Improved the in vitro cell compatibility and apatite formation of porous Ti6Al4V alloy with magnesium by plasma immersion ion implantation

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Abstract

In this study, three dimensional porous Ti6Al4V (TC4) with porosity (74%) and elastic modulus (0.83 ± 0.7 GPa) was manufactured by electron beam melting method. To improve the biofunction, plasma immersion ion implantation was adopted to implant magnesium ions into it. EDX and XPS spectrums confirmed the effectiveness of implantation and magnesium existed in the form of MgO at the surface. The in vitro cell experiments of Mg-implanted TC4 alloy showed enhanced cell adhesion, spreading and proliferation, attributing to the released magnesium ions. The 14 days immersion in simulated body fluid also leaded to the formation of hydroxycarbonate apatite (HCA), and the Mg-implanted TC4 alloy was more favorable for hydroxyapatite nucleation. In short, magnesium ions implantation was a desirable method to improve the in vitro cell compatibility and apatite formation ability of bio-inert TC4 alloy.

Keywords: Porous Ti6Al4V, Mg implantation, Biocompatibility, Surfaces, Metals and alloys

1. Introduction

Although adequate biocompatibility and suitable physico-chemical properties of TC4 have made it one of the most frequently used metallic materials for hard tissue replacements, there are still some unresolved issues such as excessive elastic modulus and poor osseointegration. It has been well proved that TC4 scaffolds with a porous structure favor tissue ingrowth, the mass-transportation of nutrients and metabolic wastes, osteointegration with the host tissue, as well as long term stable fixation of bone implants [1]. In this study, we designed a porous structure with diamond unit to achieve a proper elastic modulus to match the nature bone and Electron Beam Melting (EBM) method had been used to manufacture the TC4 samples.

In addition to the porous structure, surface characteristics such as surface roughness, topography and chemistry also play an important role in mediating the interactions between implants and host tissue because cells always interact with the outermost atomic layers of implants (0.1–1 nm thickness) [2]. One technique for altering the implant surface chemistry is ion-implantation, incorporating bioactive trace elements within the atomic network of the surface. For example, calcium, zinc, magnesium and strontium are frequently used to stimulate bone growth and bone healing by promoting osteoblast activity [3]. As one of the most abundant elements in human body, magnesium is essential for metabolism and stimulates new bone formation, as well as interacting with integrin of osteoblasts to enhance cell adhesion and viability [4]. Plasma immersion ion implantation had been proved to be favorable for the treatment of medical implants with complex surfaces and three-dimensional scales [5]. Therefore, plasma immersion ion implantation was adopted to incorporate biologically active magnesium (Mg) into the surface of Ti6Al4V alloy in the present study.

2. Materials and methods

Cylindrical porous TC4 scaffold (diameter 6.0 mm, length 9.4 mm) based on a diamond unit cell was designed by computer-aided design (CAD) software (Solidworks 2012). After converting data into STL format, the three dimensional model files were sliced into 2-D levels by software and finally imported into the EBM Q10 system (Arcam AB, Sweden) to rapidly prototype the porous implants layer by layer. Before plasma immersion ion implantation (PIII) processes, the samples were resin ultrasonically with acetone, 100% alcohol, and distilled water for 30 min, respectively. The surface morphology and chemical composition

http://dx.doi.org/10.1016/j.matlet.2017.05.088

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were characterized using scanning electron microscopy (FE-SEM, S-4800, Hitachi) coupled with energy-dispersive X-ray spectrometry (EDS) and X-ray photoelectron spectroscopy (XPS, Kratos, U.K).

After sterilizing by ethylene oxide gas, MC3T3-E1 cells were cultured and incubated on the samples. These cells were cultured in Alpha-Minimum Essential Medium (α-MEM) supplemented with 10% FBS and 1% penicillin/streptomycin. Considering the porosities and cell gravitational effects, droplets of culture medium containing $5 \times 10^4$ cells were put onto the scaffolds slowly and uniformly. After culturing 6 and 12 h, cell morphologies on the samples were observed by SEM at an acceleration voltage of 10 kV. After 1, 3 and 5 days of incubation, the cell proliferations on the scaffolds were assessed using Cell Counting Kit (CCK-8; Dojindo, Japan) and the absorbance was measured at 450 nm. As an alternative, the cells were stained with fluorescent reagent and laser scanning confocal was adopted to visually examine the cell number and distribution on scaffolds.

Supersaturated simulated body fluid, such as 1.5×SBF with 1.5 times of ion concentration of SBF solution, has been adopted to detect the bioactivity of materials by detecting the apatite formation on their surfaces [6]. In this study, porous alloys with and without ion implantation were immersed in 10 ml 1.5×SBF at 37 °C for 5 and 14 days, and the solution was refreshed every other day [7]. The formation of apatite on the surfaces of scaffolds was observed by SEM equipped with EDX, and FT-IR was further adopted to analyze the composition of apatite.

3. Results and discussion

The top and axial views (Fig. 1a and 1b) clearly show that the EBM-manufactured samples possessed fully interconnected pores and the porosity obtained by CT scan was 74%. The inserted SEM images indicate that the strut diameter (Fig. 1d) and pore size (Fig. 1e) were 540 ± 10 μm and 840 ± 20 μm, which were reported to be beneficial for bone ingrowth and osteointegration[8]. According to axial compression test, the yield strength (68 ± 7 MPa) and elastic modulus (0.83 ± 0.07 GPa) of samples before and after ion implantation were the same, similar to trabecular structure [8]. The EDX spectrum (Fig. 1c) and XPS survey spectrum (Fig. 2) clearly detected the presence of magnesium peak. The Ti 2p spectrum was ascribed to the spin-orbit splitting of Ti 2p3/2 (458.8 eV) and Ti 2p1/2 (464.4 eV) in TiO2 as described in previous literature [9]. The intensities of the Mg 2p core level signals at 50.37 eV correspond well to MgO [10]. Taken together, this may indicate that an outer oxidized layer composed of TiO2 and MgO had been formed due to the exposure to air.

The adhesion of MC3T3-E1 to porous TC4 samples was determined in vitro (Fig. 3a). It can be seen that that the cells seeded onto the Mg-implanted TC4 appeared to show better extension and spreading than those seeded onto the non-implanted TC4. After 12 h incubation, many cell tentacles had contacted with the microspheres or extended across the micropores, even cell networks were formed in some parts of the scaffolds. It was obvious

![Fig. 1.](image-url)
Fig. 2. XPS survey spectrum of TC4 (a) and TC4 with Mg implantation (c), (b) XPS Ti 2p of TC4, (d) XPS Ti 2p and (e) Mg 2p of Mg-implanted TC4.

Fig. 3. (a) Morphology of MC3T3-E1 on TC4 and Mg-implanted TC4 observed by SEM after 6 and 12 h incubation and inserted magnified sections showing the filopodia and connections between cells; (b) cell proliferation results determined by CCK8 assay; (c) CLSM projections of representative areas (the middle position of sample at the same height.) and the inserted graphs show the cross-section of cell morphologies.
that the attachment and spreading of cultured cells were significantly enhanced on Mg-implanted surface, which could be attributed to the increasing concentration of magnesium ions [11]. Fig. 3b shows that cells on different samples all proliferated exponentially with the extension of time. Also, after 3 and 5 days incubation, cell proliferation of Mg-implanted TC4 was significantly higher than TC4 control group. The CLSM images (Fig. 3c) of three dimensional cell proliferation and distribution on porous structure further supported this result. Besides, the cells could evenly distribute on the porous scaffolds after 5 days incubation.

Fig. 4 shows the surface morphology and chemical composition of samples after exposure to 1.5 \( \times \) SBF for 5 and 14 days. Clearly, there was only a few small granular sediments after 5 days exposure, while more deposits could be found on the Mg-implanted with significantly larger size. After 14 days immersion, the surfaces of TC4 and Mg-implanted TC4 were evenly covered by the sediments and the magnified figures show that the microstructure of covered layers were needle-like and porous. The results indicate that ion-implanted surfaces possess a higher ability to nucleate calcium phosphate than the control non-implanted TC4. The FT-IR results revealed that the deposited product was poorly crystallized B-type HCA. The bands at 559 and 1018 cm\(^{-1}\) were typical absorption bands of PO\(_4^{3-}\) groups, and the characteristic peaks at 863 cm\(^{-1}\) also indicate the presence of HPO\(_4^{2-}\). In addition, the phase composition of precipitates were also identified as hydroxyapatite of low crystallinity by XRD (Fig. 4f). For visible signals centered at 2-theta of 26.08° and 32.08° could be detected in the XRD pattern, corresponding to HCA crystallites [13].

4. Conclusions

In this study, we have designed a porous three-dimensional Ti6Al4V with similar macro structure to natural bone and appropriate nano/micro-scale surface topographies. PIII was adopted to implant Mg into the EBM-manufactured Ti6Al4V, and our results revealed that: (1) The porosity and elastic modulus of porous TC4 alloy were 74% and 0.83 ± 0.7 GPa, which were similar to trabecular bone structure. (2) The XPS spectra indicated that the surface composition of Mg-implanted porous TC4 alloy samples were MgO and TiO\(_2\). (3) The osteogenic MC3T3-E1 cells of Mg-implanted porous TC4 alloy showed better adhesion, spreading and proliferation than non-implanted alloy. (4) In vitro immersion test in SBF solution showed that porous TC4 alloy with Mg implantation possessed better ability of apatite formation.

Acknowledgements

This work was supported by National Natural Science Foundation of China (Grant No. 51431002), Beijing Municipal Science and Technology Project (Z131100005213002), NSFC/RGC Joint Research Scheme (Grant No. 51361165101 and 5161101031), NSFC-RFBR Cooperation Project (Grant No. 51611130054) and the National Key Research and Development Program of China (Grant No. 2016YFC1100604).

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