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In vitro bioactivity evaluation of titanium and niobium metals with different surface morphologies ☆

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Abstract

Current orthopaedic biomaterials research mainly focuses on designing implants that could induce controlled, guided and rapid healing. In the present study, the surface morphologies of titanium (Ti) and niobium (Nb) metals were tailored to form nanoporous, nanoplate and nanofibre-like structures through adjustment of the temperature in the alkali-heat treatment. The in vitro bioactivity of these structures was then evaluated by soaking the treated samples in simulated body fluid (SBF). It was found that the morphology of the modified surface significantly influenced the apatite-inducing ability. The Ti surface with a nanofibre-like structure showed better apatite-inducing ability than the nanoporous or nanoplate surface structures. A thick dense apatite layer formed on the Ti surface with nanofibre-like structure after 1 week of soaking in SBF. It is expected that the nanofibre-like surface could achieve good apatite formation in vivo and subsequently enhance osteoblast cell adhesion and bone formation.

Keywords: Bioactivity; Titanium; Niobium; Apatite; Simulated body fluid (SBF)

1. Introduction

The surface properties of implant materials play a critical role in the long-term stability and functional performance of the implants. The first event that occurs after implantation of a biomaterial is formation of hematoma around the implant surface, which contains various signalling molecules. On the one hand, giant cells communicate with fibroblasts and the latter produce fibrous tissues to wall off the implant. On the other hand, cytokines and growth factors released from the hematoma stimulate the recruitment of mesenchymal cells, which differentiate into osteoblast (bone formation cells). Osteoblasts secrete collagen, which mineralizes into new bone. The nature of the bone–implant interface is a result of the competition between bone regeneration and fibrous tissue formation [1–3]. For some bioactive materials, a thin bonelike apatite layer deposits on the implant surface after implantation into living tissues. Due to their chemical similarities, bone may not recognize the apatite layer as foreign, and therefore bonds directly with the implant [4].

Currently used titanium and titanium alloy implants typically develop a thin layer of fibrous tissue at the interface with bone. Many surface treatments have been performed to improve the bioactivity of Ti implants, such as sol–gel coating [5,6], H_2O_2 oxidation [7] and alkali-heat treatment [8–16]. It was reported that Ti after alkali-heat treatment even induced apatite layer deposition in vivo [17]. When alkali-heat-treated Ti and Ti alloys were implanted into a canine femur, the implants showed direct bonding to bony tissue without intervening fibrous tissue and showed significantly higher bond strength than their untreated counterparts [18].

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Nb is one of components of the low modulus β-Ti alloys but, unlike Ti, its bioactivity has not been extensively investigated. Miyazaki et al. [19] compared the apatite deposition behaviour on the surfaces of sol–gel-derived niobium oxide gels and NaOH-treated Nb metals, and found that the sol–gel niobium oxide achieved better apatite formation than the NaOH-treated Nb. In the present study, NaOH-treated Nb with different surface morphologies were prepared and their in vitro bioactivity was subsequently evaluated. The rate of osseointegration is directly related to the efficiency of apatite formation on the implants. Therefore, it was hypothesized that a specific surface, which had a high apatite-inducing ability, could achieve rapid osseointegration. The current work explored the efficiency of apatite formation on Ti and Nb surface with different morphologies.

2. Materials and methods

2.1. Sample preparation

The Ti and Nb discs were prepared by arc-melting the metal (purity > 99.9) in an argon atmosphere. The discs taken out from the furnace were cut into smaller discs (∅10 mm × 2 mm) and consecutively ground with silicon carbide papers with grits of 240, 600, 800 and 1200. The discs were then ultrasonically cleaned in acetone and distilled water for 15 min at each stage.

The Ti discs subjected to alkali treatment were immersed in a 5 M NaOH solution (50 ml per disc) and charged into a Teflon-lined autoclave. The autoclave was oven-heated at 60, 110 and 150 °C for 24 h, respectively. The Ti discs after alkali treatment were washed with distilled water and diluted HCl (0.05 mM). When Nb specimens treated with 5 M NaOH, extensive corrosion occurred and small particles were observed to peel off from the sample surface. The concentration of NaOH was accordingly adjusted to 0.5 M. The Nb discs were immersed in 0.5 M NaOH solution (50 ml per disc) in a sealed container and oven-heated at 60 and 80 °C for 24 h. Wash procedures were the same as for the Ti samples. Both Ti and Nb discs after alkali treatment were then heat-treated in a vacuum furnace using a heating rate of 5 °C min^{-1} to 600 °C and holding for 1 h. Ti samples after alkali treatment at 60, 110 and 150 °C are referred as Ti60, Ti110 and Ti150, respectively. Nb samples after alkali treatment at 60 and 80 °C are referred as Nb60 and Nb80. Ti and Nb discs without any surface treatment were used as control counterparts.

2.2. Apatite deposition

A modified simulated body fluid (SBF) recipe proposed by Oyane et al. [20] was used in the present study. The SBF was prepared by dissolving the following chemicals in the sequence of NaCl (5.403 g), $NaHCO_3$ (0.504 g), Na_2CO_3 (0.426 g), KCl (0.225 g), $K_2HPO_4 \cdot 3H_2O$ (0.230 g), $MgCl_2 \cdot 6H_2O$ (0.311 g), $CaCl_2$ (0.293 g) and Na_2SO_4 (0.072 g). The solution was buffered to pH 7.40 with HEPES and 1 M NaOH at 37 °C. Each Ti sample was placed in a PE Petri dish with 30 ml SBF and kept in an incubator at 37 °C. To keep the ion concentration stable, the SBF solution was refreshed every 2 days. The ion concentrations in the SBF are given in Table 1.

Table 1
Ion concentrations of blood plasma and SBF

Ion	Concentration (mM)	
	Blood plasma	m-SBF
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	103.0	103.0
HCO_3^-	27.0	10.0
HPO_4^-	1.0	1.0
SO_4^{2-}	0.5	0.5

2.3. Characterization

The morphologies of the surface layers after alkali-heat treatment and SBF-soaking were observed by scanning electron microscopy (SEM). X-ray diffraction (XRD) was used to identify crystal structure of the oxidation layer and apatite layer. The XRD measurements were performed using a Cu Kα (wavelength 1.54056 Å) X-ray source with a step rate of 0.01° s^{-1} and 3 s analysis time per step. The scanning angle ranged from 20° to 60°.

2.4. Contact angle measurement

Contact angle analysis was performed on the surfaces for all samples 7 days after preparation, using a KSV Cam101 contact angle and surface tension meter. To minimize effects of temperature and humidity, all samples were put in the testing room 48 h in advance. Drops of ultrapure distilled water were delivered onto the specimen surface by a syringe with a set drop size.

3. Results

3.1. The morphology and structure of the Ti, Nb alkali-heat-treated surface layer

Fig. 1 shows the morphologies of Ti and Nb surface layer after alkali-heat treatment. A nanoporous structure was observed on the surface of Ti60. The pores were interconnected with sizes of 200–300 nm diameter. The sodium titanate layer on Ti110 had a nanoplate structure and the thickness of the nanoplates ranged from 100 to 200 nm. Needle-shaped crystals were observed on the surface of Ti150 and the diameters of these nanofibres were between 50 nm and 100 nm. From this it is clear that the morphologies of the sodium titanate layers can be tailored by con-

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