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Fabrication, characterization and in vitro biocompatibility evaluation of porous Ta–Nb alloy for bone tissue engineering

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ABSTRACT

Porous Ta–Nb alloys were fabricated using the sponge impregnation technique and the powder metallurgy technique (P/M) in combination. All porous Ta–Nb alloys displayed interconnected open cell structures with porosities around 64% and pore sizes in the range of 300–500 μm. No carbide, oxide, or intermetallic-related phases were detected by the X-ray diffraction (XRD). Porous Ta–Nb alloys displayed sintering neck growth, smoother surface of the particles and more shrinkage of the micropores, with Nb contents increasing from 5% to 15%. The compressive strength and Young's modulus of the Ta–Nb alloys agreed well with the requirements of trabecular bone. The normalized compressive plateau stress and Young's modulus increased from 52.27 MPa to 85.43 MPa and from 1.850 GPa to 2.540 GPa, respectively, with Nb contents increasing from 5% to 15%. Porous Ta–Nb alloys had no cytotoxicity and possessed the excellent biocompatibility similar to porous Ta scaffolds.

1. Introduction

The appropriate mechanical properties, good biocompatibility and bioactivity are three important properties of the materials used as bone substitute [1]. Additionally, a highly porous structure is of significance to bone tissue engineering application as it can promote the ingrowth of the mineralized tissue into the porous network and then make the material anchor to the surrounding bone tissue [2]. The porous feature can adjust the elastic modulus of the material to fit in the similar range of human bones (0.5–30 GPa) in order to reduce the stress-shielding effect and prevent the movement and loosening of the implants [3]. The artificial bone substitute possessing the suitable balance of the properties mentioned previously has long been sought to match the behavior of human bones.

Hence, several new series of highly porous ceramics and polymer have been studied as potential bone graft scaffolds [4–6]. However, the poor mechanical properties of these porous bioactive ceramics and polymer seriously restrict their applications, especially under load-bearing conditions [7]. Hence, porous metallic orthopedic implants with excellent mechanical properties have attracted great attention recently, including titanium [8,9], titanium alloys [10,11], and tantalum [12].

Tantalum (Ta) is known as one of the most promising metal biomaterials due to its excellent in vitro and in vivo biocompatibility [13,14], and good corrosion resistance [15]. Tantalum-based implants have displayed an extraordinary biocompatibility and clinical safety record in several orthopedic applications including bone graft substitute, spine surgery, and hip and knee arthroplasty, etc. [13]. The basic structure of the porous tantalum scaffolds offers great features such as low modulus of elasticity, relatively high surface frictional characteristic and excellent osseointegration properties [16,17]. However, the extremely high melting temperature (3017 °C), powerful affinity for oxygen, and high cost of raw material have limited the widespread application of tantalum biomaterials.

Therefore, several new techniques have been developed to prepare porous tantalum for orthopedic applications. Zimmer Inc. (Warsaw, IN, USA) fabricated Trabecular Metal™ by depositing commercial pure tantalum onto a low density vitreous carbon skeleton [18,19]. However, the insufficient mechanical strength of products and relatively high cost of manufacture limit the widespread acceptance of this method [20]. Zhou et al. fabricated porous Ta foams by replication of NaCl space-holders, but the residual contamination and low control of the pore structure and porosity restrict the application of this method [21]. Recently, the sponge impregnation technique and the powder metallurgy (P/M) technique in combination has gained wide attention due to its low cost of manufacture, simple procedures, low residual contamination, and the adequate mechanical properties and controllable porosities of products.

Niobium (Nb) has excellent corrosion resistance, good biocompatibility and osteogenesis as one of the five elements which produce no adverse tissue reaction (Ta, Nb, Zr, Pt and Ti) [14,22–25]. Ta and Nb belong to the same group in the periodic table of elements, and the Ta–Nb system is a complete solid solution. Nb has lower melting temperature (2477 °C), Young's modulus, and cost of raw material than Ta. Hence, porous Ta–Nb alloys possess a low Young's modulus without

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inhibiting its corrosion resistance or introducing any cytotoxic element and reduce the manufacturing cost, compared to porous Ta scaffolds. The study about porous Ta–Nb alloys has not been reported at present.

In the present study, porous Ta–Nb alloys were fabricated using the sponge impregnation technique and the powder metallurgy technique in combination. The microstructure and mechanical properties of the obtained porous Ta–Nb alloys were investigated. A comparative study of biocompatibility on porous Ta–Nb alloys, dense Ta–Nb alloys, and porous Ta scaffolds was performed by MTT cytotoxicity and cell proliferation tests.

2. Materials and method

The starting tantalum powder (DongFang Tantalum co., Ltd. China) and niobium powder (ZhuZhou Cemented Carbide Group co., Ltd. China) were used as-received in this work. The chemical compositions and other characteristics of the starting elemental powders were shown in Table 1. These elemental powders were blended for 4 h in a roller mixer to produce powder mixtures with different compositions of Ta–5Nb, Ta–10Nb and Ta–15Nb. All the compositions in this study are given in wt.%. The polyvinyl alcohol (PVA) solution (6% PVA + distill water) were prepared as the powder binder and slurry viscosity controller. The Ta–Nb powders were then mixed with the PVA solution and kept on a magnetic stirrer to obtain the well-dispersed Ta–Nb slurries. The commercial polyurethane sponges (Dongguan Inoac Polymer Co., Ltd., China) with the open porosity of 50 pores per inch (ppi) were employed as original scaffolds and shaped to the size of $\Phi 10 \times 20$ mm.

The shaped sponges were pretreated by washing in 20% NaOH solution for 15 min at 60 °C, and then cleaned with deionized water. After being squeezed out the extra liquid, the sponges were impregnated in the Ta–Nb slurries and repeatedly compressed to force the Ta–Nb powders to homogenously migrate into the pores.

Subsequently, the specimens were dried at 40 °C for 24 h and heated at 400 °C for 2 h in vacuum. Cylindrical green compacts of Ta were produced by uniaxial cold pressing at 500 MPa. Sintering was carried out in high vacuum ($\leq 9 \times 10^{-3}$ Pa) at 1950 °C for 2.5 h with the heating rate of 10 °C/min and then furnace-cooled to room temperature. A schematic of the process was shown in Fig. 1.

Bulk densities and apparent densities of the specimens were determined by the mass and dimensional measurements and by the liquid displacement method using Archimedes principle, respectively. The total and open porosities of the specimens were calculated from the bulk and apparent densities.

The pore microstructure and the crystal phase of the porous Ta–Nb alloys were tested by the scanning electron microscope (SEM, Nova Nano SEM230, USA) and X-ray diffraction (XRD), respectively. The impurity element contents of carbon and oxygen were measured by CS-444 infrared carbon–sulfur analyzer (LECO, USA) and TC-436 oxygen–nitrogen analyzer (LECO, USA), respectively.

Compression tests for mechanical property evaluation were carried out at room temperature using a MTS machine with 50 kN capacity at a constant crosshead speed of 1 mm/min. Young's modulus and the compressive strengths of porous Ta–Nb alloys were determined from the strain–stress plots recorded during compression testing.

Table 1
The chemical compositions and other characteristics of the starting elemental powders used in the Ta–Nb scaffold preparation.

Characteristic	Particle size (μm)	Impurities (%)	Morphology
Ta	10.40	O (0.10); C (0.020); Fe (0.0010); Mg (0.0054); H (0.0080); Ni (0.0006)	Angular
Nb	40.52	O (0.27); C (0.024); Fe (0.0065); N (0.0400); Ta (0.0500)	Angular

MC3T3-E1 rat osteoblasts cells were cultured in α-MEM containing 10% (*v*/*v*) fetal calf serum (FCS), 0.2 M L-glutamine and ascorbate 2-phosphate, and subsequently used to make cell suspension for seeding with the cell density of 1×10^5 cells/mL. The specimens ($\Phi 7 \times 1$ mm) were machined from the porous Ta–Nb scaffolds and Ta scaffolds. All specimens were sterilized in an autoclave at 120 °C for 30 min prior to the cell culture experiment.

MTT cytotoxicity tests: For evaluating the cytotoxicity, Ta–Nb and Ta leaching liquors were prepared as ISO 10993-5 described. The sterilized porous Ta–10Nb and Ta specimens were immersed into α-MEM containing 10% (*v*/*v*) fetal calf serum (FCS), 0.2 M L-glutamine and ascorbate 2-phosphate (3 cm^2/mL), and incubated for 72 h at 37 °C, 95% relative humidity and 5% CO_2 with moderate shaking to obtain the leaching liquor. After seeding the 96-well cell culture plate with the MC3T3-E1 rat osteoblast cell suspension (200 μL/well) and incubating for 24 h, the medium of each well was replaced by different leaching liquors or other medium. Wells added leaching liquors were divided into three groups (i.e., porous Ta group, dense Ta–Nb group and porous Ta–Nb group). Meanwhile, wells added MEM containing 0.64% phenol were used for positive group, and wells added MEM only were used for the negative group. The MTT solution (20 μL/well, 5 mg/mL) was added in the cells cultured 24 h, 48 h and 72 h after seeding. After removing the medium from the wells, DMSO (150 μL) was added to each well to ensure the complete dissolution. All experiments were carried out in octuplicate. Finally, the optical density of the solution in each well was measured at a wavelength of 570 nm using a microplate reader (Wellscan MK3, Doragon, Finland). The relative growth rate (RGR) was calculated as: $RGR = (OD_{\text{specimens}} / OD_{\text{negative control}}) \times 100\%$.

Cell proliferation test: The cell proliferation was assessed by acridine orange fluorescence staining at 24 h, 48 h, 72 h and 96 h post-seeding. One milliliter of the cell suspension containing 1×10^4 cells/mL was seeded in wells of the 24-well cell culture plate (1 mL/well), and the cells were incubated at 37 °C and 5% CO_2. Prior to seeding, the wells were equipped with porous Ta scaffolds, porous Ta–Nb alloy or dense Ta–Nb alloy samples. After incubation, the cells were washed with PBS and fixed with 95% ethanol (500 μL/well) for 10 min. The fixed cells were dried and stained with 0.01% acridine orange for 5 min, and then rinsed with PBS for 1 min. After color separation by calcium chloride solution (0.1 mmol/L) for 1 min, cells were washed again with PBS and sequentially viewed with an inverted fluorescent microscope. All experiments were carried out in triplicate. The data were captured with Image-ProPlus6 (IPP6) software. Statistical analysis of the results was performed with SPSS17.0 software. All results were presented as means ± standard deviation (SD) and differences at $p \leq 0.05$ were considered statistically significant.

3. Results and discussion

Fig. 2a shows the morphology of the porous Ta–Nb alloy. The porous Ta–Nb alloy exhibited a three-dimensional interconnected and open cell structure. The pore size (300–500 μm) and structure of the porous Ta–Nb alloy closely match those of human cancellous bone and were similar to those of the polyurethane sponge. The interconnected porous structure and appropriate pore size (100–500 μm) are important factors for protein absorption and mineralized bone ingrowth [26–28]. Hence, the porous Ta–Nb alloys prepared in this study provided appropriate pore structure and size for seeded cells growing, the bone tissue ingrowth, and the biomedical implantation.

Fig. 2b shows the XRD patterns of the porous Ta–Nb alloys with different chemical compositions (Ta–5Nb; Ta–10Nb; Ta–15Nb). As the Ta–Nb system is a complete solid solution, the XRD patterns of the porous Ta–Nb alloys remained constant with the increase of the Nb content. No carbide, oxide, or intermetallic-related phases were detected.