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Bone integration properties of antibacterial biomimetic porous titanium implants

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Abstract: A novel antibacterial biomimetic porous titanium implant with good osseointegration was prepared by freeze-casting and thermal oxidation. Bone integration properties of the porous titanium implant were evaluated by cell proliferation assay, alkaline phosphatase activity assay, X-ray examination and hard bone tissue biopsy. The in vitro cell proliferation and the level of differentiation of the group with a modified nano-porous implant surface were significantly higher than those in the group without surface modification and the dense titanium control group ($P<0.05$). In vivo, bone growth and osteogenesis were found in the experimental groups with modified and unmodified porous titanium implants; osteoblasts in the modified group had more mature differentiation in the pores compared to the unmodified group. Such implants can form solid, biologically compatible bone grafts with bone tissues, exhibiting good osseointegration.

Key words: antibacterial function; surface modification; porous titanium implant; osseointegration; freeze-casting

1 Introduction

Titanium and titanium alloys, as bone substitute materials, have good biocompatibility, mechanical properties, and many other advantages. However, in vivo titanium and titanium alloy implants only mechanically but not biologically integrate with bone tissues after an operation. In particular, loosening, peripheral bone inflammation, and severe complications caused by bacterial growth on the implant surface increase the risk of failure after bone implantation [1,2].

New bone implants require a special response and interaction with living cells of the bone tissue, thereby inducing osteoblast development into viable new bone tissue or organs in the physiological environment, i.e., good osseointegration [3,4]. Structures with anisotropic gradient distribution of porous metals prepared by freeze-casting are very similar to natural human skeletal materials. Optimization of freeze-casting may change the pore morphology and size of a porous titanium implant, rendering them similar to human bone tissues [5−7]. Our previous study has shown that thermal oxidation helps the formation of nanoscale, nanospike structures on the porous surface of titanium implants that effectively avoid bacterial growth [8]. But osseointegration of these implants requires verification.

The formation of a good bone−implant interface is the key to successful grafting, and osteoblasts are critical components of that interface. Studies on the interaction between osteoblasts and implant materials, as well as the impact of implant materials on the biological properties of osteoblasts, can directly reflect the effectiveness of implant materials for osseointegration [9,10]. In the present work, freeze-casting and thermal oxidation were used to prepare a novel, surface-modified biomimetic porous titanium implant with good antibacterial properties and osseointegration. In vivo and in vitro osteogenic assays were used to evaluate the osseointegration of the implant to determine its feasibility for future clinical applications.

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2 Experimental

2.1 Materials

Specimens in the two experimental groups were porous titanium with surface modification (i.e., the modified experimental group) and porous titanium without surface modification (i.e., the unmodified experimental group). Dense titanium was used as specimen in the control group.

2.2 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 fraction of titanium powder in the slurry was 10%. The mold was placed in the water bath 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 being cured for 4 h, the mold was placed overnight 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 °C. After being sintered for 1 h, the dried body was naturally cooled down to room temperature, and then the preparation of the porous titanium was thereafter completed.

Samples were placed in the centre of a horizontal alumina tube furnace. After Ar was purged into the tube, the temperature of tube 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 of 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.

2.3 Porous structures and mechanical properties

The porous structures (porosity, degree of interconnection) of the porous Ti scaffolds 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 epoxy resin. The compressive strength of the porous Ti scaffolds with a diameter of 16 mm and a height of 20 mm was 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.

2.4 Proliferation and differentiation of in vitro human osteosarcoma cells, MG63

2.4.1 Detection of human MG63 osteosarcoma cell proliferation

Porous titanium specimens in the modified and unmodified experimental groups, as well as dense titanium specimens in the control group, were transferred into 24-well culture plates after sterilization and drying. Each group contained three parallel samples. RPMI−1640 culture medium (500 μL) containing 10% fetal bovine serum (FBS) was added into each well. After hydrating the specimens for 24 h, the culture medium was discarded followed by gently rinsing the specimens twice in phosphate buffered saline (PBS). Trypsinized and passaged human MG63 osteosarcoma cells were diluted to a density of 1×10^4 cells/μL per well, and the cell suspension was slowly transferred dropwise into each well on the specimen surface, followed by incubating at 37 °C in an incubator containing 5% CO_2 and 95% humidity for 8 h. Once the cells began to grow, 1 mL culture medium was added slowly into each well. The cells were further incubated with the medium changed once every two days.

Cells were assayed after 1, 3, and 5 days of incubation by discarding the culture medium and gently rinsing with PBS twice, followed by adding 500 μL RPMI−1640 culture medium containing 10% FBS and 50 μL cell counting kit (CCK)-8 reagent, and then cultured at 37 °C in the incubator for 4 h. Cell supernatants from wells co-cultured with corresponding specimens in the three different groups were transferred to a 96-well plate to measure the optical density in a microplate reader at 450 nm wavelength.

2.4.2 Detection of alkaline phosphatase (AKP) activity in human MG63 osteosarcoma cells

Cells were prepared in 24-well culture plates exactly as described in Section 2.4.1. Cell were assayed after 1, 3, and 5 days of incubation by discarding culture medium and gently rinsing with PBS twice, followed by adding 2.5 g/L trypsin to digest the cells for 2 min. Cell lysate (500 μL 0.2% TritonX−100) was added into each well to cover the cells, and then they were incubated overnight at 4 °C. An AKP assay kit (microtitration method) was used to calculate the AKP activity of the

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