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Long-term surveillance of zinc implant in murine artery: Surprisingly steady biocorrosion rate

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ABSTRACT

Metallic zinc implanted into the abdominal aorta of rats out to 6 months has been demonstrated to degrade while avoiding responses commonly associated with the restenosis of vascular implants. However, major questions remain regarding whether a zinc implant would ultimately passivate through the production of stable corrosion products or via a cell mediated fibrous encapsulation process that prevents the diffusion of critical reactants and products at the metal surface. Here, we have conducted clinically relevant long term *in vivo* studies in order to characterize late stage zinc implant biocorrosion behavior and products to address these critical questions. We found that zinc wires implanted in the murine artery exhibit steady corrosion without local toxicity for up to at least 20 months post-implantation, despite a steady buildup of passivating corrosion products and intense fibrous encapsulation of the wire. Although fibrous encapsulation was not able to prevent continued implant corrosion, it may be related to the reduced chronic inflammation observed between 10 and 20 months post-implantation. X-ray elemental and infrared spectroscopy analyses confirmed zinc oxide, zinc carbonate, and zinc phosphate as the main components of corrosion products surrounding the Zn implant. These products coincide with stable phases concluded from Pourbaix diagrams of a physiological solution and in vitro electrochemical impedance tests. The results support earlier predictions that zinc stents could become successfully bio-integrated into the arterial environment and safely degrade within a time frame of approximately 1–2 years.

Staement of significance

Previous studies have shown zinc to be a promising candidate material for bioresorbable endovascular stenting applications. An outstanding question, however, is whether a zinc implant would ultimately passivate through the production of stable corrosion products or via a cell mediated tissue encapsulation process that prevented the diffusion of critical reactants and products at the metal surface. We found that zinc wires implanted in the murine artery exhibit steady corrosion for up to at least 20 months post-implantation. The results confirm earlier predictions that zinc stents could safely degrade within a time frame of approximately 1–2 years.

1. Introduction

Coronary stents are an important and beneficial component of assisted vascular remodeling for patients suffering from coronary heart disease [1,2]. Stents are used alongside balloon angioplasty to support the damaged artery and promote revascularization, while lessening the chance of restenosis and other complications. Traditional coronary stents are composed of corrosion-resistant metals such as stainless steel [3]. While these materials meet the mechanical requirements for use in stents they also remain present throughout a patient's lifetime, which can result in complications long after stent deployment. These include chronic inflammation [4], late stage thrombosis [5], and stent strut fracture and damage to local vasculature [6], events that can contribute to restenosis.

In order to mitigate these long-term side effects inherent to current generation coronary stents, a new generation of stents using bioabsorbable material is being developed. Such stents have the potential to match the early benefits of traditional coronary stents while avoiding many of their long-term health risks [7].

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Specifically, an ideal bioabsorbable stent would remain mechanically viable in the patient long enough for arterial regeneration to occur, and then be harmlessly broken down and bio-absorbed once its function had been served and before late stage complications could arise [8]. One area of such focus is polymeric stents made of bioabsorbable polymers such as poly L-lactic acid (PLLA). But while polymeric stents have demonstrated acceptable biocompatibility [9], they have significant unavoidable mechanical limitations [10,11] and similar restenosis rates as traditional coronary stents [12].

In recent years attention has increasingly shifted to the development of bioabsorbable metallic stents. In particular, there has been a great deal of focus on magnesium (Mg) [13,14] and iron (Fe) [15,16] and their alloys as candidate materials. While pure Fe stents possess promising mechanical properties and biocompatibility [17], it has also been observed to produce a voluminous oxide layer that repels neighboring tissue and compromises the luminal cross section of the artery in some studies [18], overall potentially falling short of the necessary requirements for a bioabsorbable stent material [19]. Despite additional alloying and processing, Fe corrosion rates are still lower than the benchmark ideal [17,20,21]. For example, recent long-term studies on biocorrosion of nitride iron scaffolds in rabbit abdominal aorta suggest completion of the degradation process in approximately 4 years [22].

In contrast, Mg has achieved greater success. Both uncoated and coated Mg stents have shown excellent biocompatibility [23], avoiding significant inflammatory responses [24]. Excellent biointegration is also seen for Mg materials deployed as bone healing assist devices [24,25]. However, it is well known that the base Mg corrodes too quickly *in vivo* for bioabsorbable stent applications, often experiencing extensive material loss even within 1 month [18] and complete degradation as early as 3 months [24]. Mg is also susceptible to corrosion fatigue and mechanical failure [26–28] and generates a rapid buildup of hydrogen gas that can complicate tissue regeneration [29,30]. Alloying has successfully addressed some of these concerns. For instance, Mg-Zr-Sr devices exhibit lower degradation rates [31,32], while MgZnCa glasses have reduced levels of hydrogen gas evolution [33].

Mg based stents have also seen some success in humans. The DREAMS (Drug Eluting Absorbable Magnesium Scaffold) stents from Biotronic have shown promising results in the clinical setting. Clinical trials for both DREAMS 1G [34] and the later DREAMS 2G [35,36] iteration were associated with low thrombosis rates, low incidence of cardiac death and myocardial infarction, and less late lumen loss compared to bare metal and polymer stents, with DREAMS 2G having greater strength, flexibility, and lower neointimal formation than its predecessor. Such trials lasted for up to 12 months. However, early DREAMS designs were also associated with rapid degradation and subsequently high rates of restenosis [37]. DREAMS 2G mitigated this problem with the introduction of a drug-eluting PLLA coating to increase corrosion resistance. However, it is still estimated that ~95% of the magnesium scaffold will be degraded within 12 months, the lower end of the 1–2-year ideal degradation benchmark, with a late lumen loss that is higher than comparable stent systems such as ABSORB and DESolve [35,36].

A third candidate material that has emerged in recent years is zinc (Zn) and it's alloys [38]. Zn lacks the critical flaws observed for Mg- and Fe-based alloys, including premature degradation (Mg) and tissue displacement (by iron-oxide corrosion product), and shows promise for coronary stent application [19]. Additionally, pure Zn exhibits a tensile strength of 80–120 MPa, similar to the strength of Mg (86 MPa), but also about three times lower than that of pure Fe (290 MPa) [19]. The elongation to failure of pure Zn (60–80%) is, on the other hand, far superior relative to Mg (13%) and Fe (18%) [19]. Alloying of Zn can improve its strength to the level of Fe and above [39–41], with elongation to failure remaining above values characteristic for pure Mg and Fe [19]. Studies so far on Zn as an experimental stent material have shown good biocompatibility and a rate of degradation between 10 and 20 μm/yr, which is similar to the 20 μm/yr benchmark value for an ideal bioabsorable material [42,43]. Zn implants were also found to remain intact in the murine arterial wall even after 6 months of residence, with the first 3–6 months after implantation being considered a critical period for coronary stent scaffolding [19]. Finally, Zn is considered relatively nontoxic for humans and an essential trace element. Acute Zn toxicity is associated with symptoms such as abdominal pain, nausea and vomiting, diarrhea, lethargy and light-headedness. Excessive Zn can also cause copper deficiency, resulting in leukopenia, anemia, and neutropenia [44]. However, moderate symptoms only occur at intake rates of 100–300 mg/day, with acute toxicity at 225–450 mg/day and a median lethal dose (LD50) of 27 g. For the complete degradation of a pure Zn stent, it is estimated that daily intake would be only 150 μg/day [38].

As a novel stent material, no metallographic studies have been conducted to quantify long-term corrosion of Zn implants *in vivo* out to 20 months. It is of utmost importance in an early exploration phase to study the short-term corrosion of a bioabsorbable stent candidate material to confirm its feasibility in a coronary stent setting. Then, once the material has been validated, it is essential to investigate its long-term corrosion behavior as it progresses toward complete break down and tissue clearance. This is especially true for Zn, with earlier studies suggesting possible acceleration in biocorrosion at later time points [38]. To this end, in the present study we report on the corrosion of pure Zn wires implanted in the murine artery wall for up to 20 months. The results reveal consistent and relatively steady corrosion rates for Zn. This was unexpected due to the formation of passivating corrosion products and fibrous encapsulation of the wire, each of which would be expected to retard continued zinc corrosive activity.

2. Experimental

2.1. In vivo implantation and sample preparation

Zn wire from Goodfellow (99.99 + % purity) with a diameter of 0.25 mm was used in this study. The wire was cut into 2 cm – long segments and sanitized in 70% ethanol before being surgically implanted into live Sprague-Dawley rat arteries. Each wire was implanted into the wall of the abdominal aorta of a rat following the surgical protocol reported previously [18]. Briefly, the adventitial layer of the abdominal aorta was penetrated by the wire, which was then advanced into or near the arterial media for the entire length of the specimen. The animal study was approved by the Michigan Technological University Institutional Animal Care and Use Committee (IACUC) and was performed in accordance with the Panel on Euthanasia of the American Veterinary Medical Association.

At specified end points, rats were euthanized and arteries containing the degraded wires removed every month for 12 months, along with two additional samples at 14 and 20 months. Approximately half of each implanted wire was used for histological analysis (briefly presented in this publication and more thoroughly characterized in a recent publication [45]), while the remaining half was used for imaging and metallographic analysis.

Wire segments used for metallographic analysis were received still embedded within the rat artery, although an effort was made to trim the surrounding tissue. Samples were stored in absolute ethanol at room temperature for several days and then placed in a dessicator until the remaining biological material dried. Each