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Fe–Mn alloys for metallic biodegradable stents: Degradation and cell viability studies ☆

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ABSTRACT

Biodegradable stents have shown their potential to be a valid alternative for the treatment of coronary artery occlusion. This new class of stents requires materials having excellent mechanical properties and controllable degradation behaviour without inducing toxicological problems. The properties of the currently considered gold standard material for stents, stainless steel 316L, were approached by new Fe–Mn alloys. The degradation characteristics of these Fe–Mn alloys were investigated including in vitro cell viability. A specific test bench was used to investigate the degradation in flow conditions simulating those of coronary artery. A water-soluble tetrazolium test method was used to study the effect of the alloy's degradation product to the viability of fibroblast cells. These tests have revealed the corrosion mechanism of the alloys. The degradation products consist of metal hydroxides and calcium/phosphorus layers. The alloys have shown low inhibition to fibroblast cells' metabolic activities. It is concluded that they demonstrate their potential to be developed as degradable metallic biomaterials.

1. Introduction

Stents, tiny tubular mesh-like metallic structure implants, have proved their effectiveness in treating narrowed arteries [1,2]. Nevertheless, they present two remaining complications: subacute stent thrombosis [3] and in-stent restenosis [4]. Two approaches have been proposed to deal with these limitations: the drug eluting and the biodegradable stents. The current technology of drug eluting stents is still facing the problem of late stent thrombosis [5]. Meanwhile, the implantation of biodegradable stents hopefully will leave behind only a healed arterial vessel, preventing late stent thrombosis, in-stent restenosis and the prolonged antiplatelet therapy [6]. They are expected to degrade within a reasonable period (12–24 months) [7] after the stented artery has been remodelled (6–12 months) [8,9]. Biodegradable stents made of metals were clinically implanted to treat congenital heart disease in babies [10–12] and to treat critical limb ischemia in adults [13]. Recently, a non-randomized multi-center clinical trial on biodegradable magnesium stents was conducted with encouraging initial results [14].

Two classes of materials have been used to prepare biodegradable stents: polymers, from the lactic acid, glycolic and caprolactone families [15–18], and metals, either magnesium alloys [13,19–25] or pure iron [26–28]. Metallic biodegradable stents were more developed than their polymeric counterparts. In fact, metals have superior mechanical properties than polymers for replicating the properties of stainless steel 316L (SS316L), the reference material for coronary stent [29]. Nevertheless, improvements are needed mainly to decrease the degradation rate of magnesium alloys [14,19,23,25] or to accelerate that of iron-based stents [26,27]. Some attempts have been made by developing new magnesium alloys including Mg–Zn–Mn [30], Mg–Ca [31,32] and Fe–Mn alloys [33,34]. Fe–Mn alloys, containing between 20 and 35 wt.% manganese, exhibited mechanical properties comparable to those of SS316L alloy [33,34]. They possess a similar austenite (γ) structure, even though the γ forming elements are different as nickel was used for the SS316L and manganese for the Fe–Mn alloys. The presence of this austenitic phase reduces the magnetic susceptibility compared to SS316L alloy which will give an enhanced compatibility with the magnetic resonance imaging (MRI). From a biological point of view, the presence of an alloying element such as manganese for a biodegradable iron-based alloy appears more appropriate than nickel, the former being essential to human [35,36] the latter classified as toxic and carcinogenic [37]. An overdosage of manganese could lead to intoxification and neurotoxicity [38]; however, due to the extensive plasma

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protein binding that counteract the effect of manganese toxicity, excess of manganese is not reported to be toxic in cardiovascular system [39,40]. Moreover, considering the very light weight of a stent which is 50–100 mg depending on design, the release of element from the alloy during a controlled degradation could be expected to be lower than their toxic level in the blood.

In developing materials for biodegradable stents, studies on degradation behaviour and degradation products' cytotoxicity have to be considered. The degradation study investigates the mechanisms of decomposition and rate. A specific test bench simulating conditions encountered in human coronary artery has been developed by Levesque et al. [20]. The test bench was able to show the important role of shear stress on degradation behaviour of metals which cannot be assessed with the common immersion or electrochemical test methods [21]. This so-called dynamic degradation test method was suggested to be used for evaluating materials proposed for biodegradable stents [21].

The cytotoxicity study can assess the early signs for biocompatibility such as acute cytotoxicity. At present time, it is difficult to find literature on cytotoxicity study of degradable metals for cardiovascular applications. However, methods used in studies on magnesium alloys dedicated for bone implants could be transposed to assess the early sign of cytotoxicity for other metals proposed for biodegradable stents. Li et al. have conducted an evaluation of cytotoxicity of alkali heat treated pure magnesium using marrow cells [41]. The same indirect contact cytotoxicity test method was carried out by Li et al. [31] on Mg–Ca alloys using L-929 cells. The ISO 10993-5:1999 standard was used as a guide for preparing the extracts [42]. Therefore, in the present study, the new iron-based alloys containing manganese (Fe–Mn) were assessed for their degradation characteristics and their early sign of cytotoxicity using a dynamic degradation test bench and using a cell viability test, respectively.

2. Materials and methods

2.1. Materials

Materials used in this study were iron-based alloys containing 20–35 wt.% manganese denoted as Fe20Mn, Fe25Mn, Fe30Mn and Fe35Mn. The alloys were prepared through powder sintering process from high purity elemental powders of iron and manganese followed by a series of cold rolling and resintering cycles resulting in a highly dense material. The details about these alloys including fabrication process, structure and properties are described elsewhere [33,34]. The Fe20Mn and Fe25Mn are constituted of $\gamma + \varepsilon$ phases, whereas Fe30Mn and Fe35Mn are composed of single γ phase [34]; therefore for degradation tests, specimens of Fe25Mn and Fe35Mn alloys representing two different microstructure conditions were chosen. Specimens with an exposed surface area of $\sim$300 mm^2 were mounted in acrylic resin. They were then polished using abrasive papers #1000, ultrasonically cleaned in 75% ethanol, air-dried and stored for 24 h in a desiccator prior to use.

For cell viability tests, powders were used in order to provide an extreme condition of high surface area. Powders of Fe–Mn alloys with size of -53 μm were prepared by means of mechanical filing and sieving. This size was chosen based on preliminary experiments with different powder size where it was found that -53 μm powders induced the most severe effect to the cells. Powders of commercial iron, manganese and SS316L were also used for comparison purposes (Atlantic Equipment Engineers, Bergenfield, USA). The powders were sterilized with 75% ethanol and rinsed with phosphate buffered saline prior the tests. The Fe35Mn alloy was considered for further tests as it contains the highest manganese content among the other Fe–Mn alloys under study.

2.2. Degradation test

Test solution was prepared from modified Hank's solution having ionic composition and concentration considerably similar to those of human blood plasma. A pseudo-physiological-like shear stress of 4 Pa was generated by a predetermined laminar flowing solution in a test bench designed to mimic blood flow condition in human coronary artery. More details on the test bench and test parameters are reported elsewhere [21]. The specimens were taken out from the test bench after 1 week, 1 month and 3 months and were then characterized. The temperature of the test solution was kept at 37 °C and its pH was also recorded every 24 h.

Solution samplings were carried out during the test and their concentration of iron and manganese was measured by a Perkin Elmer 3110 atomic absorption spectrometer (AAS). A Siemens D5000 X-ray diffractometer (XRD) with Cu Kα radiation at a scanning rate of 0.02°/1.2 s^{-1} was employed to identify the structure of degradation products of the specimens. The microstructure was studied with an Olympus PME3 optical microscope (OM) and a Jeol JSM-840A scanning electron microscope (SEM). Images obtained from OM were analysed using a quantitative image analyser software (Clemex Vision) to measure corroded depth on at least 5 fields to obtain averages and standard deviations. The chemical composition of degradation products was analysed using X-ray photoelectron spectroscopy (XPS) in a Phi VersaProbe and using an energy dispersive spectrometer (EDS) which was coupled with the SEM. A Cameca SX100 electron probe micro-analyser (EPMA) was used to map iron, manganese and oxygen. Specimens for OM, SEM and EPMA were mounted in acrylic resin to expose their cross-section area and were then mechanically polished using abrasive papers up to #1200 and 0.1 μm diamond paste.

2.3. Cell viability test

Cell viability tests were carried out by indirect contact with the 3T3 mouse fibroblast cell line (3T3). They were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 100 U ml^{-1} penicillin and 100 mg ml^{-1} streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. The cells were incubated in 24-well tissue culture plates at the density of 50,000 cells/well for 24 h to allow attachment.

Samples to be tested were then added into 3 μm tissue culture inserts in the same medium as for the cells. The content of samples in the medium was varied and expressed as concentration (mg ml^{-1}). At least 6 replicates were studied for each condition. After 48 h of incubation, the cell viability was assessed using water-soluble tetrazolium based assay (10% WST-1, 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene-disulfonate) for 2 h. The dissolved degradation products were rinsed out as the medium was aspirated after the treatment with the samples. The absorbances of the solutions were measured spectrophotometrically at 440 nm using a VersaMax microplate reader (Molecular Devices, Sunnyvale, USA) and were analysed using the Prism 5 software (GraphPad Software Inc., San Diego, USA).

3. Results

3.1. Degradation tests

Fig. 1 shows cross-sectional profiles of Fe–Mn specimens before and after dynamic degradation test. The degradation (corrosion) took place over the entire surface and then went deeper into the