Published in final edited form as:

Mater Sci Eng C Mater Biol Appl. 2015 November 1; 56: 467–472. doi:10.1016/j.msec.2015.07.022.

Metallic Zinc Exhibits Optimal Biocompatibility for Bioabsorbable Endovascular Stents

Patrick K. Bowen^{2,*}, Roger J. Guillory II¹, Emily R. Shearier¹, Jan-Marten Seitz^{1,2}, Jaroslaw Drelich², Martin Bocks³, Feng Zhao¹, and Jeremy Goldman^{1,*}

¹Department of Biomedical Engineering, Michigan Technological University, Houghton, MI 49931

²Department of Materials Science and Engineering, Michigan Technological University, Houghton, MI 49931

³University of Michigan Congenital Heart Center, Division of Pediatric Cardiology, Ann Arbor, MI 48109

Abstract

Although corrosion resistant bare metal stents are considered generally effective, their permanent presence in a diseased artery is an increasingly recognized limitation due to the potential for long-term complications. We previously reported that metallic zinc exhibited an ideal biocorrosion rate within murine aortas, thus raising the possibility of zinc as a candidate base material for endovascular stenting applications. This study was undertaken to further assess the arterial biocompatibility of metallic zinc. Metallic zinc wires were punctured and advanced into the rat abdominal aorta lumen for up to 6.5 months. This study demonstrated that metallic zinc did not provoke responses that often contribute to restenosis. Low cell densities and neointimal tissue thickness, along with tissue regeneration within the corroding implant, point to optimal biocompatibility of corroding zinc. Furthermore, the lack of progression in neointimal tissue thickness over 6.5 months or the presence of smooth muscle cells near the zinc implant suggest that the products of zinc corrosion may suppress the activities of inflammatory and smooth muscle cells.

Keywords

zinc; stent; bioabsorbable; biocompatible; corrosion; hyperplasia

*Co-corresponding authors: Jeremy Goldman, Ph.D., Associate Professor, Biomedical Engineering Department, Michigan Technological University, Houghton, MI, 49931 USA, Ph: (906) 487-2851, Fax: (906) 487-1717, jgoldman@mtu.edu, Patrick Bowen, B.S., Ph.D. Candidate, Department of Materials Science and Engineering, Michigan Technological University, Houghton, MI, 49931 USA, Ph: (906) 487-2615, pkbowen@mtu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Corrosion resistant stents, including bare metal stents (BMS) and drug eluting stents (DES), are commonly used to treat stenotic or occluded vessels in both adult and pediatric populations. Whether being used in adults to treat atherosclerotic coronary or peripheral vascular disease or in children to treat congenital heart conditions—such as coarctation of the aorta or pulmonary artery stenosis—traditional corrosion-resistant stents remain a permanent fixture inside the artery once deployed [1]. In adults, the permanent presence of corrosion-resistant stents in small diameter arteries can contribute to late-stage complications, such as thrombosis, altered flow dynamics, and neo-atherosclerosis/restenosis [2–10]. In infants and children, use of permanent stents in a pulmonary artery or descending thoracic aorta leads to relative restriction to blood flow during somatic growth, necessitating serial redilation for stent expansion, potentially dangerous “unzipping” of the stent during attempted over-dilatation, and, in many cases, surgical removal of the stent [11–13]. Other problems common to BMS in pediatric patients include stent fracture and the occasional loss of integrity, making it difficult to re-access the stent and distal vessel for further dilatation procedures. Recent clinical studies with fully bioabsorbable stents have demonstrated that a stent is only needed temporarily as mechanical scaffolding to enable arterial wall healing and remodeling [14–16]. Bioabsorbable stents possessing the ductility and mechanical strength of conventional stents and the ability to harmlessly disappear when their scaffolding task has been completed hold promise for avoiding the chronic deleterious effects associated with permanent metal stents [17].

Two general categories of bioresorbable stents are currently in development and clinical use worldwide; polymeric stents and biocorrosible metallic stents. Fully bioabsorbable polymeric stent technology has progressed considerably more relative to their metallic counterparts. This success may be due in part to the pre-existence of numerous well-characterized, FDA-approved polymeric materials from which fully or partially bioabsorbable stents may be manufactured, including the most frequently used polymer, poly(L-lactic acid) (PLLA) [18, 19]. Polymeric materials have the advantage of degrading predominantly via a simple hydrolysis reaction with predictable byproducts, and degrading through similar mechanisms whether evaluated *in vitro* or *in vivo* [20].

In contrast to polymeric stents, the development of biocorrosible metallic endovascular stents, though promising at times, has generally fallen short of expectations [21]. Reasons for the relative lack of progress include the lack of suitable pre-existing materials, as well as the high cost and complexity of developing new materials. For instance, metallic materials often corrode via complex mechanisms that produce a wide range of degradation products, and the rates and products of corrosion can differ fundamentally between *in vitro* and *in vivo* conditions [22–25]. This has made it difficult to translate success on the bench top into success in a pre-clinical or clinical model. Consequently, the scientific and industrial community has engaged in a decade-long focus on magnesium and iron [26] as base materials for stent development without achieving the level of success realized by fully biodegradable polymeric stents.

Despite the challenges faced in their development, stents manufactured from metallic material possess several important advantages over competing polymeric stents. First, absorbable metallic stents possess greater mechanical strength at lower profiles (ductility) than competing polymers, and are more similar to traditional, non-absorbable metallic stents. This similarity affords clinicians a greater degree of familiarity and expectation of outcomes when using a biocorrosible metallic stent. The lower profile allows for greater flexibility and variability in stent design and a wider range of expandable diameters during deployment. The reduced radial strength and ductility of polymeric stents have necessitated substantially larger struts and, in some models, the introduction of a “locking” mechanism to maintain luminal cross sectional area following deployment. This larger profile of the polymer stents necessitate a larger introducer sheath and catheter for delivery relative to metal stents, which can result in an increased risk of vascular injury and blood flow disruptions [27]. This may preclude their use in younger infant and pediatric populations [13]. The larger stent struts may also increase susceptibility to early and midterm thrombosis [28]. The presence of a locking mechanism further constrains stent design flexibility and the freedom to control the final stent diameter during deployment. It may also be a concern from a device safety standpoint, as this complex feature may increase the risks of device failure. Even in a successful deployment, lower material ductility may also affect the clinician’s willingness to expand a polymer stent sufficiently to achieve full deployment. This effect was hypothesized to have led to significantly lower post-procedure luminal gains with a polymeric stent relative to the metallic stent control in the Absorb II clinical trial [16, 28].

In an effort to reduce the considerable obstacles present in the developmental path of new metallic materials, we have recently developed a simplified approach for evaluating candidate stent materials *in vivo* [24, 25, 29–31]. In this model, a wire of the selected material (simulating an individual stent strut) is implanted into the rat abdominal aorta. With this approach, we have shown that magnesium corrodes too rapidly to be used as the base material for a stent without first undergoing considerable metallurgical modification to safely reduce the corrosion rate [24]. Similarly, we have demonstrated that iron undergoes a harmful mode of corrosion, as it produces a voluminous iron oxide that repels neighboring cells and matrix [29]. Consequently, and in similar fashion, we tested the biocorrosion properties of zinc and demonstrated the near-ideal corrosion rate and behavior of pure zinc [32] compared to iron and magnesium. Zinc was shown to corrode at average rates of < 50 $\mu\text{m}/\text{yr}$ for 6 months, and generated corrosion products that had elemental profiles consistent with zinc oxide and zinc carbonate [32]. In this study, we present follow-up data on the biocompatibility of pure zinc for use as the base material for bioabsorbable metallic stents by demonstrating a benign and stable cellular response to its presence over 6.5 months inside the lumen of the rat abdominal aorta.

Materials and Methods

Six Sprague Dawley rats were used in the animal experiments. All animal experiments were approved by the animal care and use committee (IACUC) of Michigan Technological University.

Aortic implantation

We employed a recently developed *in vivo* model for the simplified evaluation of candidate stent materials [29]. Briefly, sterile candidate stent materials drawn into a wire are punctured and advanced into the lumen of a rat abdominal aorta. Approximately 10 mm length of the wire remains in contact with flowing blood within the aorta to simulate the presence of a stent strut with some regions of the wire in direct contact with the arterial wall and some regions of the wire not in contact. We implanted a 0.25 mm diameter wire of 99.99+ wt. % zinc (Goodfellow Corporation). After 2.5, 4.0, and 6.5 months (2 specimens per time point), the rats were euthanized and aortas containing the implanted wires were harvested for histological and immuno-fluorescence analysis.

Histology and Immuno-Fluorescence

Rat aortas containing the zinc wire implants were snap-frozen in liquid nitrogen and cryo-sectioned for histological analysis. Cross sections were ethanol fixed and then stained with hematoxylin and eosin (H&E) and imaged using an Olympus BX51, DP70 brightfield microscope. Cross sections were also stained with antibodies specific for endothelial cells (CD31; Abcam – ab64543) or smooth muscle cells (alpha actin; Abcam - ab5694) and imaged using an Olympus BX51, DP70 fluorescence microscope. Cell populations were analyzed using standard cell counting methods and the Student's *t*-test to identify significant differences.

Results

The implantation of high purity zinc wires into the rat abdominal aorta allowed for a pathological evaluation of the localized host response to metallic zinc and the products of zinc corrosion in an *in vivo* preclinical model. Unfortunately, histological preparation techniques are not amenable to quantitative measurements of corrosion due to deformation of the zinc metal and frequent dislodging of the metal and corrosion products. Determination of a precise degradation rate was therefore impossible in this study. However, the observed corrosion was in qualitative agreement with a previous report which described average cross sectional area reductions of approximately 7%, 25%, and 40% after 3, 4.5, and 6 months, respectively, in the arterial environment [32].

H&E staining of the wire/artery cross sections post implantation (Figure 1) revealed complete neo-endothelialization by 2.5 months. The neointimal tissue surrounded the zinc wire and was also well integrated into the arterial wall. The neointima contained a thin layer of smooth muscle cells (SMCs) and a region of low density inflammatory cells near the zinc metal and within the corrosion layer. The SMC and inflammatory cell layers were thickest at sites of wire contact with the aortic wall. There was no evidence of necrosis. These findings suggest that the local endothelial response to metallic zinc involves non-fibrotic tissue encapsulation with minimal smooth muscle cell infiltration, minimal tissue necrosis, and evidence of modest local cell proliferation.

As expected, the thickness of the neointimal tissue layer tended to decrease with increasing distance from the mural endothelium (Figure 2A). Importantly, the thickness of the

neointimal layer did not increase over time despite clear evidence of extensive zinc corrosion progression at 6.5 months. Furthermore, the thickness of the tissue at the luminal side of the wire never exceeded 100 μm at any time point, for any of the specimens.

High magnification microscopy at 6.5 months revealed cell migration and matrix synthesis inside the biocorrosion area, which is the space the zinc wire had previously occupied on the 2.5- and 4-month specimens. The presence of nucleated cells extending into the biocorrosion area and the synthesis of extracellular matrix suggests a non-fibrotic tissue regenerative host response to the zinc material. This type of regeneration is clearly lacking in the 2.5- and 4-month specimens and likely occurred due to the porosity generated within the implant as corrosion progressed. Importantly, there is no evidence of cell hyperplasia or chronic inflammation in this biocorrosion area being filled with regenerating tissue.

Figure 1 also demonstrates a lower cell density within the neointimal tissue at locations near the zinc implant compared to the area near the blood interface (Figure 2B). A similar neointimal layer with low cell density and without signs of necrosis was also seen around the portions of the zinc wire that were not in contact with the mural endothelium (Figure 3). The 6.5-month specimen exhibits inflammatory infiltrates along the wire length that is mild in nature and predominantly localized to the corrosion product, while the 4-month specimen exhibits less inflammatory cell infiltration, which is also localized to the biocorrosion area. The thin neointimal tissue and low cell density stands in marked contrast to what was seen with biodegradable iron, in the same animal model [29].

Endothelial cell (EC) fluorescence images show a complete endothelial layer at the outer edge of the neointima by 2.5 months and stable appearance at 6.5 months (Figure 4). Similarly, smooth muscle cell (SMC) fluorescence imaging demonstrates a layer of SMCs at 2.5 months which, again, remains stable at 6.5 months. The SMC layer is thickest ($\sim 50 \mu\text{m}$) within the neointima closest to the mural surface and is nearly absent both at the luminal interface and within the biocorrosion areas. These results highlight both the limited SMC proliferation and the persistence of a stable EC layer in response to high purity zinc and its products of biocorrosion. Note that the area around the zinc implant is observed to fluoresce in this series of images. This is due to a combination of zinc corrosion product fluorescence [33] and/or other incidental fluorescence sources.

Despite extensive corrosion of zinc over 6.5 months, we detected no discernable major chronic inflammatory response, necrosis, or hyper-proliferative response to the zinc implant in any of the six specimens evaluated. In contrast, substantial cell necrosis was evident in histological cross sections made directly at the wall puncture site (data not shown) as expected in association with transmural arterial injury.

Discussion

In this murine preclinical model, high purity zinc wires implanted within the abdominal aorta exhibited excellent biocompatibility. Specifically, we did not observe a significant chronic inflammatory response, localized necrosis, or progressive intimal hyperplasia; all of which are mediators of stent restenosis. On the contrary, local tissue response to the

implants included evidence of early tissue regeneration within the original footprint of the implant within the biocorrosion area.

We were able to demonstrate that there was a mild inflammatory response to the implants with minimal to no local cellular necrosis. Inflammatory infiltrates were limited at the early time points and increased at the 6.5-month mark, but were restricted to the biocorrosion area. At all three time points, there was no cellular hyperplasia or progressive thickening of the neointimal layer. This finding, in conjunction with the observed low cellular density and near-total lack of smooth muscle cells (SMCs) near the implant, suggests a possible suppressive effect from zinc or its corrosion products on the activity of SMCs and inflammatory cells. A similar effect of reduced cell density near an implant in the absence of necrosis has not been reported for any other stent material to our knowledge, whether polymeric or metallic. The results for zinc stand in sharp contrast to what was found for pure iron, which experienced an extensive intimal hyperplasia with uniform cell density in the same animal model, progressing to near-complete arterial occlusion by 9 months [29]. Furthermore, magnesium-based materials corrode too rapidly to compare the neointimal host response at the time points evaluated here. The results demonstrate the general stability of neointimal tissue in the presence of zinc and suggest that a zinc-based stent may not experience a substantial reduction in luminal cross sectional area due to progressive intimal hyperplasia.

This inference is supported, at least in part, by previous studies which have shown that zinc therapy may reduce neointimal hyperplasia following angioplasty [34]; zinc may regulate inflammatory cytokines [35]; and zinc deficiency increases the incidence of cardiovascular disease [36]. Together, these findings raise the exciting possibility that zinc stents may suppress localized cellular activity and effectively limit intimal thickening. This apparent localized effect of zinc requires further investigation, but, if confirmed, would make zinc and its alloys the ideal material family for the future of intravascular stenting. It may ultimately reduce the need for a drug-eluting polymer coating, and thereby avoid the harmful side effects of delayed healing and any increased risk of late-stage thrombosis. Future studies will need to be undertaken to clarify any cell-suppressive mechanism and the specific cell types affected by zinc.

The study is limited in that the model makes use of a single wire implant to simulate the presence of a stent strut within the vascular space. The application of radial force on the arterial wall with the potential for more extensive endothelial injury may result in different localized host tissue response to the material. However, although the geometry and amount of a wire is different between a full stent and this single wire, this model allows for reproducible and detailed investigations at the interface between the candidate metal and the arterial endothelium and circulating blood cells. This experimental model has been used to prescreen other candidate stent materials prior to proceeding along the more challenging path of stent manufacturing and large animal studies. Compared to the data using this preclinical model with implanted magnesium and iron wires, these results strongly support the need for the next phase of testing, including zinc-based stent manufacturing, large animal stent implantation, and associated degradation and biocompatibility studies.

Conclusions

Histological examination of zinc wires implanted in the abdominal aortas of rats indicated excellent biocompatibility with the arterial tissue. None of the major contributors to restenosis— inflammatory response, localized necrosis, and progressive intimal hyperplasia—were observed. It was found that tissue regenerated within the original footprint of the implant after partial degradation. Low cellular density and a distinct lack of smooth muscle cells adjacent to the implant interface indicates that zinc may exhibit an antiproliferative effect and guard against restenosis after stent implantation.

Acknowledgements

The Michigan Initiative for Innovation and Entrepreneurship (Technology Commercialization Fund, Grant #3093231) and U.S. National Institute of Health (National Institute of Biomedical Imaging and Bioengineering, Grant #1R21EB019118-01A1) are acknowledged for funding this work. PKB was funded by an American Heart Association (Midwest Affiliate) predoctoral fellowship. RJGII was supported by a Michigan Space Grant Consortium undergraduate research fellowship.

References

- Hascoët S, Baruteau A, Jalal Z, Mauri L, Acar P, Elbaz M, Boudjemline Y, Fraisse A. Arch. Cardiovasc. Dis. 2014; 107:462–475. [PubMed: 25128078]
- Farb A, Heller PF, Shroff S, Cheng L, Kolodgie FD, Carter AJ, Scott DS, Froehlich J, Virmani R. Circulation. 2001; 104:473–479. [PubMed: 11468212]
- Virmani R, Liistro F, Stankovic G, Di Mario C, Montorfano M, Farb A, Kolodgie FD, Colombo A. Circulation. 2002; 106:2649–2651. [PubMed: 12438288]
- Carter AJ, Aggarwal M, Kopia GA, Tio F, Tsao PS, Kolata R, Yeung AC, Llanos G, Dooley J, Falotico R. Cardiovasc. Res. 2004; 63:617–624. [PubMed: 15306217]
- Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E, Kutys R, Skorija K, Gold HK, Virmani R. J. Am. Coll. Cardiol. 2006; 48:193–202. [PubMed: 16814667]
- Barlis P, Virmani R, Sheppard MN, Tanigawa J, Di Mario C. Eur. Heart J. 2007; 28:1675–1675. [PubMed: 17615084]
- Nakagawa Y, Kimura T, Morimoto T, Nomura M, Saku K, Haruta S, Muramatsu T, Nobuyoshi M, Kadota K, Fujita H, Tatami R, Shiode N, Nishikawa H, Shibata Y, Miyazaki S, Murata Y, Honda T, Kawasaki T, Doi O, Hiasa Y, Hayashi Y, Matsuzaki M, Mitsudo K. Am. J. Cardiol. 2010; 106:329–336. [PubMed: 20643241]
- Centemero MP, Stadler JR. Expert. Rev. Cardiovasc. Ther. 2012; 10:599–615. [PubMed: 22651836]
- Kang S-J, Song H-G, Ahn J-M, Kim W-J, Lee J-Y, Park D-W, Lee S-W, Kim Y-H, Lee CW, Park S-W. JACC Cardiovasc. Imaging. 2012; 5:1267–1268. [PubMed: 23236978]
- Park S-J, Kang S-J, Virmani R, Nakano M, Ueda Y. J. Am. Coll. Cardiol. 2012; 59:2051–2057. [PubMed: 22651862]
- Zartner P, Buettner M, Singer H, Sigler M. Catheter. Cardiovasc. Interv. 2007; 69:443–446. [PubMed: 17295281]
- Peters B, Ewert P, Berger F. Ann. Pediatr. Cardiol. 2009; 2:3–23. [PubMed: 20300265]
- Alexy RD, Levi DS. Biomed. Res. Int. 2013; 2013:137985. [PubMed: 24089660]
- Haude M, Erbel R, Erne P, Verheye S, Degen H, Bose D, Vermeersch P, Wijnbergen I, Weissman N, Prati F, Waksman R, Koolen J. Lancet. 2013; 381:836–844. [PubMed: 23332165]
- Patel N, Banning AP. Heart. 2013; 99:1236–1243. [PubMed: 23474621]
- Serruys PW, Chevalier B, Dudek D, Cequier A, Carrié D, Iniguez A, Dominici M, van der Schaaf RJ, Haude M, Wasungu L, Veldhof S, Peng L, Staehr P, Grundeken MJ, Ishibashi Y, Garcia-Garcia HM, Onuma Y. Lancet. 2015; 385:43–54. [PubMed: 25230593]
- Barlis P, Tanigawa J, Di Mario C. Eur. Heart J. 2007; 28:2319–2319. [PubMed: 17485484]

18. Grube E, Sonoda S, Ikeno F, Honda Y, Kar S, Chan C, Gerckens U, Lansky AJ, Fitzgerald PJ. Circulation. 2004; 109:2168–2171. [PubMed: 15123533]
19. Tamai H, Igaki K, Kyo E, Kosuga K, Kawashima A, Matsui S, Komori H, Tsuji T, Motohara S, Uehata H. Circulation. 2000; 102:399–404. [PubMed: 10908211]
20. Onuma Y, Serruys PW. Circulation. 2011; 123:779–797. [PubMed: 21343594]
21. Heublein B, Rohde R, Kaese V, Niemeyer M, Hartung W, Haverich A. Heart. 2003; 89:651–656. [PubMed: 12748224]
22. Witte F, Fischer J, Nellesen J, Crostack H-A, Kaese V, Pisch A, Beckmann F, Windhagen H. Biomaterials. 2006; 27:1013–1018. [PubMed: 16122786]
23. Willumeit R, Fischer J, Feyerabend F, Hort N, Bismayer U, Heidrich S, Mihailova B. Acta Biomater. 2011; 7:2704–2715. [PubMed: 21382530]
24. Bowen PK, Drelich A, Drelich J, Goldman J, Biomed J. Mater. Res. A. 2015; 103:341–349.
25. Bowen PK, Drelich J, Goldman J. Acta Biomater. 2014; 10:1475–1483. [PubMed: 24296127]
26. Zheng YF, Gu XN, Witte F. Mater. Sci. Eng. R. 2014; 77:1–34.
27. Onuma Y, Ormiston J, Serruys PW. Circ. J. 2011; 75:509–520. [PubMed: 21301138]
28. Di Mario C, Caiazzo G. Lancet. 2015; 385:10–12. [PubMed: 25230592]
29. Pierson D, Edick J, Tauscher A, Pokorney E, Bowen PK, Gelbaugh J, Stinson J, Getty H, Lee CH, Drelich J, Goldman J, Biomed J. Mater. Res. B. 2012; 100B:58–67.
30. Bowen PK, Gelbaugh JA, Mercier PJ, Goldman J, Drelich J, Biomed J. Mater. Res. B. 2012; 100B: 2101–2113.
31. Bowen PK, Drelich J, Goldman J. Mater. Sci. Eng. C. 2013; 33:5064–5070.
32. Bowen PK, Drelich J, Goldman J. Adv. Mater. 2013; 25:2577–2582. [PubMed: 23495090]
33. Choi M, McBean K, Ng P, McDonagh A, Maynard P, Lennard C, Roux C. J. Mater. Sci. 2008; 43:732–737.
34. Berger M, Rubinraut E, Barshack I, Roth A, Keren G, George J. Atherosclerosis. 2004; 175:229–234. [PubMed: 15262178]
35. Foster M, Samman S. Nutrients. 2012; 4:676–694. [PubMed: 22852057]
36. Tomat AL, Costa MdlÁ, Arranz CT. Nutrition. 2011; 27:392–398. [PubMed: 21074972]

Research Highlights

- Arterial biocompatibility of zinc was evaluated in the abdominal aortas of rats.
- Risk factors for in-stent restenosis were not observed.
- Tissue regenerated following corrosion of the zinc.
- Lack of smooth muscle cells near zinc indicated that it might be antiproliferative.

Figure 1.

H&E stained sections from excised high purity zinc wires (250 μm nominal diameter) after residence in the arterial lumen for 2.5, 4, and 6.5 months ($n = 2$ per time point), showing benign neointimal formation and a healthy artery. Black arrow at 10 $\times$ magnification identifies the position of the zinc wire, which is surrounded by a neointima (wire cross sections were dislodged during sectioning). Black arrow at 60 $\times$ magnification identifies the neointima on the luminal side, which never exceeds 100 μm in thickness in any of the six specimens examined. Red stars in the images identify the position of the zinc wires. Yellow

stars in the images identify regions of low cell density near the zinc wire, which contrast strikingly with the high-cell density regions further away from the zinc wire. Dark green arrows identify cell and tissue regeneration inside the zinc implant. Light green arrowheads identify cells within the corrosion layer, highlighting the excellent biocompatibility of zinc corrosion products. Tissue regeneration can be seen at 6.5 months. Scale bars: 10 $\times$ = 500 μ m, 20 $\times$ = 200 μ m, 60 $\times$ = 100 μ m, and 100 $\times$ = 50 μ m.

Figure 2.

Neointimal tissue thickness at the luminal vs. mural side of the implant (A) and cell density near the implant vs. near the blood interface (B). Significance values were determined via the Student's *t*-test.

Figure 3.

H&E stained cross sections of wire implants showing typical regions of wire that were not in contact with the arterial wall. Upper images show 4- (A) and 6.5-month (B) implants at 40× magnification. Lower images (C & D) show high magnification images (100×) of the 6.5-month cross section shown in panel B. Note that the wire cross-section was dislodged during cryo-sectioning.

Figure 4.

Cross sections were stained for endothelial cells (red, CD31, left panels), smooth muscle cells (red, α -actin, right panels), and cell nuclei (DAPI counterstained blue, both panels) at 2.5 and 6.5 months. The green arrow in each panel identifies a characteristic region of positive staining within the neointimal tissue. Note that the corrosion layer impregnating the center of the neointimal tissue is excited and fluoresces red in these images.