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Study of biocompatibility of medical grade high nitrogen nickel-free austenitic stainless steel in vitro

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ABSTRACT

Adverse effects of nickel ions being released into the living organism have resulted in development of high nitrogen nickel-free austenitic stainless steels for medical applications. Nitrogen not only replaces nickel for austenitic structure stability but also improves steel properties. The cell cytocompatibility, blood compatibility and cell response of high nitrogen nickel-free austenitic stainless steel were studied in vitro. The mechanical properties and microstructure of this stainless steel were compared to the currently used 316L stainless steel. It was shown that the new steel material had comparable basic mechanical properties to 316L stainless steel and preserved the single austenite organization. The cell toxicity test showed no significant toxic side effects for MC3T3-E1 cells compared to nitinol alloy. Cell adhesion testing showed that the number of MC3T3-E1 cells was more than that on nitinol alloy and the cells grew in good condition. The hemolysis rate was lower than the national standard of 5% without influence on platelets. The total intracellular protein content and ALP activity and quantification of mineralization showed good cell response. We conclude that the high nitrogen nickel-free austenitic stainless steel is a promising new biomedical material for coronary stent development.

1. Introduction

For the cardiovascular system, coronary heart disease is the largest threat and the main reason of death and impairment of health [1,2]. The treatment of coronary heart disease includes three categories: drug therapy, surgical treatment and interventional treatment. Percutaneous coronary intervention is an important treatment for coronary heart disease due to small trauma, less pain, and achievement of rapid revascularization due to acute coronary syndrome in case of emergency [3–5]. At present, about 95% of patients have interventional therapy with stents [6]. Stent placement has become the main technology of interventional therapy.

At present, SUS316L, Co–Cr and platinum–chromium (Pr–Cr) are the most widely used coronary stent platforms [7]. SUS316L and nitinol alloy may release nickel ions, which may be considered a kind of toxic element, leading to anaphylactic reaction and even cancer [8,9]. SUS316L and nitinol alloy may cause thrombosis due to incompatibility with blood [10,11].

Nickel ion precipitation in environment and failure caused by degeneration of material properties are the main problems for use of stainless steel materials [12,13]. Nickel is added to these metal alloys to increase their flexibility [14,15]. However, nickel is known to induce biological responses in animals. For instance, nickel allergy is major cause of contact hypersensitivity which affects millions of people worldwide [16,17]. Nickel allergy is also associated with higher risks of restenosis after PCI [17–19], though conflicting data has been reported [20]. Ni^{2+} solubilized in sweat and other body fluids serves as a sensitizing allergen in Ni-induced contact hypersensitivity, and Ni^{2+} has been shown to directly activate inflammatory processes. For instance, Ni^{2+} triggers rapid expression of surface adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) on endothelial cells, as well as chemokines such as MCP-1 [21,22]. Due to the negative effects of nickel, it has been proposed to substitute it with nitrogen. However, homogenous injection of N into stainless steel is not feasible using classic methods such as N adsorption [23]. Our group and another group therefore developed nickel-free austenitic stainless steel with high N content fabricated using an electroslag remelting (P-ESR) method under pressurized N gas atmosphere [24,25].

In this study, the paper was examined the biocompatibility of medical grade high nitrogen nickel-free austenitic stainless steel in vitro. Nitinol alloy was chosen as reference. Cell adhesion test and cytotoxicity were used to examine the cytocompatibility of these materials. Furthermore, platelet adhesion and hemolysis rate test were used to examine the blood compatibility, and total intracellular protein content and ALP activity and quantification of mineralization were used to examine

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Table 1
Chemical compositions of the medical grade high nitrogen nickel-free austenitic stainless steel (wt.%).

Element	Cr	Mn	Mo	C	Ti	N	Fe
Materials	17.62	15.34	2.10	0.0056	0.013	0.42, 0.54, 0.60	Bal.

the cell responses. Finally, we evaluated whether the new stainless steel can be used as a base material for intravascular stents [26].

2. Materials and methods

2.1. Materials

Nickel-free austenitic stainless steel sheets with high nitrogen and nitinol alloy were provided by Chongqing Institute of Materials, China. The ossification cell line-MC3T3-E1 was purchased from Shanghai Institute of Cell Biology, China. -MEM medium was obtained from Hyclone Company, USA, and fetal bovine serum (FBS) was from Gibco Company, USA. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2 h-tetrazolium bromide (MTT) was purchased from ICN, USA. Dimethylsulfoxide (DMSO) was obtained from Sigma Chemical, USA.

2.2. Characterization of austenitic stainless steel

Vacuum induction melting (VIM) and electroslag remelting (ESR) double smelting processes were used in the test. Table 1 lists the chemical composition of the analyzed austenitic stainless steel. The sheets were solid solution treated at 1373 K for 1 h, followed by water quenching to obtain homogeneous austenitic. The tension specimens (53 × 45 mm) and charpy notch impact specimens (10 × 10 × 55 mm) were machined in accordance with Chinese standards GB228-1987 and GB/T 229-1994 standards.

In the metallographic observation experiment, we chose appropriate sizes of the specimens, sandpaper grinded to mirror surface after 120#, 400#, 800#, 1000#, 2000# and 4000# dry wet amphibious abrasive papers, respectively. The specimens were then mechanically polished with a mixture of anhydrous ethanol and glycerin (3:1 V/V), and etched with 7% nitric acid methanol. Finally, a metallographic microscope (Neophot 30) was used to observe the microstructure and the grain size was calculated by ASTM E112.

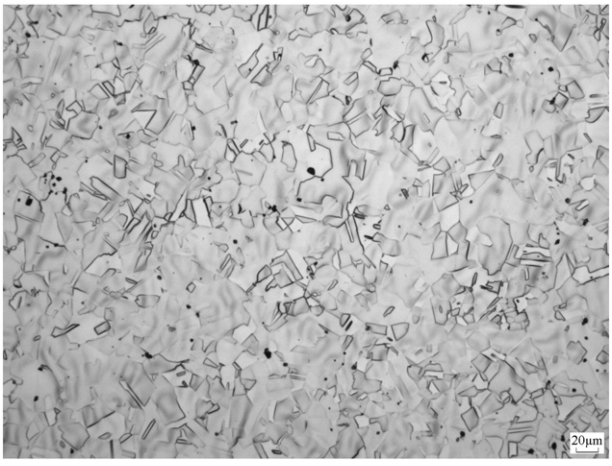

Fig. 1. Microstructure of medical grade nickel-free austenitic stainless steel with high nitrogen (200× magnification).

2.3. Cell culture

MC3T3-E1 cells were cultured in -MEM medium supplemented with 10% FBS at 5% of CO_2 and 37 °C.

2.4. Cytotoxicity test

The relative growth rate (RGR) of MC3T3-E1 cells was evaluated by MTT assay to evaluate cell proliferation. The stainless steel surface in contact with the cells produced corrosion products in the cell culture medium which affected the MTT test and increased the measurement error. Therefore, the indirect contact method [13,27] was chosen to assess cell proliferation. At first, MC3T3-E1 cells at a density of (1–2) × 10^4 cells/cm^2 were co-cultured with different nitrogen content samples in 96-well plates. The control groups involved the use of -MEM medium as negative controls and 0.64% phenol RPMI medium as positive controls. Three duplicated treatments were done. After incubating the cells in a humidified atmosphere with 5% CO_2 at 37 °C for 1, 3 and 5 days, respectively, 20 μl MTT solution (5 mg/ml) was added into each well. The plates were placed in an incubator at 37 °C for 4 h. Then, 150 μl DMSO was added after the supernatant medium was removed [28]. The absorbance was examined and recorded by an enzyme micro-plate reader (Bio-tek ELX800, Vermont, USA) at a wavelength of 490 nm. The math model of RGR was computed as:

$$RGR(\%) = As/Ac \times 100\ \%$$

where As is the absorbance of the treatments and Ac is the absorbance of the control.

2.5. Cellular morphological characterization by SEM

MC3T3-E1 cells were co-cultured with materials on glass slides for 24 h. After washing with PBS solution three times, cells were fixed with 2.5% glutaraldehyde at 4 °C for at least 30 min. The treatment samples were dehydrated with 20, 50, 75, 90, 95 and 100% ethanol for 15 min in consecutive order. After freeze-drying for 24 h, the samples were coated with an Au thin film. Scanning electron microscopy (SEM, Vega-LMH, Tescan, Czech Republic) was used to observe cell morphology with austenitic stainless steel and nickel titanium as the control.

2.6. Platelet adhesion

The 3.8% sodium citrate anticoagulant was added to fresh healthy human blood (so as to conform to the human ethics requirements) at a ratio of 1:9. Platelet-rich plasma (PRP) was prepared by subjecting whole blood to centrifugation at 1200 rpm for 10 min. The samples were immersed in physiological saline, placed into the PRP, and incubated at 37 °C for 1 h. The samples were gently rinsed with phosphate buffered saline (PBS) to remove non-adherent platelets and fixed in 2.5 vol.% glutaraldehyde solution at 4 °C for at least 1 h. This was follow-

Table 2
Mechanical properties of medical grade nickel-free austenitic stainless steel with high nitrogen.

Stainless steel material	Tensile strength (MPa)	Yield strength (MPa)	Nominal strain (%)	Reduction in area (%)	Impact property (J/cm^2)
Cr17Mn15Mo2N0.42 wt.%	1010	800	34.5	62.5	220
Cr17Mn15Mo2N0.54 wt.%	1020	855	29	63.5	232
Cr17Mn15Mo2N0.60 wt.%	1060	905	27.5	60	254
316L	550	252	64	72	290