Available online at www.sciencedirect.com
ScienceDirect
Trans. Nonferrous Met. Soc. China 27(2017) 1815−1821
Transactions of Nonferrous Metals Society of China
www.tnmsc.cn

Nanospike surface-modified bionic porous titanium implant and in vitro osteogenic performance

Guo-hui WANG[1] , Hua FU[1] , Ke-chao ZHOU[2], Yan-zhong ZHAO[1], Shai-hong ZHU[1]

1. Third Xiangya Hospital of Central South University, Central South University, Changsha 410013, China;
2. State Key Laboratory of Powder Metallurgy, Central South University, Changsha 410083, China

Received 4 May 2017; accepted 30 July 2017

Abstract: This work aimed to prepare the nanospike surface-modified bionic porous titanium implants that feature favorable osteointegration performance and anti-bacterial functions. The implant was prepared using freeze casting, and nanospike surface-modification of the implant was performed using thermal oxidation. The pore morphology and size, mechanical properties, and osteogenic performance of the implants were analyzed and discussed. The results showed that when the volume ratio of titanium powder in slurry was set to be 10%, the porosity, pore diameter, compressive strength, and elastic modulus of the porous samples were (58.32±1.08)%, (126.17±18.64) μm, (58.51±20.38) MPa and (1.70±0.52) GPa, respectively. When the porous sample was sintered at a temperature of 1200 °C for 1 h, these values were (58.24±1.50)%, (124.16±13.64) μm, (54.77±27.55) MPa and (1.63±0.30) GPa, respectively. The nanospike surface-modified bionic porous titanium implants had favorable pore morphology and size, mechanical properties and osteointegration performance through technology optimization, and showed significant clinical application prospect.

Key words: nanospike surface-modification; bionic porous titanium; osteogenic performance; freeze casting; thermal oxidation

1 Introduction

Since the elastic moduli of titanium and titanium alloy implants are higher than those of natural cortex bone [1], there is merely mechanical integration between the implants and bone rather than biological integration when it is implanted into a human body as a substitute material for bone. This, to some extent, has significantly increased risks of failure of bone transplantation, and is thus restricted in clinical application [2,3]. Porous metal materials serving as a new type of bone implants have become a key research topic in improving the osteointegration performance of implants [4,5]. Anisotropy and gradient distribution of porous metal materials by freeze casting were found much similar to those of natural human bone materials. Thus, it is highly possible to prepare bionic porous titanium implants that feature both favorable mechanical properties and similar pore morphology and sizes to human bone tissue through technology optimization [6−8].

Special responses and interactions between bionic bone implants with favorable osseointegration performance, as well as living bone tissue cells are required to form bio-integration by inducing osteoblasts to grow in the implant's porous structure. Compared with traditional porous materials, nanostructured porous implants are able to alter cell behavior on material surfaces by regulating the adsorption of macromolecules, e.g., proteins, as well as the cells' biological responses, thereby improving the adhesive and multiplication capacities of osteoblasts [9,10]. In previous research [11], an anti-bacterial nanospike was formed on the porous surface of titanium implants by applying a thermal oxidation method. And this structure showed effective performance in preventing bacteria from developing and multiplying on the implant surface, which can cause implant looseness, reoperation, and serious complications. Thus, this nanospike surface-modified structure showed significant clinical application value.

Foundation item: Projects (51290295, 51305464) supported by the National Natural Science Foundation of China; Project (2016JJ6156) supported by the Natural Science Foundation of Hunan Province, China; Project (2016JC2064) supported by the Key Research and Development Program of Hunan Province, China; Project (20130162120094) supported by the Specialized Research Fund for the Doctoral Program of Higher Education, China

Corresponding author: Shai-hong ZHU; Tel: +86-731-88618234; E-mail: zhushaihong@medmail.com.cn; Yan-zhong ZHAO; Tel: +86-731-88618669; E-mail: yanzhongzhao@163.com

DOI: 10.1016/S1003-6326(17)60204-8

However, further studies are needed to investigate the pore morphology and size, mechanical properties, and osteointegration performance of the nanospike surface-modified bionic porous titanium implants.

In the present work, the bionic porous titanium implant was prepared using freeze casting, and nanospikes modification of the surface was performed using a thermal oxidation method to obtain proper mechanical properties while ensuring porous structure and pore size, thereby improving osteointegration and physical antibacterial performance of the bionic implant.

2 Experimental

2.1 Preparation of porous titanium and nanospike surface-modification

Titanium powder with a particle size of <25 μm (Sigma-Aldrich, USA) and camphene (Fisher Scientific, UK), a solvent medium, were magnetically stirred in a water bath for 2 h at a temperature of 60 °C and a stirring rate of 800 r/min. Polyvinyl alcohol (PVA) was used as binder. The stirring vessel was covered to reduce solvent evaporation. The volume proportion of titanium powder in the slurry was 5%−20%. The mold was placed in the water bath case for precooling for 30 min at 20 °C, after which the prepared slurry was slowly poured into a precooled cylindrical mold with a thermally insulated top and bottom. After curing for 4 h, the mold was placed overnight in an environment at −20 °C for further cooling. Then, the samples were demolded and transferred to a vacuum freeze drying oven for sublimating for 24 h. The dried body was then put into the sintering furnace, where it was sintered firstly in vacuum at a heating rate of 1 °C/min until furnace temperature reached 400 °C, and then in argon atmosphere at a heating rate of 10 °C/min until furnace temperature reached 1200−1300 °C. Having been sintered for 1−4 h, the dried body was naturally cooled down to room temperature, and thereafter the porous titanium was prepared.

Samples were placed in the centre of a horizontal alumina tube furnace. After purging the tube with Ar, the temperature was increased to 850 °C at 15 °C/min. After reaching 850 °C, the Ar flow was diverted through a bubbler bottle containing acetone at 25 °C with the Ar flow rate of 300 mL/min. The temperature was kept at 850 °C for 45 min, after which the tube was allowed to cool under a flow of Ar at 500 mL/min. To remove the carbon from the as-synthesized nanospikes, the samples were heated to 600 °C at a rate of 10 °C /min.

2.2 Characterization and osteogenic performance

2.2.1 Samples cleaning and dimensional measurement

Sintered porous titanium specimens were cleaned in an ultrasonic cleaner for 15 min using analytically pure acetone (Fisher Scientific, UK), absolute ethyl alcohol (Fisher Scientific, UK), and double distilled water as cleaning agents. They then underwent drying in a high-temperature drying oven for 4 h at a temperature of 80 °C. An electronic analytical balance with an accuracy of 0.01 g was used to weigh the specimens. Finally, a Vernier caliper was used to measure 3 different positions of each specimen, and the mean value was then calculated. The accuracy was 0.01 cm.

2.2.2 Porous structures

The porous structures (porosity, degree of interconnection) of the porous Ti scaffolds and densifications of the Ti walls were characterized by scanning electron microscopy (FE-SEM, JSM−6330F, JEOL Techniques, Tokyo, Japan). The pore size was also analyzed from the SEM images of the samples prepared by infiltrating the porous Ti scaffolds with an epoxy resin.

2.2.3 Mechanical properties

The compressive strengths of the porous Ti scaffolds with a diameter and height of 16 and 20 mm, respectively, were examined using a screw-driven load frame at a crosshead speed of 5 mm/min. The stress and strain responses of the samples were monitored during the compressive strength tests. Five samples were tested to obtain the mean values and the standard deviation.

2.2.4 Adhesive function of MG63 osteosarcoma cells

The nano-modified titanium specimens, porous titanium specimens without nano-modification, and the control group of compact titanium specimens were kept in sealed preservation after sterilization and drying, and transferred and fixed to a 24-well cell culture plate. There were 3 specimens in each group. Then, 500 μL RPMI-1640 culture medium containing 10% fetal calf serum was added to each well on a clean bench. The culture solution was discarded after 24 h prewetting of specimens, and specimens were then gently cleaned twice using phosphate buffer solution (PBS). Digested MG63 osteosarcoma cells were diluted into a cell suspension with a density of 1×10^2 μL^{-1} per well, which was then dripped slowly from the specimen surface to the wells of the culture plate using a pipettor. Next, the culture plate was placed in an incubator containing 5% CO_2 for 8 h at a temperature of 37 °C and humidity of 95%.

The 24-well culture plate was taken out of the incubator on the fifth day. The culture solution was then discarded, and cells were cleaned gently 3 times using PBS buffer solution. The specimens were then transferred from the 24-well culture plate to empty containers, where they were fixed using 4 °C precooled glutaraldehyde with a volume fraction of 0.3% for 4 h. The specimens then underwent gradient dehydration for