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In vitro degradation behavior of Fe–20Mn–1.2C alloy in three different pseudo-physiological solutions

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ABSTRACT

High manganese austenitic steels such as Fe–20Mn–1.2C alloys are among the most promising candidates for biodegradable stents applications due to their high strength, high ductility and their chemical composition. In the current work, 14 day static in-vitro tests were performed in controlled atmosphere to assess the degradation behavior in three common pseudo-physiological solutions, i.e. commercial Hanks' (CH), modified Hanks' (MH) and albumin-enriched Dulbecco's modified phosphate buffered saline (DPBS) solutions. The degraded samples surfaces as well as the degradation products were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR).

Degradation of material and degradation products are shown to be strongly dependent on the test medium due to the presence of different ionic species such as HCO_3^-, CO_3^{2-}, Cl^-, Ca^{2+} or phosphate groups. In both MH and CH solutions, the increased content of HCO_3^- ions seems to promote $MnCO_3$ crystal growth on sample surfaces whereas the presence of albumin and high content of phosphate ions promotes the formation of an amorphous layer rich in phosphates, iron and manganese.

1. Introduction

Among the pathologies affecting the cardiovascular system, atherosclerosis is undoubtedly one of the most relevant with 7.3 million deaths per year in the world [1]. Significant progresses were made in the treatment of this pathology with the application of angioplasty [2]. A stent is also often deployed in the injured site to provide a mechanical support for the vessel walls [3] and to prevent its further occlusion after surgical treatment. Stents made of metallic materials such as AISI 316L stainless steel and Nitinol offer a solution to the problem of artery blockage. However, a common outcome associated with the use of permanent stents is restenosis [4]; this complication can be triggered by the release of toxic ions, for example Ni, which is a common element found in many alloys used in stent fabrication [5].

A new trend in scientific community is based on the evidence that the presence of a stent after the vessel restoration would not provide any further benefits [6]: on the contrary, it may cause additional problems, for example in pediatric patients. For this reason, biodegradable metals [6–8] emerged as a novel class of biomaterials during the last decade. Current studies focus mainly on Mg and Mg-based alloys [9], Fe and Fe-based alloys [10–12] and other metals (Zn, etc.) [13]. Metallic biodegradable materials should display suitable mechanical properties, an appropriate degradation rate matching the healing time of the injured vessel and a convenient ion release in term of chemical species, size of debris and general biocompatibility [14,15]. Fe-based alloys [16] have mechanical properties comparable to those of the reference material (AISI 316L stainless steel), but their degradation rate is still considered too slow [10–12]. Therefore, interesting strategies to improve the degradation rate are based on addition of alloying elements [10,16–18] and/or on microstructural modifications [19]. Also, improved mechanical properties would result in reduced strut thickness; a high ductility is however always needed to withstand the plastic deformation during the deployment sequence. Recently, high manganese austenitic steels with twinning induced plasticity (TWIP) effect, developed primarily for industrial applications, raised a great interest in the scientific community because of their exceptional combination of high strength and ductility [20]. The low corrosion resistance in chloride-rich environment [21,22] and the presence of Mn, reported as non-toxic for the cardiovascular system [23] for the considered concentrations, make this family of materials a promising candidate for use in cardiovascular devices. Different kinds of Fe–Mn based alloys were already investigated by several authors [17,18], in modified Hanks'

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media and in ambient atmosphere. Few studies deal with solutions in CO_2 atmosphere.

The aim of this work was to investigate the degradation behavior of Fe–20Mn–1.2C in three different pseudo-physiological solutions commonly used for in vitro tests. Characterization of sample surface and degradation products was carried out with different techniques, in order to assess the degradation pattern of this steel in a CO_2-rich atmosphere.

2. Materials and methods

2.1. Substrate material

Fe–20Mn–1.2C samples were obtained from a cast ingot produced industrially; the material was used as received in the form of rolled sheet with a thickness of 1.4 mm. The sheet was hot rolled to a thickness of 3 mm, thermally treated at 950 °C for 8 min, water quenched and finally reduced to a thickness of 1.4 mm. The nominal composition is 20 wt.% Mn, 1.2 wt.% C and bal. Fe [24].

2.2. Degradation test

Three common pseudo-physiological solutions were used for static degradation tests, to assess the degradation rate in different ion rich media, the topography and structure of the degraded surfaces and the degradation products. A commercial Hanks' solution (CH) was prepared by dissolving 9.5 g of Hanks' balanced salts (H2387, Sigma-Aldrich) and 0.35 g of sodium bicarbonate (S8875-500G, Sigma-Aldrich) in 1 L of nanopure water. A modified Hanks' solution (MH) is composed by 9.7 g of Hanks' balanced salts (H1387, Sigma-Aldrich) dissolved in 1.4 L of nanopure water with 14.169 g of HEPES acid (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (H3375-5000G, Sigma-Aldrich, USA), 16.65 g of HEPES sodium salt (sodium 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, H7006-500G, Sigma-Aldrich, USA) and 3.3 g of sodium bicarbonate (S8875-500G, Sigma-Aldrich) [25]. Dulbecco's phosphate buffered saline (DPBS) solution (D5652, Sigma-Aldrich) was prepared with the addition of bovine serum albumin (BSA) (A2153-50G, Sigma-Aldrich) by dissolving 9.6 g of DPBS and 1 g of BSA in 1 L of nanopure water. The pH of the three solutions was adjusted to 7.4 using 1 M NaOH or HCl aqueous solutions. DPBS solution pH was adjusted before adding albumin. Salt concentration and ionic composition of each solution are reported in Table 1 and Table 2, respectively. After the preparation, solutions were filtered under a biological hood with a Steritop® vacuum-driven disposable filtration system (SCGPS02RE, EMD Millipore, USA). Five samples for each solution were tested.

Static degradation tests were carried out accordingly to ASTM G31 standard. $20 \times 10 \times 1.4$ mm^3 specimens were polished with a series of abrasive papers up to 1000 grit to obtain a mirror finish, and then they were rinsed with water and ethanol, dried with compressed air and then stored in a desiccator until later use. Samples were weighted before the test with a 5 digit precision digital balance (Analytical Plus, Ohaus, USA). The static test was realized in sterilized 100 mL Pyrex bottles (Corning Incorporated, NY 14831, Germany). The degradation test was carried out in an incubator ($T_{incubator} = 37.0 \pm 1$ °C, $p_{rel.\ CO2} = 5\%$, 90% humidity) for a duration of 14 days. 95 mL of test solution were used for each sample. Bottle caps were drilled in the middle, to ensure a proper gas exchange. Samples were immersed in the solution by a nylon wire; the sample was suspended approximately in the middle of the liquid volume. The bottle and sample setup was performed in a biological hood in sterile conditions.

After the test, samples were ultrasonically cleaned for 5 min in 70% ethanol solution and weighted after each cleaning. The cleaning procedure was repeated three times, or until two following weighting did not show a weight difference lower than 1 mg. After the cleaning procedure, samples were stored in a desiccator at room temperature until their characterizations.

A part of both exhausted cleaning solution and test solutions was collected and centrifuged $v = 4000$ min^{-1} rotor speed (Marshall scientific, Beckman Allegra 6R, USA). The 70% ethanol solution used for ultrasonic rinsing was treated to analyze the particulate. The same procedure was adopted for 15 mL of exhausted test solution, that is the part at the bottom of each bottle, rich of solid suspension and particulate. The solid-rich deposit was mixed with a 50% pure ethanol-50% deionized water solution, vortexed and centrifuged again. The solid particulate was finally collected and desiccated in a 37 °C, 30 torr (Vacuum Oven Model 29) desiccator.

20 mL of the exhausted test solution were also collected and stored for AAS analysis. Degradation rate (DR) of specimens was calculated on the basis of procedures established by ASTM G31-03. This standard prescribes the use of the following parameters, that is time of exposure (t) to the solution in hour, area of the sample (A) in cm^2, mass loss (W) in grams and density (ρ) in g/cm^3, combined in the following equation:

$$DR = \frac{8.76 \times 10^4 W}{At\rho}. \qquad (1)$$

Atomic absorption spectroscopy (AAS) was performed (Model 3110, Perkin Elmer equipment), to assess the release of Fe and Mn ions. A 10% nitric solution in deionized water was used to clean glassware used during the digestion procedure, to avoid any kind of contamination.

2.3. Characterization

Sample surface and degradation products obtained after an exposition of 14 days to the three different solutions were assessed with several characterization techniques.

The microstructure of as received samples was studied with an Olympus PME3 optical microscope (OM). Fe–20Mn–1.2C foil sheets

Table 1
Solution denominations and their preparation on the basis of commercial products used; (*) salts were bought in small doses, each one corresponding to the precise amount for the needed volume of water.

Reference name	Solution composition					
	Deionized water	Salts*	Bovine serum albumin (BSA)	$NaHCO_3$	HEPES acid	HEPES Na salt
CH	1 L	9.5 g	–	0.35 g	–	–
		Sigma Aldrich Hanks' Balanced salts (H2387)	–	Sigma Aldrich S8875-500G	–	–
MH	1.4 L	9.7 g	–	3.3 g	14.16 g	16.65 g
		Sigma Aldrich Hanks' Balanced Salts (H1387)	–	Sigma Aldrich S8875-500G	Sigma Aldrich H3375-500G	Sigma Aldrich H7006-500G
DPBS	1 L	9.6 g	1 g	–	–	–
		Sigma Aldrich Dulbecco's Phosphate Buffered Saline salts (D5652)	Sigma Aldrich A2153-50G	–	–	–