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New approaches in evaluating metallic candidates for bioabsorbable stents

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A series of unconventional approaches has been developed at Michigan Technological University, which is able to screen candidate materials for use in bioabsorbable (or bioresorbable) stents by reducing the scale of necessary animal studies and the complexity of biocorrosion analyses. Using a novel in vivo approach, materials formed into a simplified wire geometry were implanted into the wall of the abdominal aorta of rodents for several weeks or months to measure the extent of in vivo degradation, quantify mechanical strength over time, characterize the resulting products, and assess biocompatibility. An in vitro method was developed to identify bioabsorbable candidate materials, reproduce the corrosion products formed in vivo, and predict the degradation rate of stent materials. To accomplish this goal, wires were encapsulated in an extracellular matrix and corroded in cell culture media in vitro. Encapsulation of the wires in vitro was necessary in order to mimic in vivo stent encapsulation within a neo-intima. Alternatively, accelerated in vitro corrosion for materials with very low corrosion rates was accomplished by exposing fibrin-coated wires to a steady flow of cell culture media. After in vivo and in vitro tests, wires were subjected to tensile testing to quantify the rate of material degradation and loss of mechanical strength.

1. Introduction and background

Coronary artery disease often culminates in vascular occlusion by way of conditions such as atherosclerosis. The occluded arteries must be repaired by coronary angioplasty, in which the blockage is compressed radially and the vessel mechanically widened to restore blood flow. Stents are commonly used in conjunction with balloon angioplasty to provide structural support and prevent collapse of the vessel after the balloon is withdrawn. Although the benefits of cardiac stents are numerous and well-documented,¹² the scaffolding is not thought to play an important role after remodeling is complete. The short-term benefit can be overshadowed by longterm complications such as chronic inflammation,³ late stage thrombosis,⁴ and stent strut disruption.⁵ In addition, restenosis often follows conventional stent implantation and is caused by neointimal proliferation and migration.⁶⁷ Newer drug-eluting stents have reduced but not eliminated restenosis relative to bare metal stents.⁸

Bioabsorbable or "bioresorbable" or "biodegradable" stents have potential as alternatives for vascular scaffolding that circumvent many of the current, long-term health risks of conventional permanent stents.^{9 10} The ideal bioabsorbable stent would retain mechanical properties for approximately 6 months before being broken down, metabolized, and excreted by the body, allowing the vessel to function and remodel naturally.¹¹ Past investigations into bioabsorbable stent materials have focused on both polymeric and

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metallic variants. Poly-L-lactic acid has been shown to possess acceptable biocompatibility,¹² but a polymeric stent requires a greater strut thickness than most metal stents because of the polymer's lower relative strength.¹³ Other limitations reported with polymer stents include the inability to expand completely with balloon dilation¹⁴ as well as restenosis and smooth muscle hyperplasia rates similar to those observed for conventional bare metal stents.¹⁵ For these reasons, bioabsorbable metal stents are being investigated.

Generally, alloys based on elements that have physiological roles in the human body are likely to be biocompatible and are therefore suitable materials for constructing bioabsorbable stents. Specifically, magnesium (Mg) and iron (Fe) have been shown to hold promise due to their mechanical properties and purported biocompatibility of the base components.16-19 Other biologically important materials, such as calcium, would degrade too quickly if placed in a physiological environment. Fe has a high mechanical strength, which allows for production of stents with a broad range of inner diameters.²⁰ Both Fe and Mg metals have higher tensile strengths and elastic moduli than biodegradable polymers^{21 22} and can therefore provide a stiffer, stronger scaffolding to support the vessel and promote healing. Both metals exhibit mechanical behavior that is more predictable than that of polymers and the stent manufacturing industry has a wellestablished record of working with metallic materials, making the task of manufacturing a stent for deployment and arterial support relatively straightforward. Mg and its alloys have the additional benefit of inherent hypothrombogenicity,²³ which may explain the lack of thrombotic complications in past clinical trials.11

While the metallic stent materials are favored in several areas, implementation of these materials is not straightforward. Unfortunately, Mg alloys of commercial purity exhibit a relatively high rate of degradation and associated evolution of hydrogen gas.^{24 25} In addition, some trials have shown that Mg completely bioabsorbs as early as 60 to 90 days postimplantation.¹¹ Many groups are currently pursuing specialized alloys, coatings, and processing techniques aimed at slowing the physiological corrosion rate of Mg. Meanwhile, Fe has been reported to possess excellent biocompatibility, with no signs of local or systemic iron toxicity, in addition to slow degradation that prevents fragment embolization.²⁰ In contrast to Mg, Fe is generally believed to degrade too slowly to be used in its pure form in bioabsorbable applications.²⁷

2. State of the art in bioabsorbable metallic materials

The industrial and academic community's current research and development efforts are largely concentrated on either accelerating or decelerating the degradation of Fe and Mg stent materials, respectively. In the case of Mg, a reduction in the corrosion rate is badly needed, as some modern works have gone as far as to dismiss the possibility of Mg implants on the basis of hydrogen evolution alone.²⁸ Multiple

approaches have been taken to limit the degradation experienced by Mg stents, with limited success.^{29–31} Mg alloys containing rare earth (RE) elements, such as WE- and AE-series alloys traditionally used in high temperature applications where creep resistance is paramount,³² have become the focus for bioabsorbable stent development. These RE-bearing alloys have exhibited reduced degradation rates relative to other, more common Mg alloys.^{33 34} Non-conventional processing methods have been used by others to produce prototypical alloys that are described as "stainless magnesium" in a recent review by Atrens *et al.*³⁵ An example of this approach is the work conducted by Hoffmann and coworkers in developing solid solution Mg-Ti alloys through non-equilibrium processing, namely high-energy ball milling.³⁶ It appears that research is currently focused on alloying, as opposed to coatings, which have traditionally been geared towards automotive applications.³⁷

Aluminum-containing Mg alloys consistently perform well in in vitro corrosion studies intended to screen candidate materials.^{23 24} However, concerns about aluminum-containing alloys have been raised in recent years, prompted by reports from toxicology and neurology researchers who have identified the prospect of aluminum toxicity.^{38 39} These toxicological concerns, however, are not founded in data related to bioabsorbable implants and often use much higher aluminum concentrations than one would expect to be released from a degrading stent. Furthermore, the aluminum introduced in many of these studies comprises trivalent aqueous aluminum, not metallic specimens that are allowed to passivate and/or dissolve naturally, as would occur in an implant scenario. Moderate doses of aluminum that produce low serum concentrations are readily eliminated via renal action, indicating that dismissal of this series of alloys may be premature.^{40 41}

In addition to alloy development work, attempts have been made to clarify the mechanism that governs magnesium degradation in a pseudophysiological environment. The work that has been done thus far points to direct participation by chloride, bicarbonate, phosphate, and calcium ions in the corrosion process as well as less direct participation by other ions, blood/cellular constituents, and dissolved gasses such as CO_2 .^{42 43} More broadly, though, magnesium deterioration depends on several parameters: makeup of the alloy, metallurgical treatments, surface treatment (coatings, ion implantation, etc.), species and concentration of the interacting electrolytes, chemistry and biology of the environment, and the transport and metabolic phenomena associated with the reactants and the products of the corrosion reaction.⁴⁴

Although there is less interest in iron stent materials, recent work on iron has aimed to increase the corrosion rate through creation of Fe-Mn alloys containing up to 35 wt.% Mn.^{45 46} This material was successful in increasing the corrosion rate of the material significantly. However, for reasons discussed in the section on in vivo evaluation of materials, the authors have evidence showing that pursuit of Fe-based stent materials may be a fruitless endeavor.

3. The need for improved testing methods for bioabsorbable materials

The enormous number of alloys and coating materials that could potentially be tested in pursuit of an ideal bioabsorbable implant material exceeds current resources for evaluating their corrosion behavior, making this screening process excessively time-consuming and costly. Because of this, the authors found it necessary to pursue the development of testing methods for evaluating candidate materials that have reduced complexity and increased cost-effectiveness. This has resulted in a suite of complimentary in vivo, in vitro, and analysis techniques, which are described together in this contribution. The methods are most useful when used concurrently, as illustrated in Figure 1.

Both in vivo and in vitro methods use a sample with a simplified wire geometry, which allows for easy implantation, explantation, sample handling, corrosion characterization, and mechanical evaluation. The wires used in this work were 0.2–0.3 mm in diameter, high-purity, and stock with an as-drawn temper (from Goodfellow; Huntingdon, England). Methods have been presented by Seitz *et al.* by which one may form wires of a comparable diameter from a billet of experimental or custom alloy.^{47 48} Other sample morphologies, such as "strips" (rectangular prisms) of material have been used in other preliminary studies,⁴⁹ as well as in producing some preliminary data presented by the authors in this report. However, the circular wire geometry is generally more conducive to batch production, testing, and analysis.

4. Methods for testing bioabsorbable materials

4.1 Arterial implantation using a rodent model Some of the most important information on bioabsorbable implants with respect to biocorrosion, biocompatibility, and localized effects of degradation (i.e. corrosion product migration, cytotoxicity, etc.) has been generated from physiologically relevant in vivo studies. To date, relatively few reports have been published on the behavior of materials in vivo in comparison to the body of literature that has grown on materials corroded in vitro. In vivo evaluations of interventional cardiology devices are more often conducted by industrial labs than by academic investigators, and involve large animals such as pigs. The unfortunate consequence of these circumstances is a lack of disclosure and interinstitutional cooperation due to intellectual property and competitive concerns. The typical cost for a 6-month in vivo study using a mini-swine subject and deployable stent would be at least \$10 000 to \$12 000 per animal.⁵⁰ Using the rodent implantation technique, by the authors' estimate, the cost is reduced to less than 10% of that for the mini-swine study including the surgical procedure, animal housing, and all postexplantation analysis. Certainly, in latestage trials where more detailed human health-related questions must be addressed, the mechanically expanded stent inside the porcine coronary artery is preferable to the implantation of wire geometries into small rodent arteries. However, the rodent model is more

accessible to most universities' materials research programs and may be a more reasonable approach for evaluating degradation behavior as a pre-screening model to identify promising stent materials prior to stent manufacturing and large animal implantation studies.

Most of the in vivo works in the scientific literature related to bioabsorbable materials investigate degradation of orthopedic implants^{34 51 52} or corrosion in a subcutaneous environment,⁵³ which can be easily accomplished in small animals such as rabbits or guinea pigs. However, these models are not suitable for determining the lifespan and behavior of a vascular device due to large differences in biological milieu between the arterial, orthopedic, and subcutaneous tissues. For this reason, the authors have developed an animal model in which a wire of the candidate material is implanted into the rat abdominal aortic artery, positioned either in direct blood contact inside the lumen or in the arterial wall, as shown schematically in Figure 2. A 0.2-0.3 mm diameter wire is preferred because it is commercially available and is approximately the same diameter as a typical stent strut. A 20-30 mm sample length is well suited for characterization and handling; 20 mm for Mg and 30 mm for Fe is now preferred for mechanical testing (discussed later). Smaller, typically 5-10 mm, segments of wire may be simultaneously implanted at a different location along the artery to provide samples for characterization with optical microscopy (OM), scanning electron microscopy and energy dispersive spectroscopy (SEM/ EDS), Raman spectroscopy, Fourier transform infrared spectroscopy (FT-IR), etc. The abdominal rat aorta can accommodate roughly 30 mm of combined wire length, although longer wire lengths than 20 mm are more challenging than smaller lengths to implant. Specific preparation, sterilization, and related procedures can be found in the authors' previous contribution.54

The position of the wire in the arterial wall or in the lumen determines the nature and composition of the wire's environment, which greatly influences the corresponding degradation that occurs. Positioning the wire in the lumen replicates the conditions a stent experiences during its early life, before it becomes encapsulated in neointimal tissue. The material is exposed to flowing blood, including circulating immune cells, platelets, constituent biomolecules, and dissolved gasses. Cellular action does not have a large effect on Mg corrosion at this stage, as the surface does not typically become encapsulated by a thrombus.²³ Conversely, implantation in the arterial wall works to mimic the environment, a stent material is subjected to after it has been encapsulated by neointimal tissue. The wall environment includes modified, generally reduced, mass transport properties, cyclic stresses, cellular action by vascular cells, and phenomena specific matrix/ionic milieu. Since the authors have found that the wire undergoes the bulk of its degradation after being encapsulated, use of the rodent wall model is preferred for simplicity in preliminary work.

During implantation in the wall environment, the wire is pushed through the adventitia and into the media. Care is taken to avoid



Figure 1. Flowchart showing how a candidate bioabsorbable material can be tested and compared with existing materials using the suite of methods (in vivo, static and dynamic in vitro, and quantitative analysis through tensile testing) presented in this contribution.



Figure 2. In situ photographs of the wire states upon implantation (a) and explantation (b), after 3 weeks' degradation, are presented. The schematic (c) shows the position of the wall implant wire in the arterial media and the luminal implant wire positioned away from the intima.

puncturing the intima during surgery, so that the sample is implanted within the media, as illustrated in Figure 2(a) and the schematic Figure 2(c). The luminal implants require that the intima be punctured during implantation so that the wire can be threaded through the artery, as in the schematic (Figure 2(c)). When Mg implants are removed from the euthanized rat, corrosion is often obvious on the wire's surface, as in Figure 2(b), but corrosion product is not typically observed in the tissue. Further discussion on this issue is presented later in regard to Figure 5. The media and adventitia tissues do not adhere well to pure, corroded Mg wire, allowing for easy extrication of the sample and preservation of the corrosion products. The relatively poor adhesion may be due to the nature of the corrosion layer, which has been hypothesized to comprise a strongly hydrated layer of amorphous and/or semicrystalline Mg(OH), when corroded in water.⁵⁵

Fe exhibits very different corrosion behavior when implanted in the arterial wall for an extended period. Elemental (EDS) maps of a 9-month wire/tissue cross section are presented in Figure 3. These maps show that a passive layer initially formed on the wire but was later "pushed away" from the Fe core. This passive layer is displayed as a bright green/yellow area on the calcium/phosphorus map. From the iron/oxygen map, one can see that a significant amount of iron oxide has developed between the passive layer and the remaining Fe. These results are indicative of the evolution of a voluminous Fe-O corrosion product, which was confirmed by histological examination⁵⁴ and by results presented later in Figure 5. The corrosion products do not appear to be excreted nor metabolized at an appreciable rate. Instead of dissolving in an apparently harmless manner, pure Fe seems to be unsuitable for application in interventional cardiology due to the accumulation of voluminous corrosion products in the arterial walls of rats.⁵⁴ Mg has not exhibited the same buildup of corrosion product, but instead appeared to dissolve harmlessly,⁵⁴ suggesting that Mg and its alloys are currently the most promising bioabsorbable materials.

4.2 Use in candidate material evaluation

The rodent arterial implant model was only recently introduced, so there is limited experimental evidence showing that the technique is useful for evaluating novel bioabsorbable materials. The authors show here, for the first time, that the arterial implant model can, in fact, be used to quantify corrosion experienced by alloyed Mg implants. A section of hot-rolled Mg-9 wt.% aluminum (A9 in conventional notation)⁵⁶ foil was examined. Due to relatively low ductility, A9 (similar to AZ91) is difficult to draw into a round wire, so a strip of material was instead prepared with a low-speed diamond saw and fine-grit SiC sandpaper. A section measuring approximately 18 mm long, 600–700 μ m wide, and about 200 μ m thick was implanted into the arterial wall in a manner identical to that described above.

After 3 months, the sample was removed and the explant was examined in whole and in cross section using OM and SEM/EDS. The results are presented in Figure 4 with an optical micrograph of the entire strip presented in Figure 4 (left), an optical micrograph of the cross section presented in Figure 4 (top), and two EDS spectra corresponding to the metallic core (left spectrum) and the corrosion product (right spectrum). Cracking was readily observable on the wide face of the explant, a feature typical of corroded Mg that is usually attributed to dehydration of the corrosion layer.⁵⁷ There also appeared to be an area of localized mineralization on the exterior surface; EDS analysis showed that the white feature was composed almost exclusively of Ca and P (data not shown). The cross section



Figure 3. Secondary electron image and elemental maps of a wire/ tissue cross section. The Fe and O elemental maps are superimposed in the top right image, and the Ca and P elemental maps are superimposed in the bottom right image. The purple areas in the Fe/O map correspond, then, to iron oxide, and bright spots on the Ca/P map correspond to some calcium phosphate product.



Figure 4. Optical micrographs of the implanted A9 strip on the wide face (left) and in cross section (top). Two schematic EDS spectra from the carbon-coated sample are shown. The left spectrum corresponds to the matrix metal and shows that Mg and AI radiation was generated. The right side corresponds to the corrosion product layer and comprises primarily Mg, AI, O, P, and Ca radiation with trace C and CI.

revealed an apparently intact Mg core, the light area, surrounded by a corrosion product layer, the dark area. The surrounding gray area on the cross section is the mounting epoxy and plastic sample clip used to secure the sample. Elemental analysis of the corrosion layer and the metallic core yielded results consistent with past experimentation. The metallic core showed evidence of Mg and Al radiation with C radiation originating from the conductive carbon coating. The corrosion layer

again showed evidence of Mg and Al radiation, but with a higher Al:Mg peak height ratio, signifying Al enrichment. The corrosion layer also revealed radiation from O, P, and Ca, with trace Cl. The C peak seemed to be more intense than the C peak originating from the metallic phase, indicating that the corrosion layer may also contain chemically bonded carbon atoms in addition to the conductive carbon coating. These results are in agreement with preliminary in vitro data that have been collected using human whole blood with regard to the P and Ca content of the corrosion layer.²³

When imageJ (National Institutes of Health, USA) was used to perform a cross sectional analysis of the explanted A9 strip, it showed an approximate cross sectional area reduction of approximately 41% after a duration of three months post-implantation. This cross sectional area reduction corresponds to a 0.06 mg/cm²/day mass loss and 0.12 mm/year rate of penetration. This degradation rate is on the same order of magnitude as in vivo degradation rates of a LAE442 alloy, which was reported to have a 0.31 mm/year penetration rate at three months in vivo as an orthopedic implant.⁵⁸ This result using A9 Mg alloy proves that the experimentation performed in vivo using the arterial implantation in rats produces degradation rates that are comparable to those shown by comparable in vivo methods.

4.3 Use in exploring other biodegradation processes Even though the rodent arterial implant model was developed for bioabsorbable material degradation studies, it has since proven useful for exploring other biological processes in the arterial environment. Calcium is a biocompatible material that may prove useful in the development of bioabsorbable stents. For instance, hydroxyapatite has already been used as reinforcement in an experimental bioabsorbable metal matrix composite in a study conducted by Witte and coworkers.59 To clarify the degradation behavior of calcium-rich materials in the arterial environment, Ti-6Al-4V wires were coated with a calcium phosphate layer by immersion in a 1.5X concentrated simulated body fluid containing 7.5 M Mg²⁺ to inhibit crystal growth for 24 hours at 37°C. A thin (<3 µm) layer of amorphous calcium phosphate was thus deposited on the implant surface. Atop this coating, a thick (30 µm) coating of crystalline calcium phosphate was grown by immersion in a supersaturated calcium phosphate solution for 48 hours at 37°C. The resulting layer, as indicated by SEM/EDS, was composed of octacalcium phosphate (OCP), Ca_sH₂(PO₄)₆·5H₂O. The OCPcoated wires were then implanted into the arterial environment for 9 months. The purpose of this experiment was to examine the modification of the OCP layer by both environments and to identify possible differences between the environments with regard to calcium-rich materials.

After initial experiments, the wires were examined via field emission (FE) SEM. The surfaces of the wires explanted from the two environments were vastly different, as shown in Figure 5. After 9

months, the OCP layer is still readily visible on the wire that was implanted in the luminal environment (Figure 5(a)), but the OCP layer was completely absent on the wire that spent 9 months in the arterial wall (Figure 5(b)). This leads to the observation that implantation of a calcium-rich material in the wall environment leads to active removal of the calcium content over several months. The mechanism for this OCP-stripping phenomenon is not currently known, but it could have important implications in bioabsorbable stent development and merits further investigation. The authors posit that the rodent arterial implant model can play a key role in this investigation.

The difference between the behavior of metallic materials in the luminal and wall environments is similarly illustrated by inspecting the degradation of candidate bioabsorbable materials (Mg and Fe) in the two locations. Whereas attack in the luminal environment was generally quite limited, samples inserted into the wall underwent substantial corrosion. Figure 5 illustrates the difference in biocorrosion of Mg in the luminal versus wall environments in Figures 5(c) and 5(d), respectively. It can be seen that the wire samples are mildly corroded in the lumen, as the surface appears tarnished in Figure 5(c), but there is little to suggest that there is any localized attack or through-thickness corrosion. However, the attack in the arterial wall is much more substantial and the fragmentation that results is evidenced by Figure 5(d). Fe extracted from the luminal environment (Figure 5(e)) appears to be lightly corroded, while the wall contacting surfaces on a portion of the Fe sample in Figure 5(f) have ballooned outwards because of the evolution of a voluminous corrosion product.⁵⁴ The precise reason for this difference between the luminal and wall environment with respect to material degradation is currently unknown. However, it will likely play a key role in the evaluation of bioabsorption, as the pre and postencapsulation degradation rate of any candidate biomaterial will differ in a manner similar to the wires presented in Figure 5.

4.4 Challenges to use of the rodent model

The rodent model shows promise in greatly reducing the cost and time required to conduct in vivo material evaluation. However, researchers must be aware of some drawbacks related to the technique. In general, the few deficiencies of the rodent model are offset by cost savings and widespread accessibility, especially in preliminary investigations.

The wire represents a small segment of a stent and does not simulate all aspects of stent/artery interaction, such as forces originating from artery-implant interaction and injury to/inflammation of the arterial wall. This model does not replicate all mechanical conditions that a stent is subjected to while in service. However, this model does allow for reliable investigation of the metal-blood and metal-matrix interface. It also allows for identification of physiological corrosion products and an estimate of the lifetime of an actual stent, barring any drastic modification of that time by effects such as stress corrosion cracking. With the relatively low level of mechanical stresses expected during service, these



Figure 5. General differences between the luminal and arterial wall environment illustrated by: two octacalcium phosphate (OCP)-coated Ti-6Al-4V wires after 9 months' time in the arterial lumen (a) and wall (b) showing presence and absence of the initial OCP layer, respectively; Mg wires corroded in the lumen (c) and wall (d) showing mild and severe attack, respectively; and Fe samples corroded in the lumen (e) and wall (f) showing negligible attack and accelerated attack, respectively.

issues, while important to the future of metallic bioabsorbable stent development, should not completely control corrosion of straight, uniform stent strut segments. As mentioned previously, a relatively expensive large animal study with a balloon-deployed stent would be required to investigate the influence of mechanical forces on degradation.

Another potential problem is that the animal model also lacks atherosclerotic lesions, which may contain considerable calcium, phenotypically modulated smooth muscle cells, and dysfunctional endothelial cells. However, the significant benefit of the rodent wire implantation approach relative to a large animal stent implantation is that it can be used to quickly evaluate the corrosion behavior of novel stent materials while incurring minimal costs. In vivo trials using small animals before further material refinement and/or large animal implantation are critical to avoid costly mistakes. Thus, this type of small animal testing, while lacking details such as atherosclerotic lesions, is an appropriate precursor to large animal studies in which these details may be replicated at greater cost.

5. Attempts to replicate in vivo results in vitro

Although in vivo studies are useful for screening late-stage material candidates (i.e. a limited number of variants within a single alloy system), in vitro methods are better suited for multi-candidate (i.e. multi-alloy system) screening experiments. In general, in vitro methods are highly flexible, as one can use simple conditions such as static submersion test conditions, or complicated conditions such as flowing media. Regardless of the conditions used, the litmus test for success should be reasonable replication of the in vivo corrosion mechanism.

Currently, there is no universally agreed-upon in vitro test that accurately predicts in vivo corrosion for metallic materials. Recent work by Geis-Gerstorfer *et al.*²³ and Schille *et al.*⁶⁰ using a dynamic environment containing human whole blood has shown markedly similar corrosion characteristics compared to corrosion experiments performed in vitro through submersion in Dulbecco's Modified Eagle Medium (DMEM) both with and without fetal bovine serum added^{43 61} and simulated body fluid (SBF).⁶² Other solutions that

have been used include Hank's balanced salt solution (HBSS, or "Hank's Solution") and phosphate buffered saline, both of which have failed to replicate corrosion in explants and samples placed in contact with human whole blood. When medical facilities are not readily accessible, the use of human whole blood may not be feasible. For this reason, DMEM and SBF will likely be the favored in vitro corrosion media in many materials labs.

The authors have developed two novel in vitro techniques with two different applications: a technique involving static submersion of fibrin-coated wires, and a second technique involving laminar flow of media over a fibrin-coated wire. The conventional wire submersion in static media was used as the *de facto* control.

5.1 Static submersion of fibrin-coated samples

Though simulation of in vivo corrosion with in vitro approaches is commonly undertaken with DMEM, SBF, and human whole blood, an important limitation of this approach is the lack of an accurate recapitulation of in vivo kinetics. An evaluation technique devoid of animal testing would be particularly useful if applied in materials laboratories where access to animal facilities is limited. Furthermore, measurements collected using bare wires submerged in the aforementioned solutions are of limited usefulness; the results can only be interpreted in a relative manner (e.g. in relation to commercially pure Mg or some in vivo data). The static in vitro method is simple, straightforward, and, when refined, will require the same amount of time as a full small animal study using rats at a reduced cost.

The disagreement in kinetics between submersion and in vivo models may be related to the reduced capacity for mass transport across the biological matrix that is, the diffusion coefficient is lower in tissue than in a liquid. Hence, in order to develop a more realistic in vitro test method that also mimics the kinetics of in vivo degradation, it was necessary to encapsulate the biodegradable metal with a diffusion-restrictive coating. Preliminary experimentation showed that fibrin, a highly networked material more effectively modulated the corrosion rate than collagen, a non-networked material. Because of this, experimentation done thus far has used a "fibrin casting" technique, details of which are presented in the appendix. This is a first step in being able to obtain an accurate estimate of the expected lifetime of a bioabsorbable device from in vitro analyses.

A metallographic cross section of a corroded wire is presented in Figure 6. The wire shown was corroded with a fibrin sheath for 4 weeks in DMEM in accordance with the protocol outlined above. The secondary electron image shows, at first glance, a non-circular cross section with odd-looking protrusions. However, when elemental mapping was performed it was quickly apparent from the Mg and Ca maps (as well as the composite map) that the circular shape of the original wire was retained to a large degree. The abnormal features on the exterior are Ca- and P-rich, indicating that localized calcification of the wire occurred. Moving inward, a bulk corrosion product containing Mg, Ca, O, P, and maybe C, occupies a sizable fraction of the cross section of the corroded wire. An intact Mg core is readily visible as a bright blue area in the elemental map. There also appears to be a slightly Mg- and O-enriched region immediately to the southwest of the Mg core, which may correspond to a second corrosion product.

The wire itself underwent uniform corrosion, as it is typically defined,⁶³ although the corrosion product is not of uniform thickness. Non-uniform distribution of fibrin around the wire allows much more rapid mass transport from one surface, just as it would in an arterial environment. Similar corrosion distribution was observed by Waksman et al. during the histological examination of an iron stent explanted from a porcine subject.⁶⁴ Thus, corrosion appears to be much more rapid on the "outwardfacing" side of the wire, resulting in higher penetration rates. A cross sectional area analysis of the wire in Figure 6 yields a degradation rate of 0.20 mg/cm²/day or a penetration rate of 0.42 mm/year. It should be noted that this value is largely a function of the fibrin encapsulation; one may modify the corrosion rate by changing the thickness or concentration of the encapsulant (fibrin). It is the expectation of the authors that this methodology may be further refined to the point where the thickness and concentration of the encapsulant are engineered to give the same corrosion rates as the in vivo implantation model. If this were to happen, accurate testing may be performed in vitro without calibration experimentation involving small animals, opening the door for material development without intensive use of animal facilities.

The degradation of iron wires is fundamentally different from the corrosion of magnesium under these conditions. In many cases, it appears that the bulk of the wire remains passive in DMEM while pitting occurs at a few sites along the length of the wire. When degradation occurs by this mechanism, uniform corrosion is of minimal interest. In addition to a schematic showing the internal structure of the circumferential pits as determined by optical examination, a typical example of Fe corrosion in static media is presented in Figure 7. The typical morphology is a large (two or three times the nominal wire diameter) "ball" of fragile, brown corrosion product under which the wire has a severely reduced cross section. Immediately adjacent to the "ball," there is often a short segment where the passive layer on the wire is apparently disrupted, resulting in blistering and discoloration. Along the wire, away from the voluminous corrosion product, the wire remains shiny and unaffected. It is likely that similar pitting mechanisms to those outlined by Jones⁶³ (for Fe pitting) and MacDonald⁶⁵ (for pit initiation) are responsible for the localized breakdown of Fe wires.

5.2 Laminar flow over encapsulated samples

In the field of bioabsorbable material development, it is desirable to use accelerated corrosion experiments with the purpose of rapidly





Figure 6. Secondary electron image (top left) and elemental maps of a magnesium wire that was corroded in vitro with a fibrin sheath for 4 weeks. The composite map (bottom left) shows three or four regions of composition: a metallic Mg core (bright blue), calcification on the exterior of the wire (gray), bulk corrosion layer (purple/gray/ blue/green intermixed), and possibly a Mg oxide-rich region near the core (blue/green). The composite map is broken into is constituent element maps to the right; all constituent map scale bars are 50 µm.

-100 μm

screening candidate compositions. A dynamic environment (i.e. with flowing media, cyclic stress, and/or other factors) does not appear to be strictly required to mimic the basic corrosion behavior (mechanism) of Mg, as that can be accomplished with static submersion. However, the use of flowing media is able to accelerate the corrosion of candidate materials to the point that evaluations of material degradation relative to a standard sample (i.e. bare Mg or bare Fe) can be completed in a matter of days. An in vitro test method has been devised using a parallel plate flow chamber that promises to be highly scalable and can accommodate many samples at once. On a small scale, this allows for testing of a few candidate compositions at once, a single composition at several time points, or a single composition and time with many replicates.

A diagram of this steady flow setup is presented in Figure 8(a). Fluid moved by the pump flowed into the top of a compliance chamber where it was allowed to settle and flow smoothly out of an outlet at the bottom. The compliance chamber served to both aerate the

solution and mitigate the "pulsing" effect of the peristaltic pump. The bottom outlet of the compliance chamber was connected to the inlet of the parallel plate flow chamber. Details regarding the design and construction of the parallel plate flow chamber are presented in the appendix. The fluid then flowed over the fibrinencapsulated sample, as in Figure 8(b), and emptied into a fluid reservoir. DMEM from the reservoir was pulled back into the pump to complete the circuit. Using this setup, the flow chamber was necessary to achieve laminar flow. At steady state, the volumetric flow rate through the pump was necessarily equal to that of the parallel plate chamber if the flow rates were unequal, accumulation in one or another chamber would have occurred. Figures 8(c) and 8(d) illustrate the in-incubator configuration (sans the peristaltic pump) and a close-up of the flow chamber assembly, respectively.

Although only preliminary data are available at this point, it appears that the steadily flowing DMEM does not significantly modify



Figure 7. Pit morphology on an iron wire corroded in vitro in static media for 2 weeks of an intact pit (left) and a broken pit (middle). A schematic of a typical pit in cross section is presented on the far right showing the disruption of the passive layer adjacent to the pit cap product as well as the empty space between the wire core and the insoluble pit cap.

the corrosion behavior (in terms of products and morphologies) relative to the static submersion testing. Mg wires corroded using this technique had similar characteristics as samples produced using the other methods, including corrosion anisotropy typical of wires encapsulated in asymmetric fibrin. It also displayed non-uniform distribution of corrosion products similar to the wire in Figure 6. Fe exhibited the same corrosion behavior in this dynamic environment as in the static media. However, unlike the static media where a pit cap was produced around an intact segment of Fe wire, no pit cap was observed in steady flow samples. The reason may be that the flowing media was able to carry away the pit reaction products before they were able to precipitate and form a cap. Strong localized attack still occurred, resulting in in situ fracture of the Fe samples after a short time. The rate at which degradation occurs using this system is much accelerated relative to the other methods discussed in this report.

Using this methodology, it is feasible that a complete assessment of the degradation of some candidate materials could be completed within a week with several replicates at each desired time point. This method would also move farther away from an absolute corrosion rate; comparison to in vivo data (or in vitro data using a refined encapsulation method) as "calibration" points would be necessary to translate the results into a real, arterial degradation rate. Large-scale experimentation with the steady flow system has not yet been done by the authors, but this system could easily be implemented in an industrial or academic laboratory setting with minimal capital investment.

6. Qualitative testing results

In this particular corrosion system, one may glean a significant amount of information from simple stereomicroscopic examination. The color of the corrosion products, the morphology of secondary and tertiary products, and any localized diametral variations carry information about corrosion progression. This crucial information is neglected by many investigators in the field of bioabsorbable materials, but is widely used by corrosionists.⁶³ Figure 9 presents several examples of Mg wires corroded using the methods described above, in addition to results from the more conventional bare-wire submersion method. It is apparent from Figure 9 that a well-defined pattern applies to the corrosion of Mg wires. Fe degradation is not discussed in this section, as the nature of degradation has already been addressed and there is no significant change in sample appearance from one time point to another. Fe wires simply begin to exhibit more severe pitting behavior as the corrosion time increases.

In general, the Mg degradation products appear to begin as a thin, coherent gray/blue layer. Soon after, a secondary golden-brown layer forms on the initial gray/blue film. The brown layer appears to spread over the wire surface in the in vivo and steady flow cases, but remains localized in "islands" in both in vitro cases, probably because of some transport-related phenomenon. Nevertheless, it is clearly present in both instances. Finally, a white, tertiary product begins to form on the brown and/or gray/blue layer. The white layer is barely visible at the end of 2 weeks in vivo and appears relatively late in steady flow, but is pronounced earlier in in vitro tests with static media. Thickening of the white layer appears to be the terminal corrosion stage in the extended in vitro studies. The layer itself becomes brittle and begins to chip away, and, as the cross-sectional area of the Mg core reduces further and further, the wires take on a brittle mechanical character. At very late stages of corrosion, the wires are extremely difficult to handle and only remain intact in the in vitro scenarios where static media is used. Fragmentation of the wires is observed in vivo and in the steady flow case after the white layer becomes dominant, precluding detailed assessment of



Figure 8. Operation of the steady flow setup used to accelerate in vitro degradation: a schematic showing the flow direction for a single circuit (a); a wire encapsulated by fibrin in the bottom half of the flow chamber prior to experimentation (b); the inside of the incubator housing all parts of the circuit except for the peristaltic pump (c); and a close-up image of the steady flow chamber with the flow direction to the left (d).

failure in those cases. At extremely long times (many weeks) in static DMEM after the Mg core has been completely dissolved and the other corrosion products apparently transform into the white product, the corrosion product remains intact but is readily broken apart by the smallest disturbance of the media. It could be argued, based on these results, that the mechanism of failure (dominance of a uniform brittle phase cracked by minute stresses) is likely to be the same in both static in vitro and in vivo/steady flow cases.

7. Quantification of metal degradation rate

As work in this area progresses, it is desirable to develop and fully characterize a single quantitative method for the evaluation of bioabsorbable candidate materials. To date, studies of Mg corrosion have relied on quantitative techniques such as monitoring the changes in sample mass,^{66–68} current density (or other electrochemical techniques),^{42 69} and/or the volume of evolved hydrogen.^{24 70} X-ray tomographic analysis has been conducted on some bioabsorbable samples explanted from animals, though the application to date has been limited to orthopedic specimens.³⁴ These quantitative techniques have their own strengths and weaknesses and provide valuable information about Mg degradation, but some are simply not feasible for use in bioabsorbable material systems. For example, the simple mass gain/loss method is difficult to apply to our in vivo approach, since explanted wires retain significant amounts of tissue,⁵⁴ which hinders estimation of the precorrosion-removal mass. The largest single drawback, common to all of these techniques, is the difficulty in applying data generated from any of these techniques directly to stent design.



corrosion without a fibrin coating, in vitro corrosion with a fibrin coating, and in vitro corrosion in the parallel plate flow chamber. Corrosion time increases going down each column. In application, the single most important aspect of a stent is that is must act as vascular scaffolding until repair is complete, and its effectiveness as scaffolding is largely dictated by its mechanical behavior. Consequently, it is advantageous to know precisely how

behavior. Consequently, it is advantageous to know precisely how the mechanical properties of a given material change (decrease) over time. If a mechanical degradation profile is well known, then a material can be specified to have a specific fractional strength at any point during its lifetime for example, 50% of the nominal strength is retained after degradation over 6 months. To be practically helpful, data gleaned from the characterization method must account for the effects of uniform corrosion (cross sectional area reduction) as well as localized attack in the form of pits and/or crevices. To this end, a method of tensile testing wires similar to that used to evaluate polymeric nanofibers⁷¹ has been developed. The method was developed to characterize the mechanical profile of materials over their corrosion lifetimes with strong statistical significance.⁶¹ The technique is easily used in conjunction with in vitro and in vivo corrosion methods described above and works well with wire sample geometry.

To accomplish this, the corroded wires are mounted to two pieces of polycarbonate substrate using general-purpose epoxy. A "gauge length" is established by maintaining a precise distance between the substrate pieces. Toothpicks (or some other support) are taped to the back of the polycarbonate in order to support the relatively fragile wires. A schematic of the tensile assembly is presented in Figure 10, showing its construction before testing is performed. Mechanical testing is conducted by gripping the two polycarbonate pieces, clipping the toothpicks, and running the test. The authors use a small tensile frame, a Test Resources 100-series instrument with wide soft tissue grips and a 250 lb_f (~1 kN) load cell, although smaller load cells can be used depending on the material being tested.

The data generated by this test is in the form of load/elongation. However, to be instructive in an engineering analysis, the data are better presented in a stress/strain configuration. The strain is simply calculated by the elongation divided by the nominal gauge length, 10 mm. Calculation of the stress in the "raw," or uncorroded, wire is also trivial, as it is the load divided by the nominal cross sectional area. Calculation of the real stress in the corroded wires, on the other hand, is a complex proposition. This is due to the unknown cross section at the point of failure resulting from the invariable presence of changes in uniform corrosion severity, pits, and crevices. Hence, the true stress is not calculated for the corroded wires, but rather an "effective" stress is calculated using the same nominal cross sectional area as the "raw" wire calculation. Typical mechanical behavior of a severely corroded Mg specimen is contrasted with that of an as-received wire sample in Figure 10. This particular sample had been corroded for 3 weeks with a fibrin coating in DMEM under cell culture conditions and has a significantly reduced effective tensile strength and total elongation (> 60% and > 80% decreases, respectively).



Figure 10. Schematic of the tensile samples used in this study (left) and an example of a "raw," or uncorroded, Mg wire compared to a severely corroded Mg wire (right). The tensile curves on the right illustrate the drastic reduction in ductility, elongation at failure, and tensile strength after corrosion of fibrin-encapsulated wires in Dulbecco's Modified Eagle Medium for 3 weeks.

When the mechanical behavior data were aggregated, the ultimate (or "effective" ultimate) tensile strength was selected for evaluation because of its good reproducibility from sample-to-sample. Other variables, such as compliance, Young's modulus, and 0-2% offset yield strength all showed larger scatter, although they also would provide some information about corrosion progression. Another reason for selecting the effective tensile strength is that, using the assumption stated previously, the ratio of the strength of a corroded wire to that of an as-received wire gives a rough estimate of the cross sectional area reduction at the point of failure. It should be noted, though, that this extension of the tensile testing method requires the assumption that the contribution of localized corrosion was negligible.

In both Mg and Fe, the mechanical data showed a considerable amount of variability, but a generally decreasing tensile strength trend was apparent for both materials. The data, plotted as a function of corrosion time for all methods (in vitro steady flow, in vitro static with and without fibrin, and in vivo), are presented in Figure 11. The statistical nature of these data is discussed in the authors' previous work,⁶¹ so the discussion here is limited to trends and preliminary data that have not yet been reported.

The Mg data show a clear downward trend with some methods resulting in considerably more scatter than others. First comparing the static in vitro techniques, the rate of decrease of the effective tensile strength in the uncoated samples is clearly greater than the rate of decrease in the fibrin-coated samples. This observation stands to reason; mass transport will occur at a reduced rate in the system containing the fibrin-coated sample, thus reducing the corrosion rate. In addition to this, the scatter in the fibrin-coated sample data is quite large, owing to non-uniform distribution of fibrin around the wire and/ or defects in some fibrin sheaths. It is noteworthy, also, that the single, preliminary in vivo data point lies remarkably close to the static in vitro trendline for samples that employed a fibrin coating. This single data point is not conclusive and has a large associated error, but it is safe to say that the degradation behavior of the coated is trending in the right direction relative to the bare wire data. An additional preliminary data point for wires corroded for 1 day using the in vitro steady flow method is presented for Mg. In a situation similar and opposite to that previously stated, the enhanced transport due to flowing media appears to accelerate corrosion, although more data are needed before conclusions can be drawn.

The Fe data exhibit a comparatively large amount of scatter at each time point, indicative of pseudo-random, non-uniform corrosion events leading to failure. In most cases, the initially passive (Ca- and P-containing) film was disrupted by the evolution of a voluminous product identified as hematite,⁵⁴ as in the rodent arterial explants. The same relationship between the uncoated static wires and those with a fibrin sheath is observed in both materials. As before, the bare wires were observed to degrade at a much faster rate. In the case of Fe, however, no significant change



Figure 11. Complete tensile strength data for Mg (top) and Fe (bottom) showing the degradation rates for wires corroded in vivo, in vitro in Dulbecco's Modified Eagle Medium with and without a fibrin coating, and in the in vitro steady flow configuration.

in the tensile strength was observed even after 4-5 months of time spent in the arterial wall. Samples were also evaluated in tension after 1-5 and 3 months of implantation, but those surface coatings of the wires possibly a passive layer caused them to separate from the epoxy at relatively low loads, resulting in no valid tensile stress data. To replicate the in vivo behavior in vitro, then, a higherconcentration fibrin coating or alternative coating method would be required. Regardless, the negligible amount of degradation in the Fe wires coupled with the voluminous corrosion product observed in vivo collectively prove that Fe is a poor choice for bioabsorbable implants.

8. Conclusions

In vivo evaluation using the rodent arterial implant model, in vitro evaluation using fibrin-coated samples in static DMEM, an in vitro steady flow method, and quantitative tensile evaluation are all viable methods for evaluating bioabsorbable materials, such as Fe, Mg, and Mg alloys.

In vivo, implantation of Mg wires in the arterial wall leads to well-behaved, typically uniform degradation, while implantation of Fe wires leads to the evolution and retention of a voluminous corrosion product. The in vivo technique developed is also useful in evaluating candidate materials and biological processes, as shown in the candidate material degradation and OCP-stripping examples. The measured degradation rate of a candidate alloy (A9) was shown to be reasonable for physiological corrosion, and the elemental makeup of the corrosion products was shown to be consistent with previously published data.

The in vitro techniques were shown to provide valuable information related to the degradation behavior of bioabsorbable Mg, and a significant amount of information about the corrosion progression was gleaned from stereomicrographs. The bare wire control method, when DMEM was used as the corrosion medium, appeared to replicate the corrosion products observed in vivo. When a fibrin coating was introduced to the system, degradation was observed to slow significantly, closer to the in vivo corrosion rate. This is likely due to restricted mass transport between the wire and the surroundings. Finally, steadily flowing media was introduced to the system, which appears to replicate the corrosion products and morphology of samples corroded using the rodent arterial implantation model. The flowing media appeared to increase the corrosion rate due to enhanced mass transport, even to the point of causing in situ fracture of Fe samples.

To quantitatively evaluate the wires, it was shown that a tensile testing method consistently produced a degradation profile for both Fe and Mg. The profile for Mg appeared to have less scatter at each time point, and the preliminary tensile data for in vivo degradation of Mg were in good agreement with data corresponding to in vitro degradation of fibrin-coated Mg wires. The qualitative observation that fibrin-coated wires degraded more slowly than their bare counterparts was supported by the quantitative data. Fe, on the other hand, exhibited a large amount of scatter within each time point. The same decrease in the rate of degradation of fibrin-coated wires relative to bare wires was observed for Fe, but the in vivo corrosion rate was apparently much lower than that of the fibrincoated wires for this material. Most importantly, though, this quantitative evaluation technique works seamlessly with all the corrosion methods presented, completing a "suite" of corrosion techniques useful in bioabsorbable material studies.

9. Appendix-details of experimentation

9.1 Fibrin encapsulation for static submersion

A mold was machined which had several rectangular grooves, each 30 mm long, 5 mm wide, and 3 mm deep. A method for suspending the wires above the bottom of the large mold slots

was needed; 0.3 mm deep semicircular grooves were added to each long end of the large grooves. The bottom and sides of the grooves were covered with parafilm to prevent adhesion of the polymerized fibrin to the mold walls. 30–34 mm wires were then positioned such that equal lengths on each end were supported by the shallow grooves. Then, fibrinogen (100 mg/mL) and thrombin (2.5 U/mL) solutions were added in equal amounts to each groove containing the wires, and the entire mold was placed in an incubator to allow the fibrinogen/thrombin mixture to polymerize and form a fibrin sheath. After polymerization was complete, the fibrin-coated wires were removed from the mold and submerged in DMEM under cell culture conditions. Recently published work⁶¹ presents a more complete protocol as well as data acquired using this methodology in contrast to data from submersion of bare wires.

9.2 Flow chamber design and construction

The parallel plate flow chamber was designed to subject wires to a shear stress representative of the arterial environment. Published ranges for arterial shear stresses were approximately 1–3 Pa,^{72 73} so a target value of 2 Pa was chosen for the chamber design. Using the design methodology of Reyes⁷⁴ and assuming a value of 0.78 mPa·s for DMEM viscosity, a plate separation of 300 μ m, and width of 5 cm, a flow rate of 115·4 mL/min was calculated to obtain the desired shear stress. The steady flow system used a peristaltic pump with an adjustable flow rate, which was set to the desired 115 mL/min. Further review of the literature suggested that the blood flow through coronary arteries could be modeled as laminar flow, meaning a Reynolds number less than 2100 was appropriate.⁷⁵ To achieve laminar flow, then, required a minimum entrance length of 9 mm.⁷⁴

With these design parameters under consideration, a parallel plate flow chamber was designed and fabricated using polycarbonate for the base plates (Lancet Glass; Houghton, MI, USA). A seal was achieved by using a 1/8 in. (3.18 mm) standard O-ring (McMaster-Carr; Robbinsville, NJ, USA) inset in symmetric 0.3 mm deep grooves on each base plate. The inlet and outlet holes were positioned on the top plate such that the entrance length was 26 mm, which provided a factor of safety of approximately three relative to the minimum length required for laminar flow. The inlet and outlet were polycarbonate tube connectors (McMaster-Carr) which connected to silicone tubing (Cole-Palmer; Vernon Hills, IL, USA). The bottom plate had a 40 mm slot machined into it with 0.5 mm grooves on each side, which allowed the fibrin casting/ wire encapsulation to be performed in situ. The assembly was held together by four hold-down toggle clamps (McMaster-Carr) attached to an aluminum base plate.

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