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Biocompatibility of TiO_2 and TiO_2/heparin coatings on NiTi alloy

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ABSTRACT

NiTi shape memory alloy has been used widely as implanted material; however, possible release of Ni ions may cause toxic, allergic, and potentially carcinogenic effects in a physiological environment. In order to solve this problem, we notice that, as shown by previous studies, anticoagulant heparin is a better material for improving blood biocompatibility. Thus a promising way is to develop new TiO_2/heparin coatings on NiTi shape memory alloy, for the purpose of reducing possible release of toxic Ni ions and hence improve hemocompatibility. In this regard, we have fabricated four kinds of hemocompatible coatings: sol–gel TiO_2, heating TiO_2, sol–gel TiO_2/heparin and heating TiO_2/heparin on NiTi shape memory alloy. Various measurements have been done against this new material, including FE-SEM, XRD and contact angle tests (used to monitor surface characters), as well as hemolytic tests, dynamic clotting time experiments, platelet binding tests, cell morphology, MTT, ALP and RT-PCR (utilized to investigate blood- and cell-compatibility). Our results reveal that this new material of heparin loaded coatings, as expected, is able to improve the hemocompatibility of NiTi surgical alloy material. Moreover, RT–PCR measurements indicate that the best coating for cell growth is the heating TiO_2/heparin. Our results suggest that deposition of TiO_2 and heparin coating on the surface of a NiTi alloy sample is a promising method to improve its hemocompatibility.

1. Introduction

Nowadays, nickel–titanium (NiTi) shape memory alloy (SMA) becomes an attractive implantable material in biomedical applications, such as intravascular stents to prevent occlusion, restenosis of coronary arteries, bone fracture fixtures, orthodontic wires and various prosthetic devices in orthopedics and dentistry [1–5]. The attractiveness of NiTi is mainly attributed to its unique shape memory properties, superelasticity and biomechanical characteristics [6–8].

However, in a physiological environment, release of Ni ions, which may cause toxic, allergic, and potentially carcinogenic effects, takes place in corrosion process of all metallic implants; in addition, the Ni content in NiTi alloy is more than 50 in percentage. Therefore, NiTi alloy still remains controversial for medical applications [8], and the biocompatibility of NiTi SMAs, especially hemocompatibility and cytocompatibility, becomes a hot topic of research. Toward solving this problem many efforts have been made in this area, with emphasis placed on the direction of surface modification. Sui et al. [9–13] fabricated diamond-like carbon (DLC), TiN and TiO_2 films on NiTi alloy, via plasma immersion ion implantation and deposition (PIIID), laser re-flow bumping and sol–gel, for the purpose of improving corrosion behavior. These coatings passivated a NiTi alloy surface and decreased release of Ni ions. Up to date, the TiO_2 coating has been regarded as the best biocompatible material on NiTi alloy [5,16]. Moreover, Heparin is a kind of anticoagulant medicament. We have verified that anticoagulant heparin loaded coating can be used to improve the blood compatibility of NiTi alloy [14,15]. In this study, four TiO_2 coatings are fabricated via simple sol–gel and heating method; and in order

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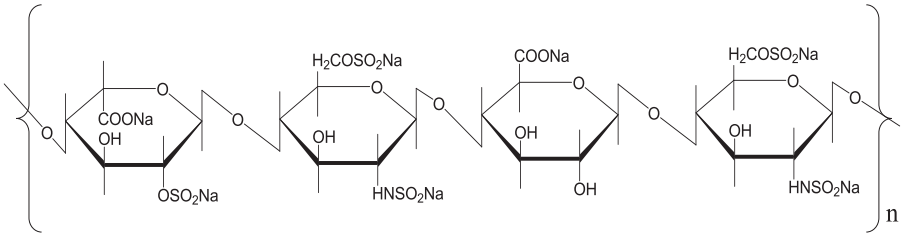

Fig. 1. The structure of heparin sodium.

to make further improvement of the blood compatibility of NiTi alloy, anticoagulant heparin can be introduced into TiO_2 coating. Our expected outcome is to achieve the coatings on NiTi alloy with better capability.

2. Experimental

2.1. Sample preparation

In this experiment, the NiTi pieces were provided by Lanzhou Seemine Company. The substrates were cut from a plate to a size of 10 mm × 10 mm × 1 mm, and were thoroughly ultrasonically cleaned with ethanol and de-ionized water, after being polished progressively with 200–1200 grits SiC emery paper to remove surface defects and contamination at macroscopic level [12,13].

The sample of tetrabutyl titanate ($Ti(C_4H_9)_4$, or $Ti(OBu)_4$) was used as a TiO_2 precursor. First, 5 mL of $Ti(OBu)_4$ was dissolved in 20 mL of ethanol by adding 1 mL hydrochloric acid. After the solution was stirred for 30 min at room temperature, 100 mL of water solution (pH 1–2) containing hydrochloric acid was added for hydrolysis. The solution was continually stirred for 1 h and subsequently incubated at 40 °C for 30 min. Then a homogeneous and clear sol–gel solution was achieved which could be used for coating [20]. For heating treatment, we put the NiTi substrate in a furnace in open air at 600 °C for 1 h, with a heating/cooling rate of 10 °C/min. After the heating TiO_2 samples were obtained, they were put into water and illuminated by ultraviolet lamp. Then the modified substrate was immersed in the heparin solution for 10 min. Heparin sodium salt (1 mg ≥ 140 unit, Fig. 1) was dissolved in sodium chloride. After natural drying in air, the heating TiO_2/heparin coatings were obtained. The samples were labeled as (a) NiTi substrate, (b) NiTi/sol–gel TiO_2 coating, (c) NiTi/heating TiO_2 coating, (d) NiTi/heating TiO_2/heparin coating (e) NiTi/sol–gel TiO_2/heparin coating.

2.2. Microstructure characterization

The surface morphology of these samples was studied by field emission scanning electron microscopy under an applied voltage of 20 kV (FE-SEM, JEOL JSM-6700F, Japan). The thin film X-ray diffraction (XRD) measurement was performed on a piece of Bruker X'pert X-ray diffractive equipment with Cu K_α source(1.5418 Å) at 40 kV (D8 ADVANCE, Bruker Company). The Raman scattering was characterized by a confocal laser Raman spectrometer (Renishaw inVia plus). The 514.5 nm line of an Ar+ laser was used for excitation, with the laser power maintained at about 20 mW. Average static contact angles were obtained from three measurements of de-ionized water droplets to characterize hydrophilic or hydrophobic properties of coatings. Water contact-angles were measured on a Dataphysics OCA 20 contact-angle system at room temperature. The surface hardness was measured by nanoindentation test system, which including a hardness tester (NHT) and a combined optical scanning force microscope (SFM) developed by CSEM instruments (Switzerland).

Table 1
Ion concentrations of SBF and human blood plasma.

	Concentration (mM)							
	Na^+	K+	Mg^{2+}	Ca^{2+}	CL^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
SBF	142.0	5.0	1.5	2.5	148.8	4.2	1.0	0.5
Human blood plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5

All samples were immersed in 10 mL of conventional simulated body fluid (SBF, Table 1), at pH = 7.2, 37 °C for 5, 10, 15, 20, 30 and 40 days. The Ni ion release was quantified by atomic absorption spectroscopy. The results were normalized by the specific surface area of each sample, and the mean value of the three measurements was calculated.

3. Hematologic experiment

3.1. Hemolysis test

8 mL blood, which was freshly collected from a rabbit and diluted with 10 mL 0.9% NaCl solution, was anticoagulated with potassium oxalate (2%, w/v). Each sample was contained in a test tube with 10 mL saline and incubated at 37 °C for 30 min. Then 0.2 mL diluted blood was added into each test tube and incubated for 60 min. After incubation, the suspensions were centrifuged at 3000 r/min for 10 min. The absorbance of the supernatant fluid was measured by a spectrophotometer (UV-2550, Shimadzu) at 545 nm [21].

3.2. Determination of dynamic clotting time

0.1 mL rabbit blood adulterated with anticoagulant of Alsevre's solution was dripped on the sample surface at 37 °C, then 0.2 mol/L $CaCl_2$ solution was added to initiate the blood clotting. After 5, 10, 20, 30, 40, 50 and 60 min, each sample was transferred into a beaker containing 50 mL of distilled water. Then the sample was rinsed gently and the absorbance of the supernatant liquid was measured at 545 nm wavelength by UV–vis [22]. The experiment was repeated three times and the optical density of each sample was expressed as the mean OD value.

3.3. Platelet binding test

Platelet rich plasma (PRP) was isolated from rabbit blood by centrifugation at 2000 r/min for 10 min. Then the material was washed up and incubated in PRP for 60 min at 37 °C. After incubation, the specimens were rinsed by sodium chloride and fixed in 2.5% glutaraldehyde for 1 h. Then the specimens were dipped in order into 30%, 50%, 70%, 90%, 100% ethanol (v/v, dissolved in de-ionized water) for dehydration, and into 30%, 50%, 70%, 90%, 100% isoamyl acetate (v/v, dissolved in ethanol) for degreasing. Then the specimens were dried up before conductive coating, and were scanned by a scanning electron microscope (SEM).