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Cold drawn bioabsorbable ferrous and ferrous composite wires: An evaluation of in vitro vascular cytocompatibility ☆

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ABSTRACT

A systematic approach is applied to quantify the impact of bioabsorbable metals on human vascular endothelial cells (EC) and aortic smooth muscle cells (SMC) with the aim of optimizing bioabsorbable endovascular stent development. Composite wires comprising novel combinations of Fe, Mn, Mg, and Zn were produced and fabricated into tubular mesh stents. The stents were incubated with primary EC in order to assess attachment and cell proliferation. Migration of SMCs from the vessel medial wall to the target lesion site following recanalization of an atherosclerotic artery is important in the process of neointimal hyperplasia. Metal ion species were assayed for their impact on cell migration and survival at concentrations ranging from 0.037 to 10 mM. An MTT-based assay was used to assess cytotoxicity after insult with various metal ion concentrations. Fe^{2+} and Fe^{3+} ion species were found to repress the migration of SMCs across a porous polycarbonate track etch membrane at concentrations of 1 mM. Mn^{2+} promoted SMC migration at a concentration of 1 mM, however, this effect was quenched when Fe^{2+} was included. Mg^{2+} was found to significantly increase SMC migration at concentrations above 1 mM. Cell survival was not reduced after 24 h insult with concentrations of Mg^{2+} up to 10 mM. LD_{50} concentrations of greater than 1 mM were found for Mg^{2+}, Fe^{2+}, Fe^{3+}, and Fe^{2+} with 35 wt.% Mn^{2+}. Significantly greater numbers of EC attached to bioabsorbable metal species compared with 316L stainless steel. Good EC coverage and proliferation were observed for all tested materials up to 120 h.

1. Introduction

Stenting is a common therapy used to maintain patency in freshly recanalized, usually atherosclerotic, arteries [1]. In its most common embodiment occluded arteries are held open by a small, corrosion-resistant metal scaffold which is permanently integrated into the vessel wall by endothelialization and neointima formation [2]. Once integration is complete the scaffold remains fixed within the body. Intimal, medial and adventitial remodeling after luminal expansion means that the mechanical support is only needed for about 3–6 months [3–5]. Despite the clinical success of modern stents compared with plain coronary artery bypass graft surgery (CABG), there remains a need for bioabsorbable stents that leave behind only a healed, prosthetic-free vessel. Analogous problems exist in the realm of orthopedic hard and soft tissue fixation, in which permanent structures may shield native tissues from mechanical loads which would otherwise promote beneficial remodeling [6,7].

Bioabsorbable materials have the potential to yield implants for clinical applications requiring only transient support, ideally resulting in only healed native tissues. As a result of their biocompatibility and process-dependent mechanical properties, transition metals, such as iron, manganese, magnesium, and zinc, may provide high strength building blocks for bioabsorbable medical implants such as vascular stents and orthopedic screws, pins and plates [7–10]. The use of metals in bioabsorbable therapy dates back to at least 1887, when magnesium was suggested by Dr. E.C. Huse for use as a bioabsorbable ligature [11]. Alloys of magnesium were unsuccessfully used in the 1930s by McBride and others for bone fixation [12]. In these early efforts magnesium was found to be well tolerated with no systemic toxicity, but rapid or inconsistent degradation and excessive degradation-related gas formation caused undesirable wound interface cavitation [7]. With the advent of durable stainless steel implant technologies in the 1940s there was little work on new biodegradable metals until the 1990s [3,13]. Metals known to exist naturally in the body have recently been examined as both pure and alloyed constituents for bioabsorbable devices because of their known biological tolerance, however, significant materials engineering and biological interaction data are needed to promote their integration into future device designs [10].

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1.1. Research aim

An ideal bioabsorbable material for vascular stent applications should provide structural stability until healing is complete while promoting the attachment and proliferation of endothelial cells. Further, this optimized material may promote the rapid formation of a natural endothelium while providing a cytostatic, suppressive influence on smooth muscle cells, reducing neointimal hyperplasia (NIH). Degradation products, such as metal ions, which accumulate on the apical side of the endothelium should not promote invasion of SMC from the vessel medium, through the endothelial basement membrane towards the lesion site. In vitro experiments can give dose-dependent information on cellular chemotaxis, proliferation and toxicity, however, it is impossible to include all intervening factors such as blood shear-mediated gene regulation [14–16], shear-mediated cell alignment [17], atheromatous plaque [18,19], etc. In vitro data are needed describing not only the dose-dependent cytotoxicity and chemotactic behavior of human vascular cells but also the impact of degradation species on cell attachment and proliferative capacity. The aim of this study is to optimize bioabsorbable materials design through quantification of the vascular cell response to insult by candidate bioabsorbable metal species and predicted metal degradation products. This work targets one facet of the challenge described by Oeveren et al. [20], namely the in vitro reactions at the tissue–biomaterial interface. The important reactions which occur at the material–blood interface will be left for future work. The response of human vascular endothelial cells (EC) and smooth muscle cells (SMC) is studied in the presence of various doses of Fe, Mg, Mn and Zn ions in comparison with stainless metal ion species, including Co, Ni, and Cr. A goal of these experiments was to determine the dose dependent cytotoxicity, ranging from innocuous to toxic, and identify a working range to serve as a design tool in conjunction with material degradation kinetics experiments, hemocompatibility studies, and theoretical models. The quantitative cytotoxicity, chemotaxis and attachment data developed in this work can be used to limit future clinical expenditure to the most promising bioabsorbable materials systems.

2. Materials and methods

Thin, visibly oxide-free, 177 μm diameter bioabsorbable wires were produced according to previously published techniques [8]. Monolithic and bimetal composite configurations were manufactured from elements including iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn). Table 1 shows the nominal chemical compositions, cold work and mean transverse grain size for each material. All wires were produced for testing from commercially available feedstock. Manufacturing processes were developed for each material system in order to facilitate the wire drawing process and improve the mechanical strength of each material to yield strengths exceeding 1 GPa. The wires were fabricated by braiding 24 filaments into a tubular structure with a diameter of 7 ± 0.5 mm and a wire crossing angle of 90 ± 5°. Test "stent" samples were cut from the tubular braided structure and affixed to provide open channel working lengths of 5 ± 0.5 mm for cell attachment and proliferation experiments. All Fe and Fe–Mn metal surfaces were annealed during wire processing and after stent fabrication under a protective high purity argon atmosphere, resulting in a bright, visibly oxide-free surface condition. 316L stainless steel was bright annealed in a reducing hydrogen atmosphere.

Table 1
Compositional and microstructural data summary for bioabsorbable wires adapted from Schaffer [8].

Wire	Composition (wt.%)	Cold work (%RA)	GS (μm)	GS location parameter	GS scale parameter
	Mg	75	n/a	n/a	n/a
1	99.95 Fe	50	2.5	0.76	0.57
2	99.95 Fe	90	1.8	0.50	0.45
3	99.95 Fe	99	0.26	−1.4	0.38
4	316L	90	0.55	−0.72	0.47
5	Fe-35Mn	99	0.20	−1.7	0.33
6	Fe35Mn-DFT-25%ZM21	75	0.25	−1.5	0.31
7	Fe-DFT-25%ZM21	50	0.27	−1.4	0.38
8	Fe-DFT-57%ZM21	50	0.29	−1.3	0.37

GS, mean grain size.

2.1. Cell culture and maintenance

Primary human aortic endothelial cells (EC) were acquired in the proliferating state at passage number 4 from Lonza (CC-2635, Lonza Inc., Walkersville, MD). EC were expanded by subculture when cells reached 80–90% confluence by splitting into T-75 cell culture flasks (353136, BD Biosciences, Sparks, MD) at a seeding density of 2500–5000 cells cm^{-2} in endothelial basal medium (CC-3156, EBM-2, Lonza Inc.) supplemented with EGM-2MV BulletKits (CC-4147, Lonza Inc.) containing fetal bovine serum (5 vol.%), hydrocortisone, basic human fibroblast growth factor (hFGF-B), vascular endothelial growth factor (VEGF), propeptide R3 insulin-like growth factor (R3-IGF), human endothelial growth factor (hEGF), ascorbic acid, gentamicin sulfate (50 μg ml^{-1}) and amphotericin B (AmpB) (2.5 μg ml^{-1}). Cells were released for subculture and experimentation by standard trypsinization using buffered trypsin (0.05% w/v) with sodium EDTA (0.02% w/v, Life Technologies, Grand Island, NY). Cells were maintained in an aseptic, 37 ± 0.1 °C, 5% CO_2 environment and used for experimentation at passage numbers 8–10. The cell media were refreshed every 2–3 days.

Primary human aortic smooth muscle cells (SMC) were acquired in the proliferating state at passage number 4 or 5 from Lonza (CC-2671, Lonza Inc.). SMC were expanded by subculture when cells reached 80–90% confluence by splitting into T-75 cell culture flasks at a seeding density of 2500–4000 cells cm^{-2} in smooth muscle basal medium (CC-3181, SmBM, Lonza Inc.) supplemented with SmGM-2 BulletKits (CC-4149, Lonza Inc.) containing fetal bovine serum (FBS) (5 vol.%), insulin, hFGF-b, hEGF, gentamicin sulfate, and AmpB. Cells were released for subculture and experimentation by standard trypsinization using buffered trypsin (0.10% w/v) with sodium EDTA. Cells were maintained in an aseptic, 37 °C, 5% CO_2 environment and used for experimentation at passage numbers 8–10. Undisturbed SMC residing in the tunica media are expected to reside in a quiescent state, being subject to less disturbance during intervention than the more disrupted endothelium [21]. Therefore, for this experiment SMC were brought to quiescence for migration studies by incubation in a low serum, growth factor-deficient medium comprising SmBM basal medium with 0.1 vol.% FBS. The cell media were refreshed every 2–3 days.

2.2. Cell behavior

Fig. 1 shows the four tissue interface investigations performed in this study. Fig. 1a shows the chemotaxis conditions for a single well and experimental condition described further below. Cell suspension was placed above and separated from a set metal ion concentration by a porous polycarbonate track etch membrane (PCTE). No known reports are available which have conclusively determined metal degradation by-product concentration gradients or magnitudes in living tissue, thus an investigation of metal ion dose dependence was carried out at concentrations ranging from low to levels expected to exert cytotoxic effects. Metal ion species were selected based on their expected oxidation states in pH 7.4 aqueous solution at an assumed near neutral surface potential from

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