. The corrosion potential, E_{Corr}, can also be determined from a tafel analysis. The following tafel plot is an example of one tafel plot constructed from the experiment:

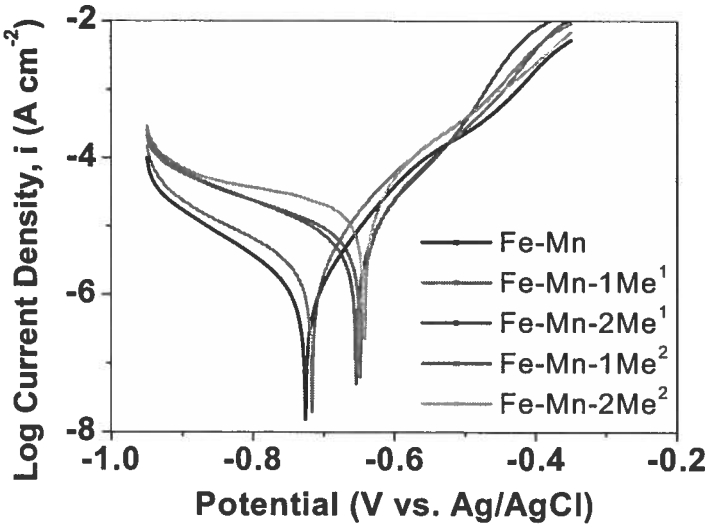


The curve shown on this graph represents the tafel plot with the left-hand curve being the anodic reaction and the right hand side representing the cathodic reaction. The line drawn on the left hand side of the graph represents the cathodic current while the line on the right represents the anodic current [3]. Cathodic and anodic constants are determined from the respective slope values of these lines. Additionally the point at which these lines intersect should represent the E_{Corr} and i_{Corr} values as such: (E_{Corr}, i_{Corr}) (gamry) [3].

After determining the value of the i_{Corr} value it is related to the corrosion current with the following equation:

$$Corrosion\ Rate\ (MPY) = \frac{0.13(i_{corr})(E.W)}{Ad}$$

In this circumstance corrosion rate is measured in millimeters per year. Variables are defined as follows; E.W represents the equivalent weight, A represents area, d represents density, and 0.13 is the metric and time conversion factor [4]. Once this calculation has been performed for all of the experimental trials the values can be averaged by sample material and then compared to each other. A final tafel plot showing these averaged results by composition is shown below:



As can be seen in this tafel plot the addition of the third alloying element did in fact increase the rate at which the sample corroded. From these results the two materials which were selected to move onto the next were Fe-Mn and Fe-Mn-1Me[1]. The other alloys were eliminated because their corrosion rates were further from the desired characteristics for bioresorbability.

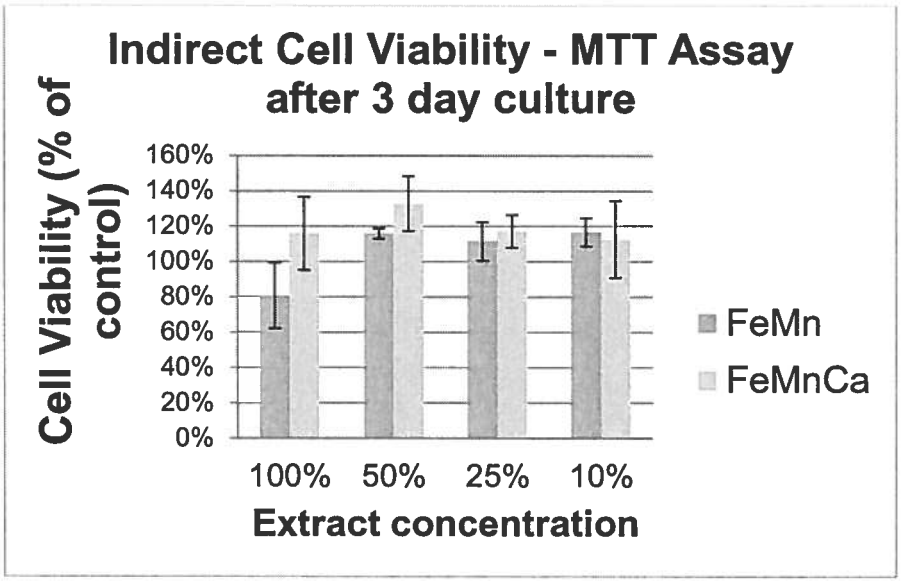
Determining Biocompatibility

When determining cytocompatability of a biomaterial the intended location is an important variable. In this study the device will come in direct contact with bone, the experimental procedures should therefore be designed to test the biocompatibility of the material with respect to bone cells. In order to stay consistent with other studies planned for this test MC3T3 cells were chosen for use in the in vitro studies. MC3T3 cells are the undifferentiated preosteoblasts of mice in this test to determine the cytocompatability of 3D printed samples comprised of the final two alloy compositions, Fe-Mn and Fe-Mn-1Me[1].

Indirect MTT assay was the first test of cytocompatibility conducted. This test involves the culture of the desired cells in a well plate as well and then preparing an MTT extract media. To prepare such a media a sample is submerged in a media replicating that of the ultimate location and then incubated for a set period of time. These procedures had to be performed according to the standards set by the international organization for standardization. Once the extract media is prepared the dilutions of varying concentrations are prepared for the testing. These different concentrations are then introduced to the cells cultured in the well plate and incubated for a set period of time. Following the duration of the experiment the viability is determined by first staining the cells and then examining them under a microscope [2]. There are multiple staining techniques, each targeting a specific function

of the cell. For example, Trypan Blue is used to stain dead cells and therefore allows the experimenter to distinguish between the two when looking under a microscope.

The results of our study were positive, proving both alloys to be cytocompatible. These results can be seen in the following graph:



This shows that even at 100% cell extraction our experimental alloy FeMn1Me[1] has no harmful effects on the viability of preosteoblast cells. Combing these results with the results from the electrochemical analysis provides significant scientific evidence that this new biomaterial is potentially bioreabsorbable when 3D printed using a binder-jetting method.

Conclusion

Having the opportunity to take part in this internship was one of my most rewarding experiences while at Robert Morris University. As a research assistant I was working directly with graduate students and conducting world class original research. This was something I never expected to experience as an undergraduate. Perhaps the most rewarding part of this experience was possessing an understanding of the experimental design as well as the theories used. Specifically my knowledge of materials

engineering, anatomy and physiology, and basic bioengineering directly impacted my success in this environment.

References

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