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Development of a Biodegradable Polymer-Metal Composite as a Novel Biomaterial

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APPROVAL PAGE

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Development of a Biodegradable Polymer-Metal Composite as a Novel Biomaterial

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Table of Contents

Table of Figures

Abstract:

Poly(lactide-co- glycolide) (PLGA) is a versatile biomaterial and is desirable for use in tissue engineering applications requiring a degradable matrix or fixation. PLGA degradation rate can be controlled through manipulation of the ratio of its constituent monomers PLA (polylactic acid) and PGA (polyglycolic acid). When PLGA degrades it can lead to the development of acidic conditions eliciting an inflammatory response. We have investigated the addition of biodegradable Magnesium (Mg) particles to a PLGA matrix, aiming to achieve neutral byproducts. Within the body Mg degrades into a basic oxide and hydrogen gas, so through varying its concentration in a PLGA scaffold it may be possible to determine an optimal concentration for pH neutrality. It was also hypothesized that the maintenance of a neutral pH during degradation would prolong degradation rate of the composite. It was found that as Mg concentration increased, the length of degradation increased from ~35 days (0%wt Mg) to ~75 days (10%wt Mg). There was also a proportional shift in pH toward the basic end of the spectrum. In order to assess neutralization a simulated in vivo degradation study was performed. It was found that the addition of Mg (5%wt Mg) prolonged degradation from ~40 days to ~80 days and kept pH in a fairly neutral range (6-8.5 versus 4-7.5 without Mg). Increasing concentration of Mg was also found to increase the modulus of elasticity of composites. During the development of the PLGA-Mg composites agglomeration of the Mg particles was observed. Agglomeration presents potential issues for the composites including variable mechanics and formation of hydrogen gas pockets in the body. In order to overcome this Mg particles were modified with a hydrophobic self assembling monolayer. The intention of the layer was to improve particle integration and add a protective coating against degradation. Assessment by micrographs revealed some degree of improvement in 10%wt Mg composites where hydrogen gas formation was reduced. In an degradation study modification wasn't found to have any consistent observable difference on degradation behavior. Further testing must performed to assess whether modification was successful.

Chapter One: An Introduction to Polymer-Metal Composites

1.1 PLGA as a Biomaterial

PLGA is clinically approved for a variety of applications including cell scaffolds, drug delivery devices, coatings, and fibers both as a reinforcement and a matrix [1]. PLGA is desirable as a material due to its tunable properties, which can be controlled through manipulation of the ratio of its constituent monomers, Lactic acid (LA) and Glycolic acid (GA) [2]. LA is hydrophobic while, GA is hydrophilic [3]. By increasing the percentage of PLA, PLGA can be made more hydrophobic resulting in a slow degradation rate. It has been demonstrated that degradation rate can be prolonged from a few weeks to a year for the same amount of PLGA by increasing LA composition [3]. In addition, PLGA is also biodegradable. When it degrades it produces Lactic Acid and Glycolic Acid, both of which are natural byproducts of metabolic processes of the body [2]. In moderate concentrations the body is able to process and remove them.

1.2 Challenges of PLGA

Although Lactic Acid and Glycolic Acid are naturally occurring in the body, they are still acids. At high concentrations they are very capable of influencing the local pH. In clinical trials where PLGA fixation devices were used in bone, the buildup of acidic byproducts triggered an immune response [4]. In some cases, the response may lead to inflammation and implant failure [4].

When PLGA degrades it does so through bulk degradation. Instead of degrading from the surface inward, water diffuses into the polymer and degrades it from the inside [5]. The breakdown of the interior leads to the development of a pocket filled with acidic byproducts [5]. The collection of these acids leads to the development of a very acidic environment inside the structure [5]. This acidic buildup accelerates the degradation due to autocatalysis [3]. Once

structural integrity has been compromised the polymer collapses and the remainder is quickly broken down [3].

1.3 Mg as a Biomaterial

Mg as a biomaterial has a range of applications. It is generally used in cardiovascular, and orthopaedic applications [6][7][8]. It is desirable as a biomaterial because of its mechanics (similar to that of bone), osteogenic benefits, and ability to be alloyed to achieve tunable physical and mechanical properties [6][7][8] In addition Mg is among a subgroup of metals that are biodegradable, the body is able to break them down since they are naturally found within the body. Some of these metals are reduced to nontoxic byproducts while others corrode leaving toxic particles such as iron oxide. Concentration is often the determining factor when it comes to the effects of metal, in low concentrations the body is able to breakdown and clear the material without eliciting an immune response or cell death. Biomaterial implants are generally on a large enough scale that concentration becomes a concern. By limiting the rate of degradation of these metals they can generally be kept within safe limits.

1.4 Challenges of Mg

Mg has several challenges as biomaterial, that become more of a concern with higher concentration. Mg, due to its chemical properties, is susceptible to a rapid and uncontrolled degradation. In the presence of oxygen or water Mg will form a passivation layer like most metals. The thickness of this layer can be categorized by the Pilling-Bedworth ratio, whereas a thickness less than 1 is considered very thin, a thickness between 1 and 2 is considered to be stable and preventative of further degradation of the metal, and a ratio of 2 or more leads to the formation of a thick and unstable layer that will break off and continue to grow, degrading the underlying metal [9]. Mg has a ratio of .8 and thus forms a thin layer that is very vulnerable to penetration by water [9]. Comparatively titanium (Ti) forms a stable layer due its ratio of 1.5 while

iron (Fe) forms a flaking layer of rust due to to its high ratio of 2.1 [9]. Since Mg undergoes rapid degradation, it leads to the rapid accumulation of its byproducts, which at high concentrations presents a challenge for the body to dissipate.

While the formation of the oxide byproduct can be potentially useful in neutralizing acidic byproducts, the formation of the other byproduct, hydrogen gas, can be problematic.

Hydrogen gas formation in low concentrations is easily handled by the body. However, when hydrogen gas is formed rapidly the body is unable to dissipate it. This can lead to the formation of gas pockets, an undesirable complication that can cause blood clotting and tissue necrosis [10]. In addition to the issue of rapid formation of byproducts, the degradation of Mg means a loss of mechanical properties. As the metal degrades it becomes weaker and weaker as would be expected. This can present issues when Mg is being used in applications where it's mechanics are required for implant integrity under loading.

1.5 PLGA-Mg Composites as a Biomaterial

The use of PLGA-Mg composites has recently become a topic of interest. Most commonly PLGA has been used as a protective hydrophobic coating on Mg in order to prevent it from degradation [11, 12, 13]. As of recently several groups have investigated the development of a three-dimensional (3D) PLGA-Mg scaffold [14, 15, 16]. Ma et al. 2015 demonstrated antibacterial effects of PLGA/Tricalcium Phosphate/Mg scaffolds [14]. Brown et al. 2015 used a cylindrical PLGA matrix containing Mg particles to prevent bone from receding in jaw void defects [15]. Liu et al. has recently patented a degradable neural guidance conduit composed of a PLGA matrix containing Mg fibers [16]. Although this composite is being adopted, there are still several challenges that need to be addressed before it can be effectively used as a biomaterial.

1.6 Challenges of PLGA-Mg Composites

Current studies on PLGA-Mg composites have yet to demonstrate control over the composites degradation behavior. With the ability to control the rate and pH at which the composite degrades, these studies will allow for development of a material than can be safely used in the body. In addition, there is little research on use of Mg to neutralize the acidic byproducts. It has not been proven whether Mg oxide can neutralize PLGA byproducts.

In our own lab we have developed PLGA films containing Mg particles.Within them we have encountered an issue with agglomeration of Mg particles (Figure 1.1). The agglomeration presents two problems for the composites. First, the clumping of Mg particles means that matrix has unbalanced consistency, which results in varied mechanics of the composite. In addition when the Mg particles degrade they will release their byproducts in one location, making it more difficult for the body to dissipate them. This can lead to the development of gas pockets as previously discussed in subchapter 1.4.

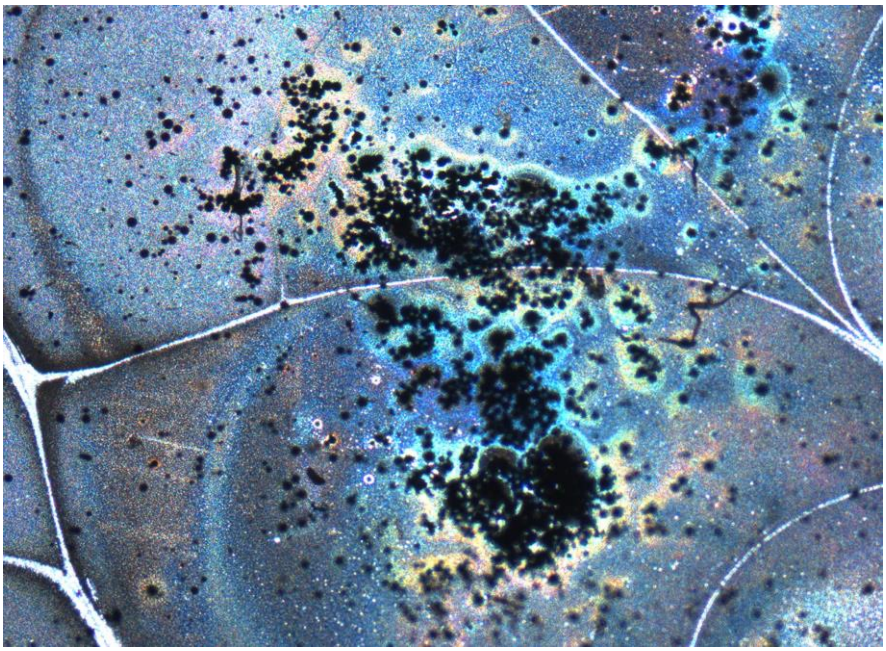

Figure 1.1- A PLGA film with Mg particle (black dots) agglomeration. The glow around the particles is caused by light reflecting off the hydrogen gas bubbles.

1.7 Mg Alloys

Mg as a metal alone has deficiencies as previously described. To overcome them and improve Mg it is frequently alloyed with other metals. A common alloy that may be beneficial as a biomaterial is Zinc (Zn) containing alloy ZK61. ZK61 is composed of approximately 95% Mg and 5% Zn with traces of several other metals (Zirconium, Manganese, Iron, Nickel, Copper, Silicon, and Aluminum) [17]. When used as a biomaterial Zn is nearly exclusively used in alloys because it has been demonstrated that the addition of Zn can bring several osteogenic benefits to biometals. Studies have demonstrated the presence of Zn directly influences mineralization, bone cell proliferation, and osteoblast adhesion along with providing antibacterial effects [18, 19, 20, 21].

1.8 Project Statement

The objective of this research was to overcome the aforementioned challenges of PLGA-Mg composites through two specific aims.

1) **To control degradation rate, mechanics, and byproduct pH of a PLGA-Mg composite through Mg concentration.**

   Hypothesis: Mg concentration directly influences composite degradation rate, mechanics, and pH.

2) **To chemically modify Mg particles to achieve uniform particle dispersion in PLGA and prolong degradation rate.**

   Hypothesis: By chemically modifying Mg particle surface with a hydrophobic layer,

particle distribution can be improved.

Chapter Two: Development of PLGA-Mg films

2.1 Film Fabrication

In the study solvent casting process was used to create PLGA-Mg composites. The process begins with dissolving (1g) PLGA beads in an organic solvent. At this point Mg particles are added for the composites containing Mg. The two or three are then vigorously mixed for 60 minutes until they have become uniformly mixed into a solution. The solution is then poured into a lined 90 mm petri dish at 4°C. This is done so that the solvent is evaporated out slowly, as compared to the rate at which it would evaporate at room temperature. It is expected that slow evaporation of the solvent reduces the mobility of the particles, it prevents the formation of solvent vapor bubble formation as well. The majority of the solvent has been found to evaporate within the first 24 hours, after this the films can be brought to room temperature. The films were kept in a fume hood for 24-48 hours to further remove the solvent while reducing their exposure to water vapor in the air. After this the films were stored in a desiccator until time for use.

2.2 Solvent Selection

In order to improve particle agglomeration in the films the manufacturing process was investigated step by step. First it was assessed if there was any influence introduced through the solvent. The standard solvent used for dissolving PLGA is dichloromethane (DCM) also known as methylene chloride [22]. In order to determine if DCM had any influence on films its performance was compared to acetone and chloroform at the same volume of 5 mL, with fixed amounts of PLGA (1g) and Mg (3% by weight). After mixing and allowing the films to dry for 24 hours at 4°C the films were observed on both the macroscopic and microscopic level. Macroscopically the DCM and chloroform films appeared similar, while the acetone film had observable agglomeration patterns (Fig. 2.1). The chloroform film appeared to have slightly more uniform particle dispersion than the DCM film. Observing the films at 100x magnification it was found all three still contained particle agglomeration (Fig. 2.2). Again, chloroform appeared

to be the most uniform in terms of particle dispersion, while acetone had the most agglomeration. To explain this phenomenon several properties of the solvents were investigated. There appears to be a correlation between dielectric constant and the behavior of the particle dispersion, as the dielectric constant decreased the agglomeration decreased. However the meaning behind this relationship is still unclear. Another possibility is the relationship between the rate of solvent evaporation and the viscosity of the films. Although the particle agglomeration is due to Mg surface charge, it may be possible that more viscous films will make particle movement more difficult and aid in keeping the particles suspended while the film solidifies. Based on the results chloroform was chosen for further testing.

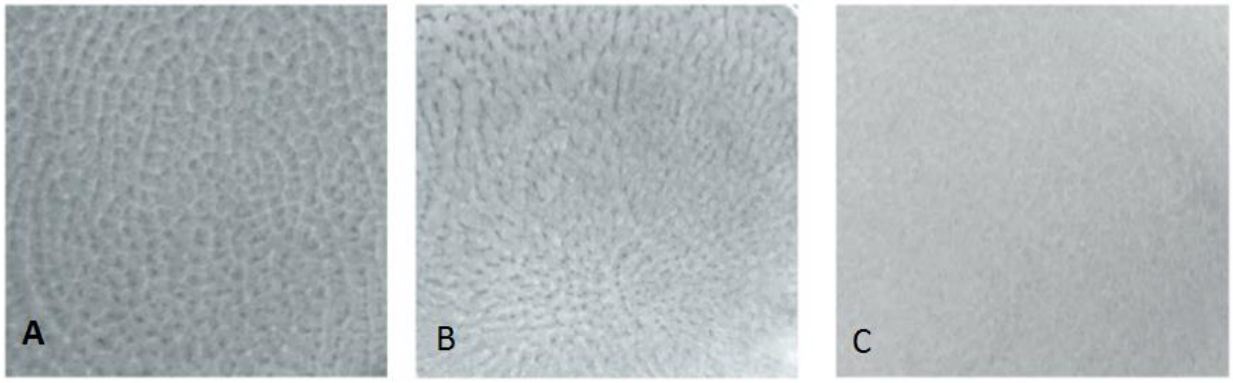

Figure 2.1- Images comparing the use of three solvents on Mg particle dispersion A) DCM B) Acetone C) Chloroform. Both the DCM and acetone dissolved samples have easily vision patterning which indicates particle agglomeration.

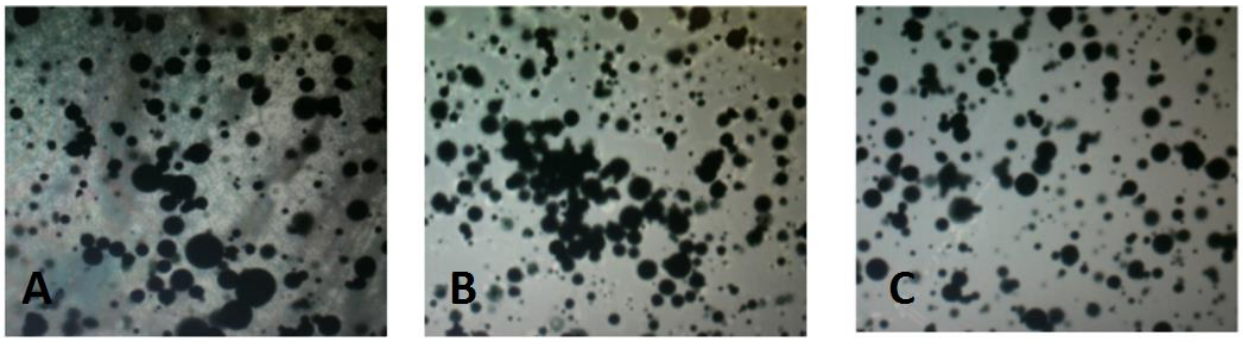

Figure 2.2- Micrographs (100x) comparing the use of three solvents on Mg particle dispersion A) DCM B) Acetone C) Chloroform. Both the DCM and acetone dissolved samples showed a greater amount of particle agglomeration compared to the chloroform sample.

2.3 Effect of Solvent Volume

After establishing that chloroform use resulted in superior particle dispersion compared to the other solvents, volume of solvent was investigated. Several volumes of solvent were compared while maintaining the same amounts of PLGA (1g) and Mg (3% by weight). Volumes of 3 mL, 5 mL, and 7 mL were compared using a 90 mm petri dish. Like in the solvent comparison, samples were compared by observation in terms of Mg particle distribution. On the macroscopic level, it was observed that films prepared with 3 mL of solvent were very thick and particle dispersion was poor. The composition itself was not even able to cover the entire petri dish, which restricted the space the particles could occupy. The 7 mL composition was observed to have the opposite effect, the film was very thin (Fig. 2.3). However due to the high amount of liquid the particles were able to more freely agglomerate resulting in observable poor dispersion. The 5 mL composition appeared to have uniform dispersion on the macroscopic level. Thus it was decided that it was clear that 5 mL was superior without the need for examination on the microscopic level. By varying the solvent volume it appears that too little solvent restrict the particles to one location while too much solvent enables particles to more easily agglomerate. An ideal volume falls somewhere in between, for chloroform that volume appears to be 5 mL per 1g PLGA.

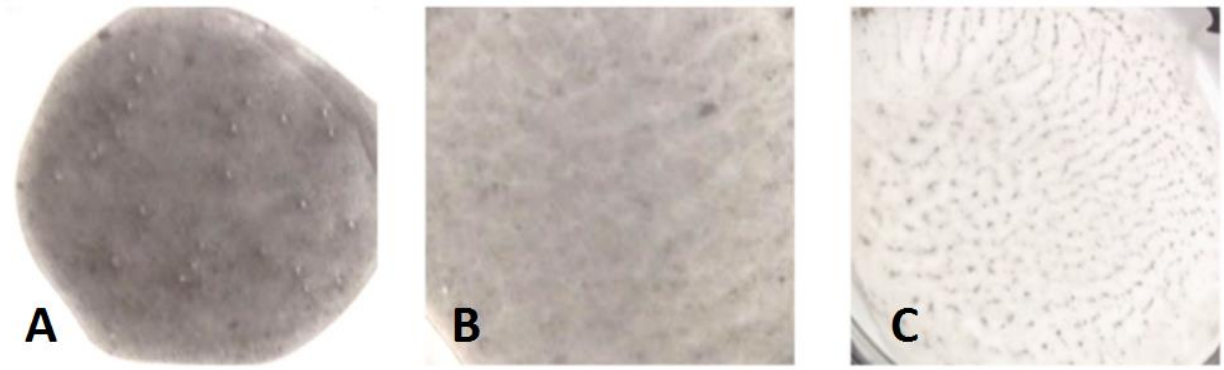

Figure 2.3- Images comparing the use of three volumes of solvent on Mg particle dispersion A) 3 mL B) 5 mL C) 7 mL. The 3 mL sample shows a very tight packing of Mg particles along with

the formation of many bubbles. The 5 mL sample has a fairly balanced appearance of particle distribution. The 7 mL sample has clear particle agglomeration indicated by distinct dots and lines of Mg.

2.4 Effects of Mg Concentration of Composites

After determining the ideal solvent and volume of solvent, in order to meet the first specific aim, the effects of Mg particle concentration were assessed. Samples containing 1% Mg by weight, 3%wt Mg, 5%wt Mg, 10%wt Mg, and 20% wt Mg were produced. These compositions were visually compared and several observations were made. First it was observed that as Mg concentration increased, there was the presence of more gas bubbles in the samples (Fig 2.4). This is likely due to a proportional increase in gas being formed, leading to bubbles compounding into larger bubbles that aren't able to escape the composite as easily. The increase in bubbles is not ideal because it weakens the composite mechanics, it is also expected the bubble release will continue at the same proportion in vivo. This indicates that the 10% and 20% films may lead to the production of too much gas for the body to handle resulting in gas pocket formation as previously discussed. The second trend observed was an increase in composite uniformity with increasing concentration of Mg. The 1% and 3% had clear patterns of particle agglomeration, while films of 5%, 10%, and 20% appeared to be very uniform in composition. This phenomenon may be due to particle saturation, it may also be due in part to increasing particle repulsion as the number of particles increases. From the comparison it was evident that the 5% sample appears to be the ideal concentration due to it offering the best trade off between bubble formation and particle distribution.

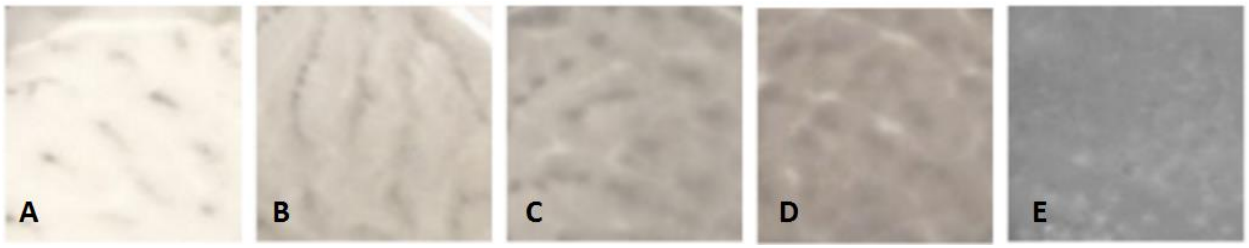

Figure 2.4- Images comparing the use of various concentrations of Mg A) 1% Mg by weight B)

3%wt Mg C) 5%wt Mg D) 10%wt Mg E) 20%wt Mg. Two trends are observed, 1) an increase in bubble formation as Mg concentration increases, and 2) a decrease in observable particle agglomeration with increase in Mg concentration.

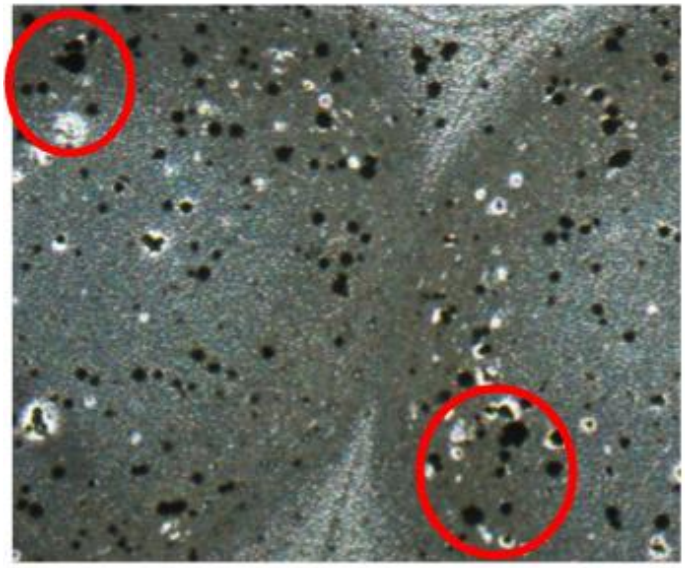

Figure 2.5- A micrograph (40x) of the 5%wt Mg sample, which despite being produced with what was determined to be the ideal solvent at the ideal volume, there is still agglomeration throughout the sample (circled in red) on the microscopic level.

2.5 Discussion

Despite the improved macroscopic particle distribution observed from using Chloroform at 5 mL, there still exists Mg particle agglomeration on the microscopic level (Fig. 2.5). Although agglomeration has been reduced it is still a concern that should be addressed. In order to overcome this issue the use of a means to better integrate Mg particles into the PLGA matrix is needed. This can likely be accomplished through a chemical mediator. This will be further discussed in Chapter 4.

Chapter Three: Influence of Composite Composition on Degradation and Mechanics

3.1 In vitro Degradation Study

Degradation studies were performed to determine the influence of Mg concentration on the properties of PLGA-Mg composites. A study was performed comparing several compositions of Mg films in phosphate buffered saline (PBS). Magnesium concentration was varied between groups while PLGA was kept constant at 1g. Groups consisted of a PBS control, PLGA (85:15), PLGA with 1%wt Mg, PLGA 3%wt Mg, PLGA 5% wt Mg, and PLGA 10%wt Mg. 12mm diameter film punches were added to 10 mL of PBS in a test tube. pH was measured at least once for every two days, and a mean pH was calculated for each time point from three replicates for each group. A pH probe was used for measurements and was calibrated prior to each measurement.

The samples were incubated in a 47°C water bath (10 degrees above physiological conditions) in order to accelerate degradation from taking approximately 1 year to only taking a couple months. It has been demonstrated that a temperature increase of 2 degrees Celsius can result in a 25-30% reduction in degradation time [5].

The results of the study (Fig. 3.1) shows a direct correlation between magnesium concentration and the rate of composite degradation. The sample containing no Mg (PLGA alone) was found to fully degrade in about 35 days, while the 10%wt Mg sample was found to fully degrade in over twice the amount of time at about 75 days. This is in part a result of an increased pH early during the degradation process causing the film to more slowly degrade, this is due to PLGA's pH influenced degradation, since PLGA degrades more rapidly in an acidic solution as previously discussed. A second trend is also observed, there is a shift in pH towards the basic end of the spectrum as Mg concentration increases. Although there is a clear change in degradation behavior, it is still not certain that Mg is actually neutralizing the acidic byproducts.

It may actually just be shifting the curve leading to a delayed degradation. In addition it is possible all the Mg degrades entirely in the first week, after which it has no influence on the solution pH. In order to assess this a second degradation study with a modified profile was performed.

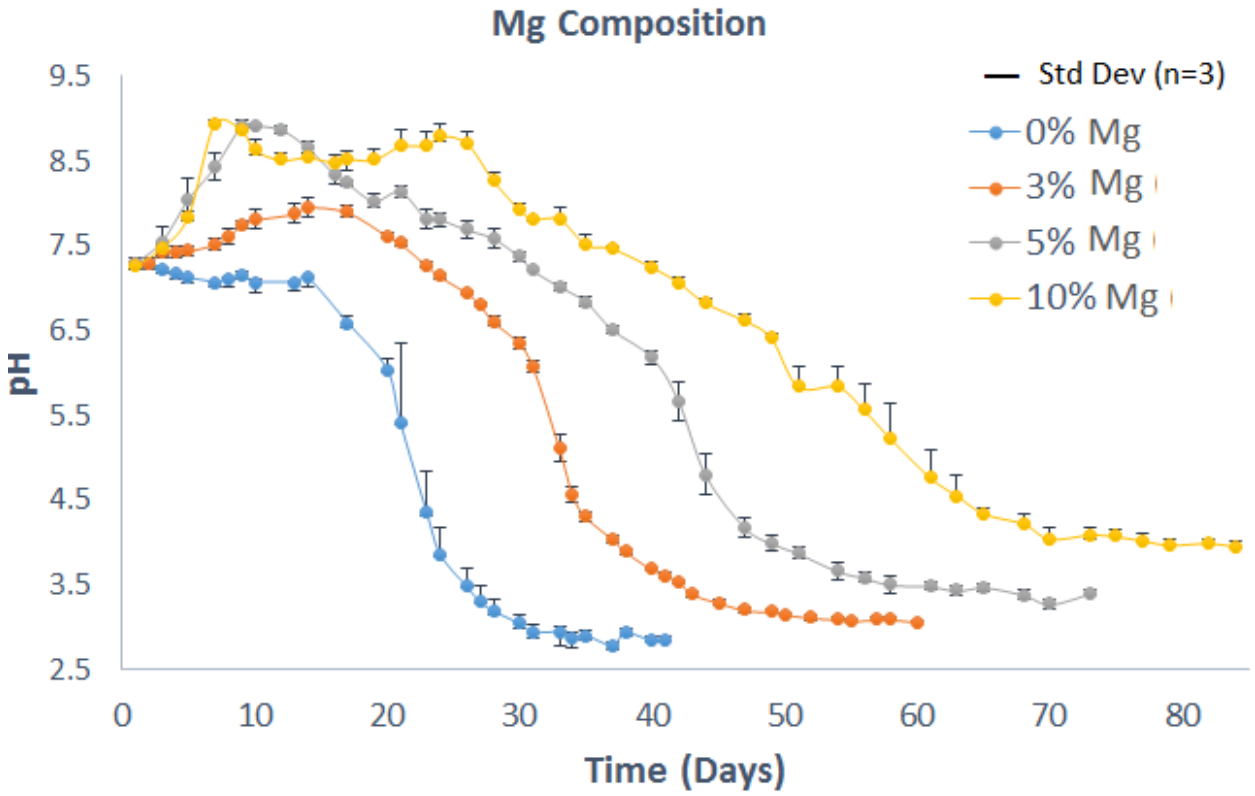

Figure 3.1-Results of the degradation study with a clear demonstration of the ability to control PLGA degradation behavior through varying Mg concentration.

3.2. In Vivo Simulation Degradation Study

In the body an implanted material is subjected to the forces of homeostasis and immune response. Materials that are capable of being broken down are exposed to constant sink conditions where the body attempts to break down and remove the foreign object through continuous cycling of the surrounding fluid. In order to simulate in vivo conditions a degradation study was carried out in which the solution was fully replaced once per week. Although this isn't a perfect model of body conditions, it is a starting point for assessing the effect of replacing

solution and is infrequent enough to allow for degradation to still occur in a reasonable amount of time.

Only one composition of Mg was selected to be compared to PLGA alone. 5%wt Mg was picked due to the observations made while observing the effects of different Mg concentrations on film properties. Plotting pH against time revealed very different behavior between the two samples. The plot shows that the addition of Mg is enough to buffer the acidic byproducts of PLGA maintaining a pH between ~6 and 8.5, while the PLGA sample had a pH range of ~4 to 7.5 (Fig. 3.2). It was also observed that the addition of Mg prolonged the rate of degradation. The PLGA sample degraded fully in 55 days, while the Mg containing sample fully degraded in 75 days. The two samples were also compared to the corresponding compositions from the original degradation study (Fig. 3.3). Just by replacing the PBS solution there was an observable change in degradation behavior. pH range was reduced, this suggested the expected, that in the body the range of pH will be reduced. It is also observed that the time for full degradation is longer in the samples where solution is replaced, also as expected. Based on these findings it can be concluded that when this composite is in the body it will degrade far more slowly and the pH range will be further compressed towards the physiological pH.

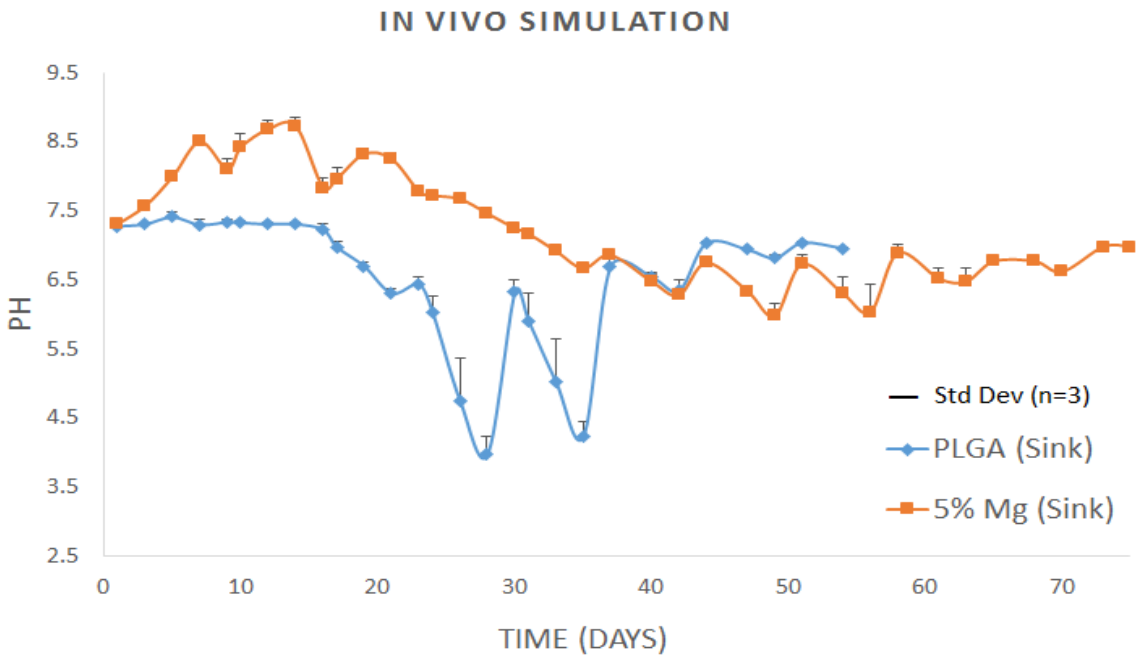

Figure 3.2- Results of the in vivo simulation degradation study. The plot shows that the presence of Mg in the composite can neutralize acidic byproducts of PLGA and slow the rate of composite degradation.

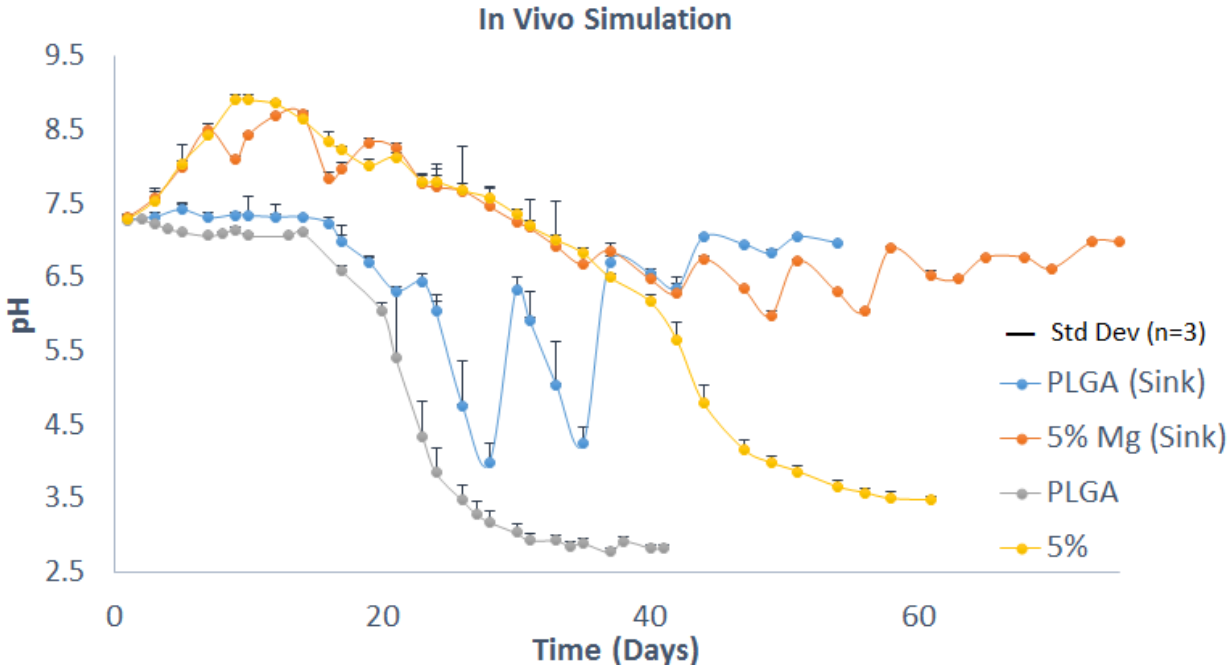

Figure 3.3- Comparison of the in vitro degradation study to the in vivo simulation degradation study results. In vivo degradation time is observably longer than in vitro.

3.3 Tensile Testing and Mg Concentration

To fulfill the first specific aim, tensile testing was performed to assess the influence of magnesium composition on film mechanics. Testing was performed on 13 mm by 6.5 mm and ~.05 mm thick samples. To prepare the samples for testing, they were lyophilized for two weeks immediately after fabrication. This was done to ensure removal of the solvent in its entirety. Presence of the solvent effects mechanics by making samples more elastic, so it is important to make sure they are properly dried. Samples containing 3%wt Mg, 5%wt Mg, and 10%wt Mg were compared, with n=6 per each composition. The strips were stretched to 50% elongation and elastic modulus was recorded. To assess the results, one way analysis of variance (ANOVA) was used to compare all three groups at once, and Tukey's Multiple Comparison Test was used to compare the groups to each other. ANOVA determined that the groups had very significant difference among them with p=.0006. Tukey's Test revealed that the elastic modulus of the 10% group was significantly greater than the 3% group, and very significantly greater than the 1% (Fig. 3.4). The 3% and 1% groups were not found to be significantly different. This demonstrates that with an increase in Mg concentration the mechanics of the films can be improved making them stronger. This also shows that it is possible to tune the mechanical properties of the composite through changing Mg concentration alone.

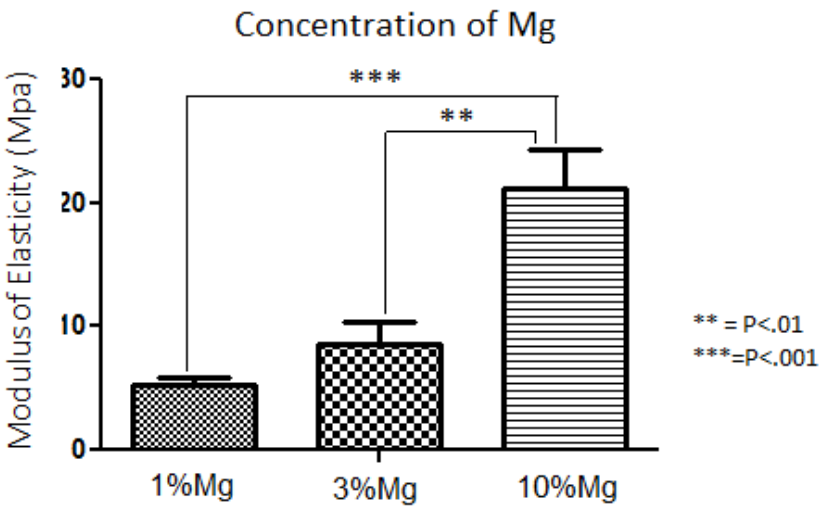

Figure 3.4-. Comparison of the modulus of elasticity between three compositions of Mg. The 10% samples show significant difference from the 3% and 1%, indicating that increase in Mg leads to increased strength of the composite.

3.4 Influence of Bubbles on Composite Mechanics

It was observed during the film fabrication process that an increasing concentration of Mg was shown to increase the formation of gas bubbles. This has several implications upon the mechanics of the composite. It is expected that an increase in number of bubbles in the composites would lead to weakening of mechanics. Having more gas pockets means the samples are structurally compromised with voids.

3.5 Discussion

Increasing Mg concentration was found to have several effects upon the properties of the composites. This demonstrates the ability for tunable degradation and mechanics of the films. By varying Mg concentration it is possible to prolong composite degradation allowing the body for a longer time to dispose of byproducts. It was also demonstrated that Mg directly influence mechanics of the composite allowing for composite strengthening. When used in osteogenic applications this could permit for fine tuning composites to be suitable for degradable bone scaffolds that degrade at the rate bone heals while matching the needed mechanics.

4. Chapter Four: Modification of Mg

4.1 Self Assembling Monolayers

To meet the second aim, Mg particle modification was investigated for the purpose of preventing particle agglomeration and prolonging their degradation. A literature search and review found a promising solution. A study by Grubac et al. demonstrated the use of alkylphosphonic acids as a self assembling monolayer (SAM) to coat the surface of a Mg alloy [23]. This coating was shown to increase the hydrophobicity of the metal surface.

Alkylphosphonic acids have long carbon chain tails that are hydrophobic, while the molecule head is hydrophilic. Since the Mg is hydrophilic as well, the alkylphosphonic acid head is attracted to the Mg particle surface. Since the hydrophilic head is in toward the particle, the hydrophobic tail extends outward. It was hypothesized that phenomenon could be utilized to form a hydrophobic coating on our Mg particles. By having the coating in place it would be expected that the particles would be protected from water exposure, and thus prolong their degradation. It is also expected that the modification would allow for the Mg particles to be better integrated into the PLGA matrix. Since the hydrophobic tail extends out from the molecule and the matrix is hydrophobic as well, it is expected that the particles will become integrated into the matrix as shown in Figure 4.1. This integration will prevent the Mg particles from being freely passed from the matrix thus preventing them from agglomerating.

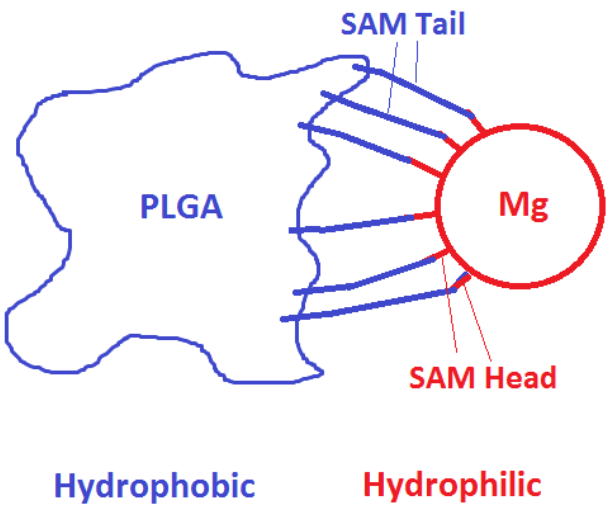

Figure 4.1- It is expected that the Mg particles will be integrated into the matrix by hydrophobic tails of the SAM layer interacting with the hydrophobic matrix.

4.2 Modification Process Overview

The modification process begins with dissolving the acid in ethanol. The Mg particles are then introduced and the solution is mixed to coat the particles. The liquid is then filtered out and the Mg particles are heated. The particles are heated at 120°C for at least six hours. The heating done to improve the strength of the modification.Grubac et al. demonstrated that heating for 24 hours improves the bond strength by converting the phosphonic acid coating to a phosphonate [23].

The acids decylphosphonic acid (DP) and octadecylphosphonic acid (ODP) were selected based on their success in the literature. DP has a 10 carbon chain, while ODP has an 18 carbon chain. ODP was demonstrated to have a greater hydrophobic effect, demonstrated by a higher contact angle with water when compared to the shorter chain acid [23]. This is likely due to the longer chain keeping water farther from the metal surface.

During the film fabrication process it was hypothesized that the exposure of chloroform to the modified particles could be disrupting the bonds. To reduce this risk, the film fabrication protocol

was modified so that the Mg particles were added 45 minutes into mixing so that they would only be exposed to the high volume of solvent for a limited time.

4.3 Modification Influence on Agglomeration

To assess if the Mg particles were successful at preventing particle agglomeration, compositions containing both the DP modification and the ODP modification were compared to a sample containing unmodified Mg particles. Mg concentrations of 1%wt Mg, 5%wt Mg, and 10%wt Mg were observed at 100x magnification (Fig. 4.2). There was no clear differences observed at 1% and 5% but at 10% there was a clear reduction in gas bubble formation with both reduced bubbles and reduced bubble size. This however is indicative that the modification may be preventing or delaying particle degradation. It was difficult to determine if particle agglomeration had actually been reduced because during the modification process it was observed that the solvent was causing Mg particles to stick together in clumps. When viewing the films micrographs it is difficult to determine the difference between these clumps and agglomerated particles.

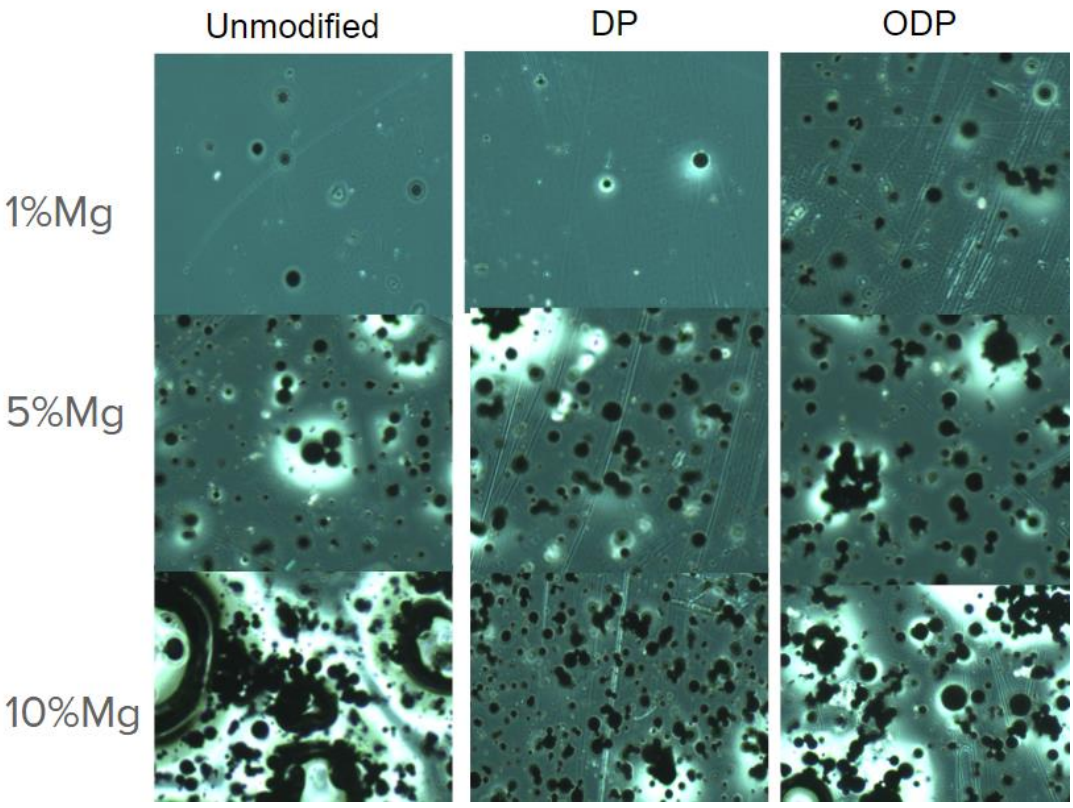

Figure 4.2-A comparison of Mg to Mg modified with DP and ODP. There was an observed reduction in gas bubble formation in the 10% samples (bubbles appear with white outlines).

4.4 Degradation Study on Modification

To determine if there was an influence of modified particles on composite degradation behavior a degradation study was performed. Samples of the same concentrations as the original degradation study were used (1%, 3%, 5%, and 10%wt Mg) for both DP and ODP. The results were compared to unmodified Mg (Fig. 4.3, Fig. 4.4, Fig. 4.5, and Fig. 4.6). The modified samples at 1% and 3% showed no clear difference in behavior from the unmodified group. At 5%, it is observed that the modified samples have slightly elevated pH during PLGA bulk degradation suggesting modification slightly delayed degradation. However this trend is reversed at 10% with the unmodified sample performing slightly more desirably. From these results it is not possible to make a conclusion about the success of modification. It is possible

that modification was successful but it is not having a strong enough effect to produce the composite behavior that was expected.

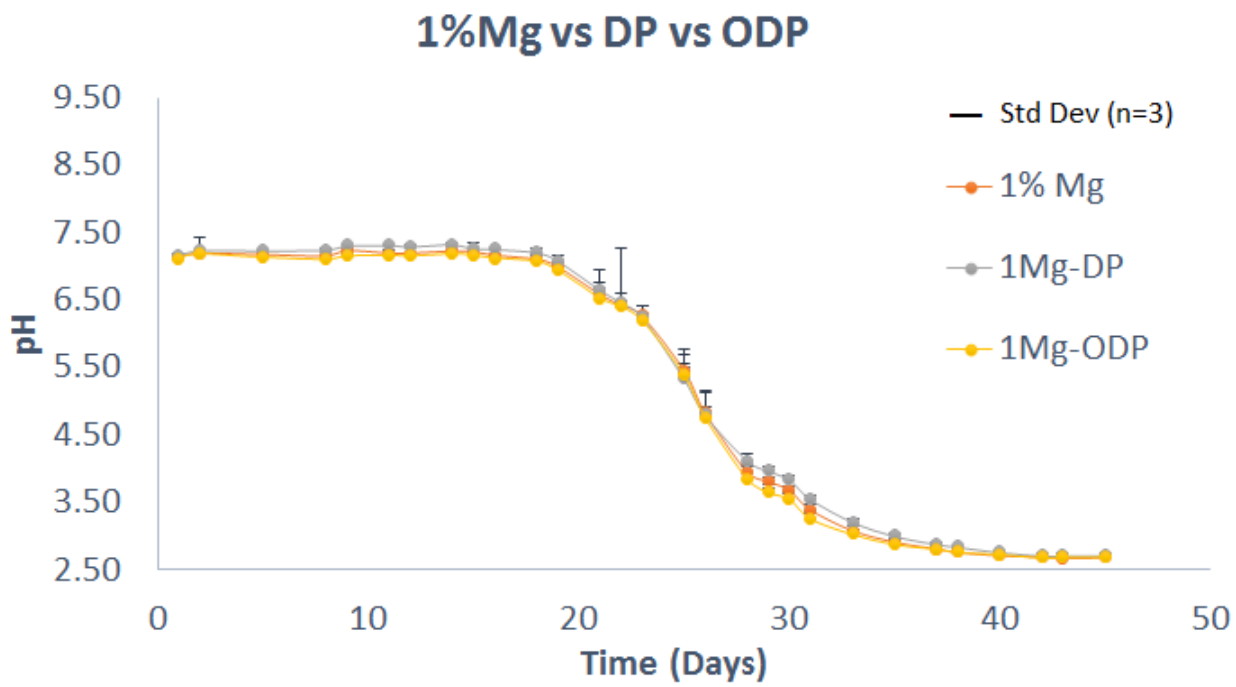

Figure 4.3- A comparison of 1%wt Mg against modified Mg samples of the same concentrations. There appears to be no notable difference.

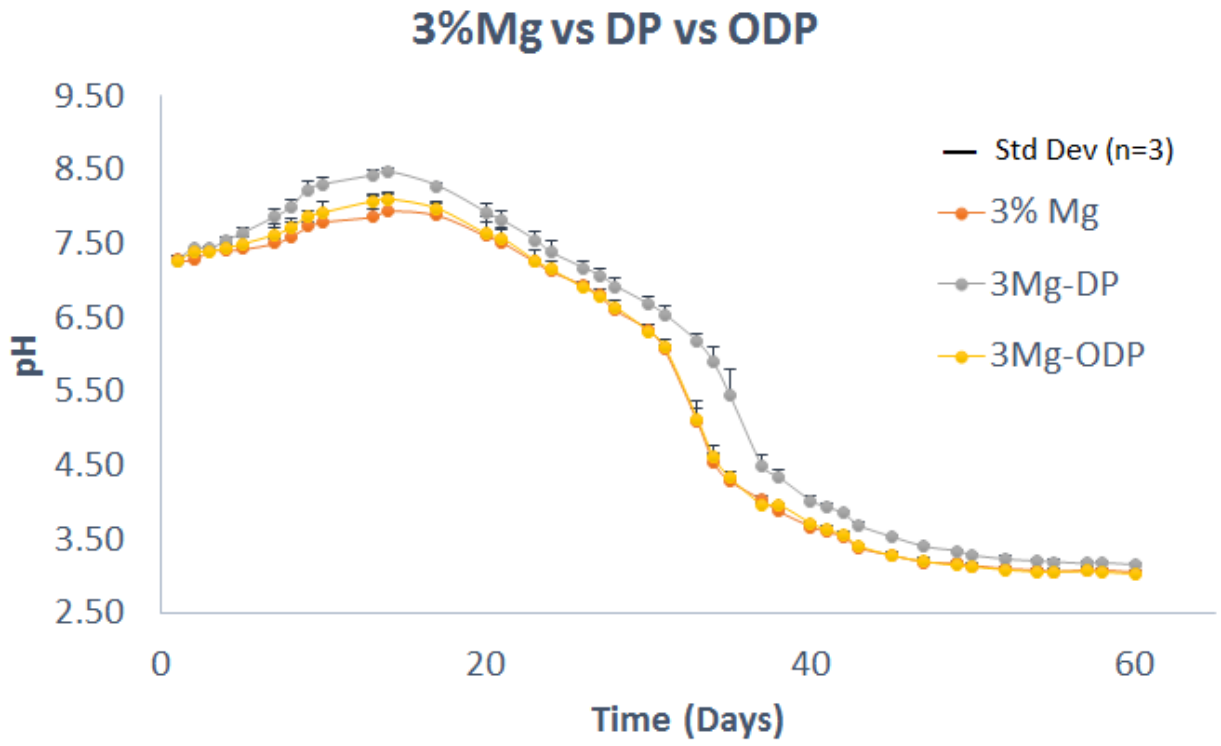

Figure 4.4- A comparison of 3%wt Mg against modified Mg samples of the same concentrations. There appears to be no notable difference.

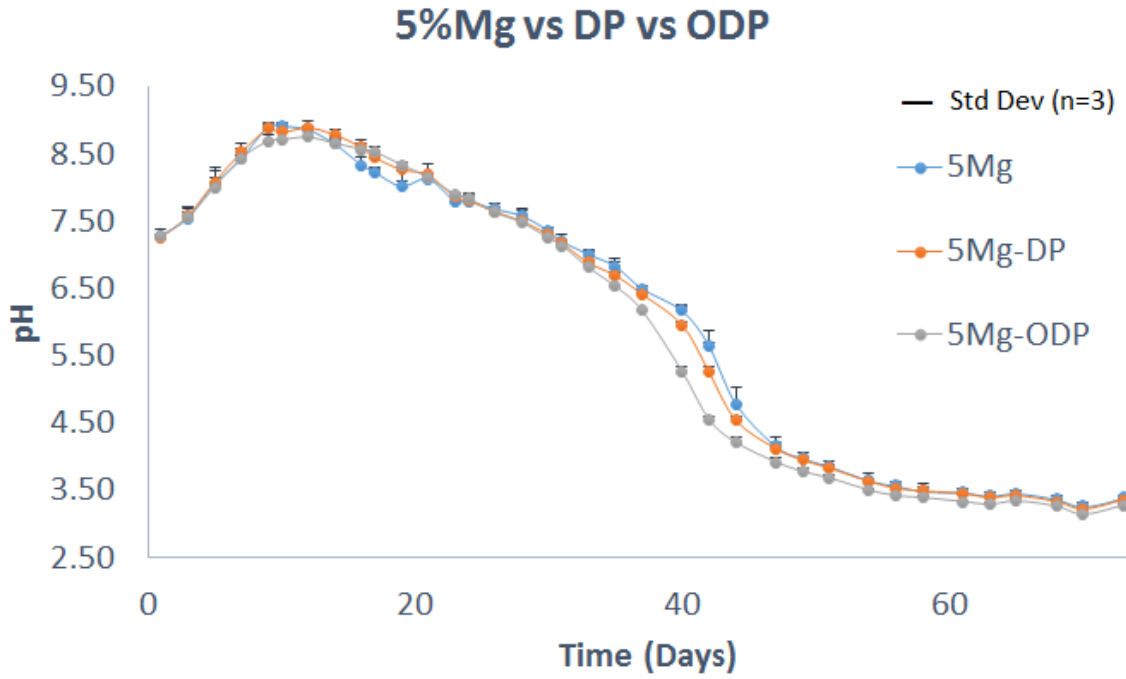

Figure 4.5- A comparison of 5%wt Mg against modified Mg samples of the same concentrations. There appears to be a slight difference during bulk degradation of PLGA (~day 40). .

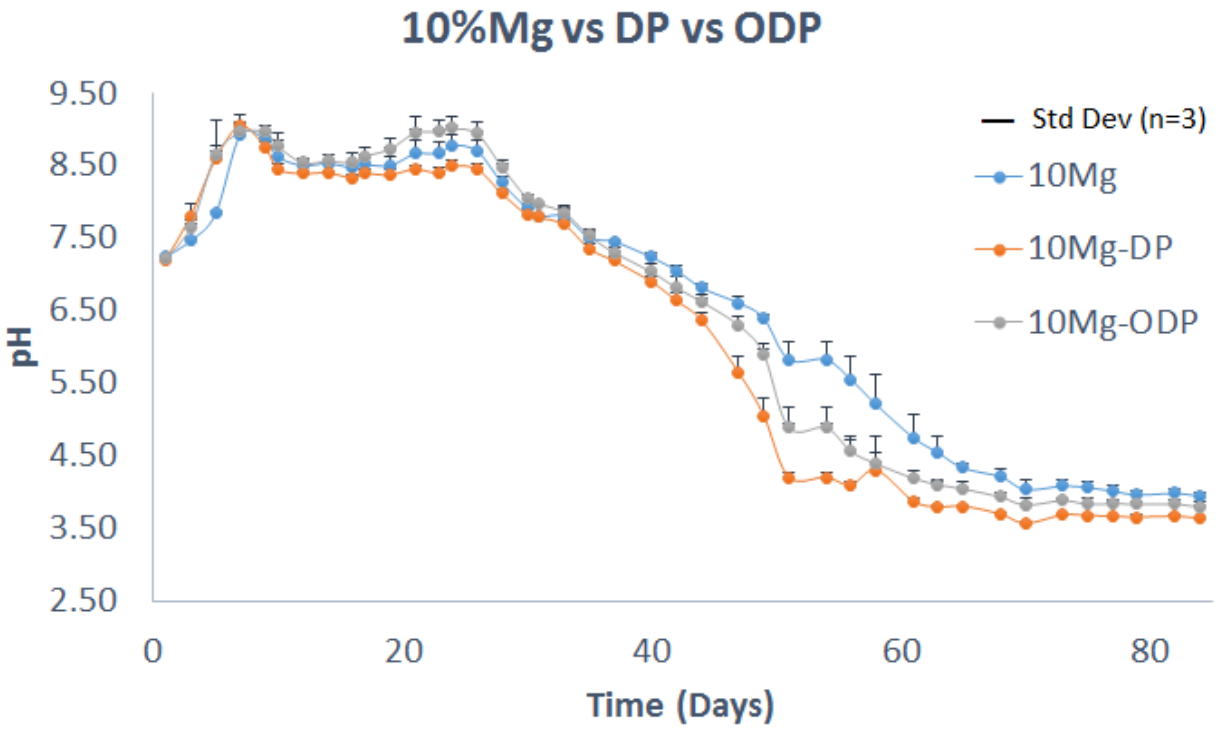

Figure 4.6- A comparison of 10%wt Mg against modified Mg samples of the same concentrations. There appears to be a slight difference during bulk degradation of PLGA (~day 50-60), the unmodified sample group has slightly higher pH.

4.6 Tensile Testing and Mg Modification

The improved integration of the particles into the matrix is expected to lead to improved composite mechanics. In addition the improved particle integration is expected to reduce particle agglomeration and improve uniform particle dispersion which would improve composite mechanics. In order to assess the influence of the modification upon composite mechanics, tensile testing was performed on strips of film. Composite compositions of 3% and 10% were compared (Fig. 4.7). Samples were compared once again using ANOVA and Tukey's Test. At 3% there was no significant difference shown by ANOVA nor by the Tukey's Test. However, at 10% there was shown to be a significant difference (p=.0004) by ANOVA, and significant differences between 10%Mg and 10%ODP, and 10%DP and 10%ODP. These results provide mixed implications, there may or there may not be an association between modification and

mechanics. There may also only be an effect at higher concentrations of Mg, further testing is needed.

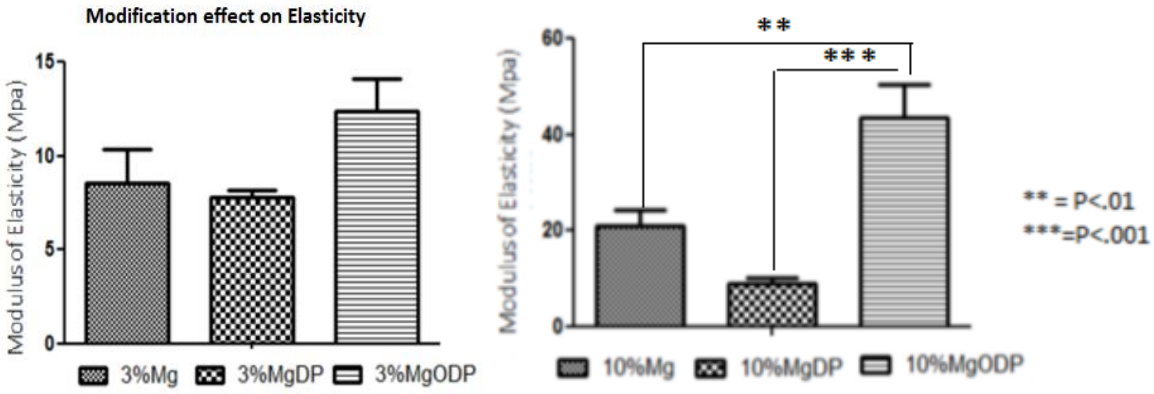

Figure 4.7- Results of tensile testing of the modification at 3% and 10%. (n=5)

4.7 FTIR

Fourier transform infrared spectrometry (FTIR) was used to assess whether modification was successful. FTIR measures the absorbance of light by a material which allows for determination of molecular composition and structure. Samples of modified Mg were analyzed and a measurement from unmodified Mg was subtracted from the reading. The resulting plots are shown below for DP modified Mg (Fig. 4.8) and ODP modified Mg (Fig. 4.9). Peaks at ~2900 and ~2850 are indicative of a methylene group. The intensities of the peaks are not very large but they still suggest the presence of some degree of successful modification. Further testing must be done with alternative methods to confirm this.

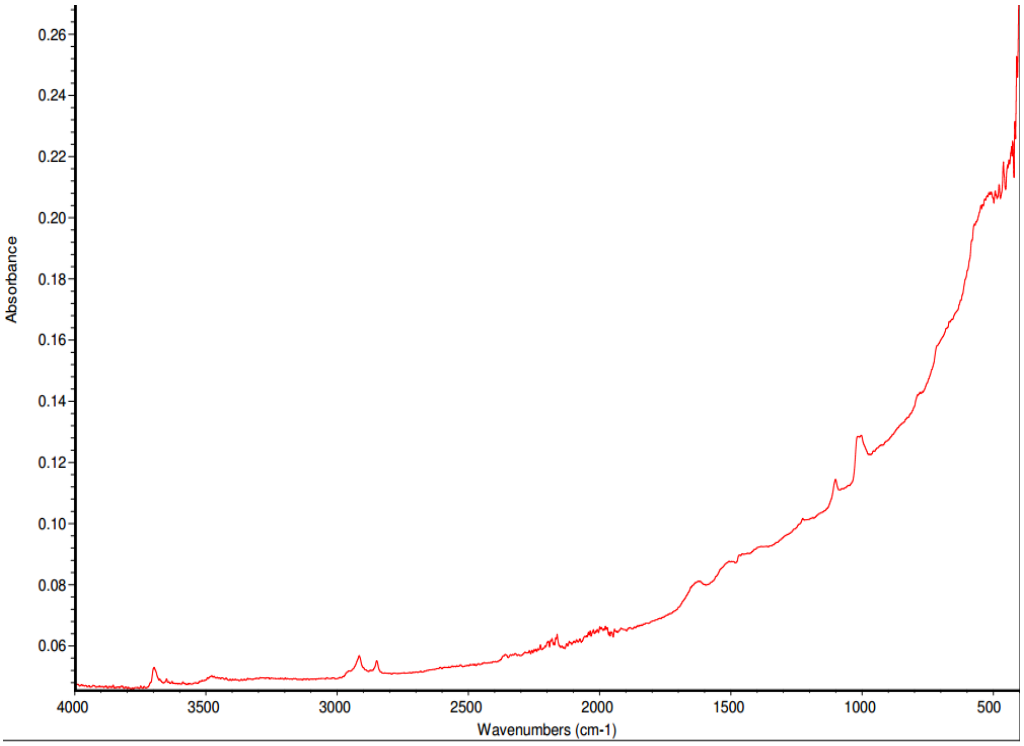

Figure 4.8-The resulting difference in FTIR spectra between Mg and Mg modified with DP.

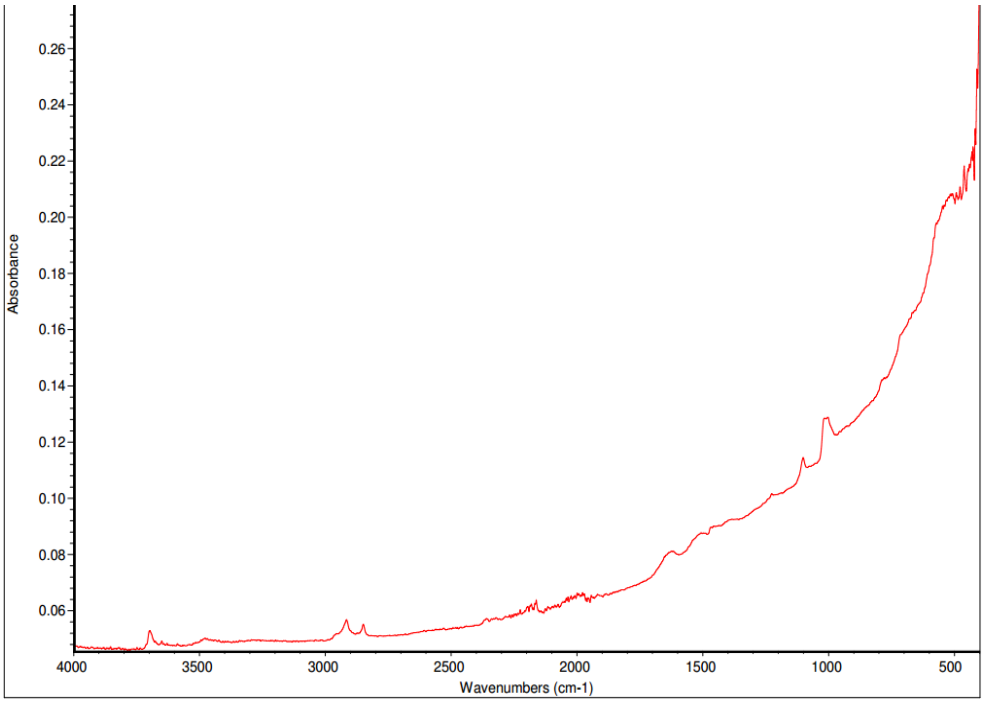

Figure 4.9- The resulting difference in FTIR spectra between Mg and Mg modified with ODP.

4.8 Saturation of Modification Solution

Since the results of the degradation study, imaging, mechanical testing, and FTIR were inconclusive, the method of modification was reevaluated. The conclusion was reached that only some of the Mg particle surfaces were being modified. It was then suggested that the factor influencing this was the concentration of the acid in the modification solution, since this variable had not been controlled during the process. ODP was found to saturate at 30 mM, DP however due to its lower molecular weight was found to easily exceed this this concentration and was able to reach 200 mM and greater. A hypothesis was developed; by using a shorter acid at a higher concentration more acid chains could be available in solution leading to more of the Mg surface getting coated (Fig. 4.10). This however leads to a tradeoff with hydrophobic strength, shorter chains would be expected to have weak hydrophobic forces.

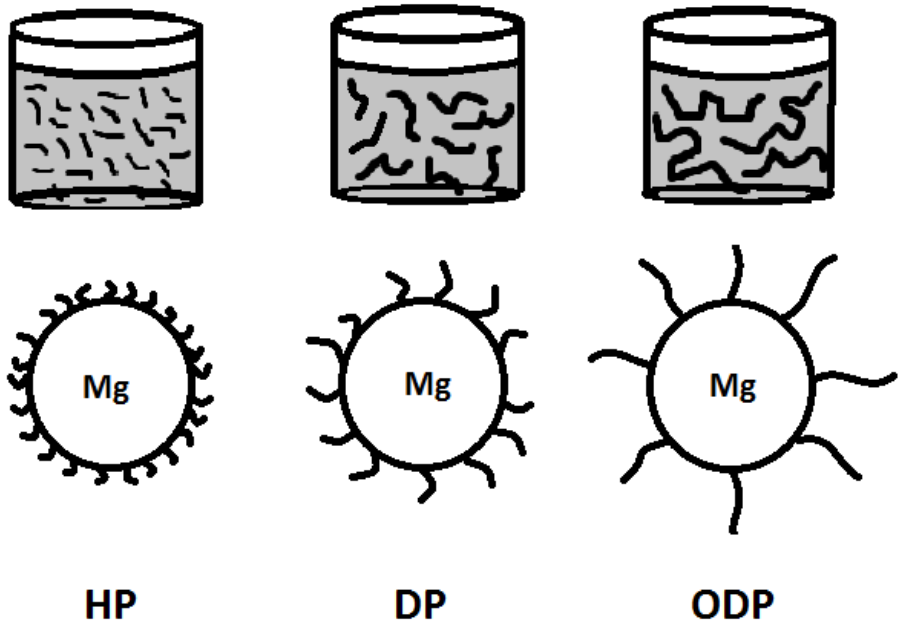

Figure 4.10- It was hypothesized that by using a shorter acid, more acid chains could be dissolved in solution (at the saturating point) allowing for more of the Mg particle surface to be coated.

To assess this hypothesis a third acid was introduced, hexylphosphonic acid (HP), with a six carbon chain. The three acids were compared by degradation, first by comparing all three at the same concentration of 30 mM (ODP saturation) then comparing HP to DP at over five times the concentration (200 mM). These two comparisons assess the influence of the hydrophobic chain length. The results of the degradation studies are shown in Figure 4.11. When plotted, the results show that at 30 mM HP and DP performed very similarly but ODP performs slightly better. At 200 mM DP performed the same as HP up until the PLGA bulk degradation period at which point it showed a slightly more basic pH and prolonged degradation. At both concentrations, when compared to unmodified Mg, all the acids had a more basic pH and a comparatively prolonged degradation. Grubac et al. demonstrated that at the same concentration, an ODP coating on a Mg surface was more hydrophobic than a DP coating [23]. This appears to support the results of the degradation study.

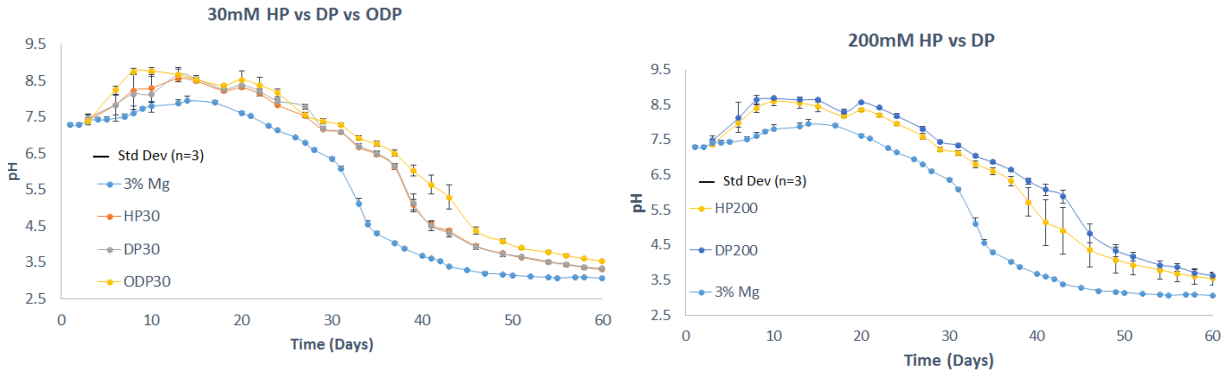

Figure 4.11- A comparison of the length of the acid and the degradation behavior.

A third analysis was then made comparing HP and DP against themselves at the two concentrations to assess if there is an influence of concentration on degree of modification. When plotted the results showed comparable degradation up until the bulk degradation phase, at which the lower concentrations both had more acidic pHs and degraded slightly faster (Fig. 4.12). Although there is a slight difference, it is hard to say whether the modification is truly

responsible. Further testing will be needed in which all three acids are brought to their saturation points during the modification process.

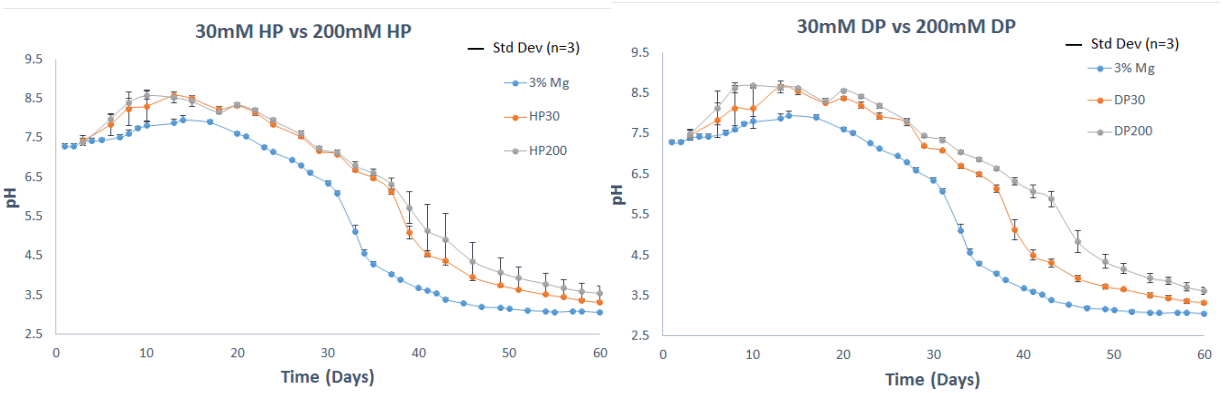

Figure 4.12- A comparison of the concentration of acid used to modify the Mg particles.

4.9 Discussion

Modification was met with mixed results. Although it appeared to reduce hydrogen gas formation and bear some influence over degradation it is still not clear whether it was successful. Advanced methods of analysis such as x-ray photoelectron spectroscopy (XPS) will be used in the future to confirm. It is also possible that modification is only in part successful, covering only a fraction of the Mg surface. Some evidence of improving degradation through manipulation of acid chain length and concentration of acid were found. More research will have to be done to compare the performance of all three acids at their solution saturation points. If the modification can be properly controlled it can allow for an extra degree of Mg particle degradation protection which will prevent Mg from degrading so quickly. This will allow for longer buffering effect against acidic byproducts of PLGA and prolong degradation of the composite.

Chapter Five: Summary

5.1 Conclusion

This study demonstrated that through varying the Mg concentration of PLGA-Mg composites, byproduct formation and degradation rate can be controlled. As the concentration increases the time it takes the composition to degrade increases as well as the overall pH is elevated. In addition, through simulating in vivo sink conditions, it was proven that Mg oxide byproduct can be used to neutralize acid byproducts of PLGA. A sample containing 5%wt Mg was compared to a PLGA only sample. The 5% degraded fully in ~75 days compared to the PLGA which degraded fully in ~35 days. The pH range was also more neutral for the Mg sample (6-8.5) while the PLGA reached an acidic pH level (4-7.5). Through mechanical testing it was found that as Mg concentration increases, elastic modulus increases as well. 10%wt Mg samples were shown to have a significantly greater mean elastic modulus compared to that of 3% Mg ($P<.01$) and 1%Mg ($P<.001$). Attempted use of particle modification to improve particle dispersion and prolong particle degradation showed mixed results. Micrographs of samples revealed a decrease in gas bubble formation in 10%wt Mg samples with modified Mg. FTIR found low intensity peaks at wavelengths indicating methyl group presence, suggesting some degree of successful modification. Degradation studies did not demonstrate any observable differences of the modified samples beyond a slightly slower bulk degradation phase of PLGA. Further investigation of the alkylphosphonic acid modification and alternative modification methods will be carried out in the future. Despite this setback the ability to control the composite's properties has been demonstrated to be feasible through other means. Through its tunable properties this composite can be used in a range of applications as a novel biomaterial without the concern for degradation related issues.

5.2 Future Goals

Moving forward there are still several important questions to address. To do this a cell study will be performed with MC3T3 (osteoblast progenitor) cells seeded onto 2D scaffolds of the composite. The initial tests will assess the performance of 5%wt Mg composites against PLGA alone, and 5%wt ZK61. Assays on cell proliferation will be performed to confirm neutral pH of the composite byproducts. A second assay will be performed to quantify mineralization to determine the osteogenic benefits of Mg. Lastly an assay will be performed to assess if the addition of Zn through the alloy introduces a significant level of antimicrobial defense.

The results of the modification were inconclusive. As previously stated FTIR may be insufficient to determine if the modification is present on the particle surface. A different means of assessment will be used, XPS. XPS will be able to provide insight into the surface chemistry of the particles, by telling whether the alkylphosphonic acids are present.

When assessing whether modification had any influence on particle agglomeration it was observed that modification process had lead to particle clumping. This clumping was nearly indistinguishable from the agglomeration so it was difficult to assess if agglomeration had truly been prevented. Future work will investigate the prevention of particle clumping during modification through careful control of the modification process. In addition alternative methods of surface modification will be investigated and compared to the use of alkylphosphonic acids.

Lastly, the hypothesis that saturation of the modification solution having influence on the degree of particle surface coating, will be further explored. Both HP and DP will be brought to their saturation points and a degradation study will be carried out where they are compared to ODP also at saturation concentration. The results of this study will provide valuable insight into modification process allowing for improvement in the development of the composite.

References

[1] Kohane DS, Langer R. Polymeric biomaterials in tissue engineering. Pediatric research. 2008 May 1;63(5):487-91.

[2] Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. Journal of controlled release. 2012 Jul 20;161(2):505-22.

[3] Gentile P, Chiono V, Carmagnola I, Hatton PV. An overview of poly (lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering. International journal of molecular sciences. 2014 Feb 28;15(3):3640-59.

[4] Böstman O, Pihlajamäki H. Clinical biocompatibility of biodegradable orthopaedic implants for internal fixation: a review. Biomaterials. 2000 Dec 15;21(24):2615-21.

[5] Pietrzak WS, Kumar M, Eppley BL. The influence of temperature on the degradation rate of LactoSorb copolymer. Journal of Craniofacial Surgery. 2003 Mar 1;14(2):176-83.

[6] Zheng YF, Gu XN, Witte F. Biodegradable metals. Materials Science and Engineering: R: Reports. 2014 Mar 31;77:1-34.

[7] Di Mario C, Griffiths HU, Goktekin O, Peeters N, Verbist JA, Bosiers M, Deloose K, Heublein B, Rohde R, Kasese V, Ilsley C. Drug- eluting bioabsorbable magnesium stent. Journal of interventional cardiology. 2004 Dec 1;17(6):391-5.

[8] Staiger MP, Pietak AM, Huadmai J, Dias G. Magnesium and its alloys as orthopedic biomaterials: a review. Biomaterials. 2006 Mar 31;27(9):1728-34.

[9] Smallman RE, Ngan AH. Physical metallurgy and advanced materials. Butterworth-Heinemann; 2011 Feb 24.

[10] Witte F. The history of biodegradable magnesium implants: a review. Acta biomaterialia. 2010 May 31;6(5):1680-92.

[11] In vitro degradation and cell attachment of a PLGA coated biodegradable Mg–6Zn based alloy J. N. Li

[12] Hornberger H, Virtanen S, Boccaccini AR. Biomedical coatings on magnesium alloys–a review. Acta biomaterialia. 2012 Jul 31;8(7):2442-55.

[13] ZHAO CL, ZHANG SX, HE CH, LI JN, ZHANG BL, ZHANG XN. Study of PLGA coating on bio-absorbable Mg alloy [J]. Journal of Functional Materials. 2008;6:030.

[14] Ma R, Lai YX, Li L, Tan HL, Wang JL, Li Y, Tang TT, Qin L. Bacterial inhibition potential of 3D rapid-prototyped magnesium-based porous composite scaffolds--an in vitro efficacy study. Sci Rep. 2015 Sep 8;5:13775

[15] Brown, Andrew, et al. "Porous magnesium/PLGA composite scaffolds for enhanced bone regeneration following tooth extraction." *Acta biomaterialia* 11 2015: 543-553.

[16] Liu H, Augello C, Zahedi A, Ethell I, inventors; THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, assignee. POLY (LACTIC-CO-GLYCOLIC ACID (PLGA) COMPOSITES WITH MAGNESIUM WIRES ENHANCED NETWORKING OF PRIMARY NEURONS. United States patent US 20,160,008,516. 2016 Jan 14.

[17] AZoM.com Staff Writers. Grade ZK61 Magnesium Alloy. http://www.azom.com/article.aspx?ArticleID=9967 (accessed 5/1/16).

[18] Li X, Li Y, Peng S, Ye B, Lin W, Hu J. Effect of zinc ions on improving implant fixation in osteoporotic bone. Connective tissue research. 2013 Aug 1;54(4-5):290-6.

[19] Chen D, He Y, Tao H, Zhang Y, Jiang Y, Zhang X, Zhang S. Biocompatibility of magnesium-zinc alloy in biodegradable orthopedic implants. international journal of molecular medicine. 2011 Sep 1;28(3):343-8.

[20] Yusa K, Yamamoto O, Fukuda M, Koyota S, Koizumi Y, Sugiyama T. In vitro prominent bone regeneration by release zinc ion from Zn-modified implant. Biochemical and biophysical research communications. 2011 Aug 26;412(2):273-8.

[21] Jin G, Cao H, Qiao Y, Meng F, Zhu H, Liu X. Osteogenic activity and antibacterial effect of zinc ion implanted titanium. Colloids and Surfaces B: Biointerfaces. 2014 May 1;117:158-65.

[22] Jain RA. The manufacturing techniques of various drug loaded biodegradable poly (lactide-co-glycolide)(PLGA) devices. Biomaterials. 2000 Dec 1;21(23):2475-90.

[23] Grubač, Zoran, et al. "Functionalization of biodegradable magnesium alloy implants with alkylphosphonate self-assembled films." *Materials Science and Engineering: C* 33.4 2013: 2152-2158.