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Improving Bioactivity of Porous β-TCP Ceramics by Forming Bone-like Apatite Layer on the Surfaces of Pore Walls

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Abstract. Bone tissue engineering provides a new way to repair the bone defect in orthopaedics. The scaffolds, porous materials with excellent biocompatibility, bioactivity and biodegradability, play an important role in bone tissue engineering. Furthermore, the bioactivity of the pore interior surfaces is very important for cell attachment, differentiation and growth, as well as new bone tissue ingrowth into pores. In this paper, β-TCP was selected as materials of scaffolds, and its bioactivity was improved by activating the interior surfaces of pore walls. The porous β-TCP scaffolds with about 50 ~ 300 μm of pore size and above 80% of porosity were obtained by 3D-gel-laminated processing. Their surfaces of the scaffolds were easily covered by a low crystallized bone-like apatite layer, which determined by XRD and FTIR, after immersing in 1.5SBF solution following pre-treatment by NaOH solution. MTT and ALP assays were performed after cells cultured on the porous scaffolds with bone-like structure, and the results showed higher proliferation rate and differentiation level than that on the scaffolds without treatment, which indicated that the porous β-TCP scaffolds with bone-like apatite layer on surfaces of pore walls possess higher bioactivity. Therefore, the bioactivity of tissue engineering scaffolds could be improved by deposited bone-like apatite layer on their surfaces.

Introduction

Bone defects are familiar to orthopaedics, but the present bone implantation materials, such as autograft, allograft and biomaterial-filling, are not perfect enough. Because tissue engineering combined the engineering and regeneration, bone tissue engineering provides a new way to solve the above problems for bone defect restoration [1]. The scaffolds, porous materials serves for cell attachment, proliferation, and differentiation, with excellent biocompatibility, bioactivity and biodegradability, play an important role in bone tissue engineering. [2] These properties were related to the structure and component of the surface. Many methods were researched to improve the bioactivity of porous scaffolds, but they mostly focus on the surface of the monolithic material, not the interior surfaces, such as pore wall surfaces. In fact, the exterior and interior surfaces both are important, because the bioactivity of the pore interior surfaces is very important for cell attachment, proliferation, differentiation and growth, as well as new bone tissue ingrowth into pores. [3]

Depositing bone-like apatite layer on the surface in physiological environment was always used as a method to characterize the bioactivity of biomaterials. After dipped into SBF (Simulation Body Fluid) solution for a period of time, a thin layer would be formed on the surface of biomaterials, such as HA, β-TCP and glass ceramics [4]. Leng Y et al. [5 and 6] identified the crystal phase in deposited layers was octacalcium phosphate (OCP) by transmitting electronic microscope (TEM), however, many of other researchers proved the layers were consisted by HA crystal, rather "bone-like apatite" [4, 7, 8]. The bone-like apatite means the crystals are calcium deficiency and carbonate contained, and their morphology are small and needle-like. Neo M et al. [9] and Ohtsuki C et al. [10] found that when

biomaterials were implanted into the bone defected position, they were bonded to bone through a thin Ca-P-rich layer consisting of fine apatite crystals demonstrated by SEM and TEM. The layers possess a low crystallization degree and good bioactivity. It was a kind of apatite containing CO_3^{2-}, and similar to those of natural bone in components and structure, also similar to the bone-like apatite layer formed in SBF solution described above. Cell or collagen fibers of the bone would firstly reach the surface of this layer, and chemical bonding between the biomaterials and the cell or bone. Hence, the bioactivity of biomaterials and scaffolds could be improved by the bone-like apatite layer on their surfaces.

In this paper, β-TCP was selected as materials of scaffolds. The present work focuses on estimating the bioactivity of the scaffolds with or without the bone-like apatite layer on their pore wall surfaces. Furthermore, the depositing procedure was intensified by pretreatment in NaOH solution.

Materials and Methods

Preparation of β-TCP Scaffolds. The β-TCP powders used in this paper were prepared by wet-chemical method from the reactant of $Ca(NO_3)_2$ and $(NH_4)H_2PO_4$ in our laboratory. Other reagents used in this paper were all grade of chemical purity. The wet green bodies of porous β-TCP were obtained by 3D-gel-laminated system [11] with foamy ceramic slurry, after drying, they were sintered at 1150°C for 2 hours in air.

Pretreatment and Soaking in 1.5SBF. The sintered β-TCP scaffolds were firstly pre-soaked in 10M NaOH solution for 4 days for pretreatment process. After washing for several times and drying, the pretreated specimens were soaked in 1.5SBF solution for 14 days. Other scaffolds without pretreatment were directly soaked in 1.5SBF solution for 14 days as control. The 1.5SBF was used to enhance and expedite the deposition speed of apatite crystal.

Cell Culture and Characterization. The MC3T3-E1 cells were seeded on the porous scaffolds and cultured in vitro. Following seeding, MTT (3-([4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetetrazolium bromide) assay was used to quantitatively evaluate the viability of cell adhesion and proliferation on surfaces of pore walls after cell culturing for 1, 4, 7 and 10 days. ALP (Alkaline Phosphatase) activity was used to characterize the osteoblastic differentiation after cell culturing for 7 and 14 days. The surfaces of the mineralized scaffolds were characterized by XRD (D/MAX-RB, Rigaku), FTIR (Spectrum GX, Perkin Elmer) and SEM (QUANTA 200, FEI Company, Netherland). The states of cell attachment, spreading and morphology were observed by ESEM (Environmental Scanning Electron Microscopy, QUANTA 200, FEI Company, Netherland).

A total of five samples were used in each experimental condition for MTT and ALP activity assays. Student's *t* test was used for statistical evaluation ($p<0.05$).

Results and Discussion

Scaffolds and Pretreatment in NaOH Solution. Figure 1 shows the fractural surface morphology of sintered β-TCP scaffolds, which exhibits about 50~300μm of pore size and above 80% of porosity. And lots of micro-pores with the sizes of 20~100 μm can be easily observed distributed at the pore walls, which appears highly interconnected like the netlike structure of natural bones. The as-fabricated structure with large pores and good interconnectivity was suitable for the materials as bone tissue engineering scaffolds. [3] However, considering of the requirement of adhesion to cells and new tissues, the sintered surfaces of pores were too smooth and faulty bioactivity to use. Hence, the interior surfaces of sintered porous scaffolds need to be modified for cell and new tissue adhesion and growth.

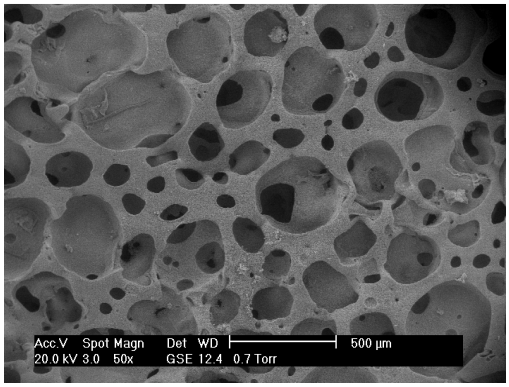

Fig.1 SEM photograph of fractural surface of sintered β-TCP scaffolds

In order to improving deposition ability of Ca-P crystals on the surfaces, the pretreatment by NaOH was applied in this paper. After soaking in 10M NaOH solution for 4 days, the surfaces of pore changed coarser. The curves of Fig.2 (B) and Fig.3 (B) show the XRD pattern and FTIR transmission spectrum of pretreated ceramics. Compared to XRD pattern of sintered β-TCP scaffolds (shown in Fig.2 (A)), there was not distinctly change in crystal phase after pretreatment. Nevertheless, two special absorption peaks at 3571 cm^{-1}, corresponding to stretch vibration of O-H bond, and 631 cm^{-1}, corresponding to bending vibration of O-H bond, were observed, which indicated that a number of –OH groups were adsorbed on the surfaces during NaOH pretreatment. Noticeably, the –OH groups was effective for bone-like apatite nucleation in body or simulation body environment [12, 13].

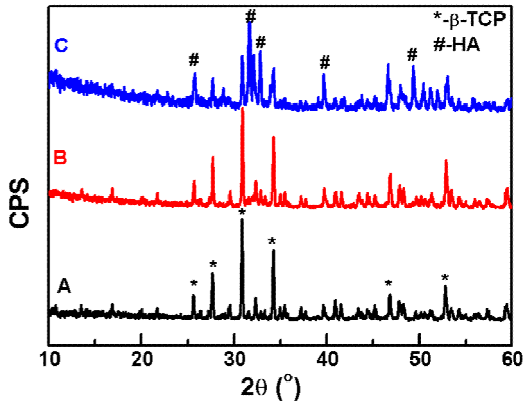

Fig.2 XRD patterns of β-TCP scaffolds. (A): Sintered scaffolds; (B): Scaffolds after pretreatment; (C): Scaffolds with pretreatment after soaking in 1.5SBF for 14days

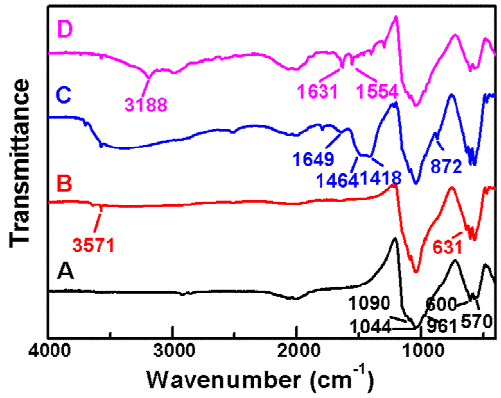

Fig. 3 FTIR spectra of β-TCP scaffolds. (A): sintered scaffolds; (B): scaffolds after pretreatment; (C): scaffolds with pretreatment after soaking in 1.5SBF; (D): scaffolds without pretreatment after soaking in 1.5SBF

Bone-like Apatite Layer. In order to form a bone-like apatite layer on surfaces of pore walls, the porous scaffold with or without pretreatment both were immersing in 1.5SBF solution. The SEM photos in fig.4 show the morphologies of pore wall surface after soaking in 1.5SBF for 14days. The particle-like crystals were found on the surfaces of scaffolds without pretreatment (shown in fig.4 (A) and (B)), and these crystals do not form a continuous layer. Correspondingly, a brushy layer was observed on the surfaces of scaffolds with NaOH pretreatment (shown in fig.4 (C) and (D)). Compared to the two layers, that on the pretreated scaffold was more uniform and denser. The layer basically covered the nearly whole surface of the porous scaffold. The larger amplificatory photo (fig.4 (D) shows the morphologies of grains, which were nano-sized plate-like.

XRD was applied to estimate the phase distribution of the specimens. In fig.2 (C), the peaks corresponding to HA phase were found, which could proved that the deposited micro-crystal layer contained HA crystal, and possessed low crystallization degree.

The curve C and D in Fig. 3 show the FTIR spectra of the scaffolds with deposited layer. In curve D, the characteristic peaks of adsorption H_2O were found at 3188 cm^{-1} and 1631 cm^{-1}, thereof, the latter generally appeared while the water adsorbed in apatite carbonate. In addition, the characteristic peak of A-type carbonate substituted apatite at 1554 cm^{-1} was found. Otherwise, in curve C of fig. 3, the peaks at 1649 cm^{-1}, 1464 cm^{-1}, 1418 cm^{-1} and 872 cm^{-1} were found. Thereof, the adsorption peak at 1649 cm^{-1} was corresponded to the bending vibration band of adsorbed water. The two peaks adsorbed at 1464 cm^{-1} and 1418 cm^{-1} were the characteristic γ3 adsorption double peaks of CO_3^{2-}. The characteristic B-substituted carbonate apatite was appeared at 872 cm^{-1}. Hence, it could be concluded that the apatite carbonate was both formed on the surface of scaffolds after immersing in 1.5SBF for 14 days, and the A type substitution for scaffolds without pretreatment and B type substitution for that with pretreatment, respectively.

Considered the results of XRD, SEM and FTIR synthetically, the brushy layer on the surfaces of porous scaffolds was bone-like apatite (HA phase, nano-sized crystal, B-substituted carbonate), and furthermore, the procedure of NaOH pretreatment could improve the deposited quantity, and promote the formation of B-substituted type.

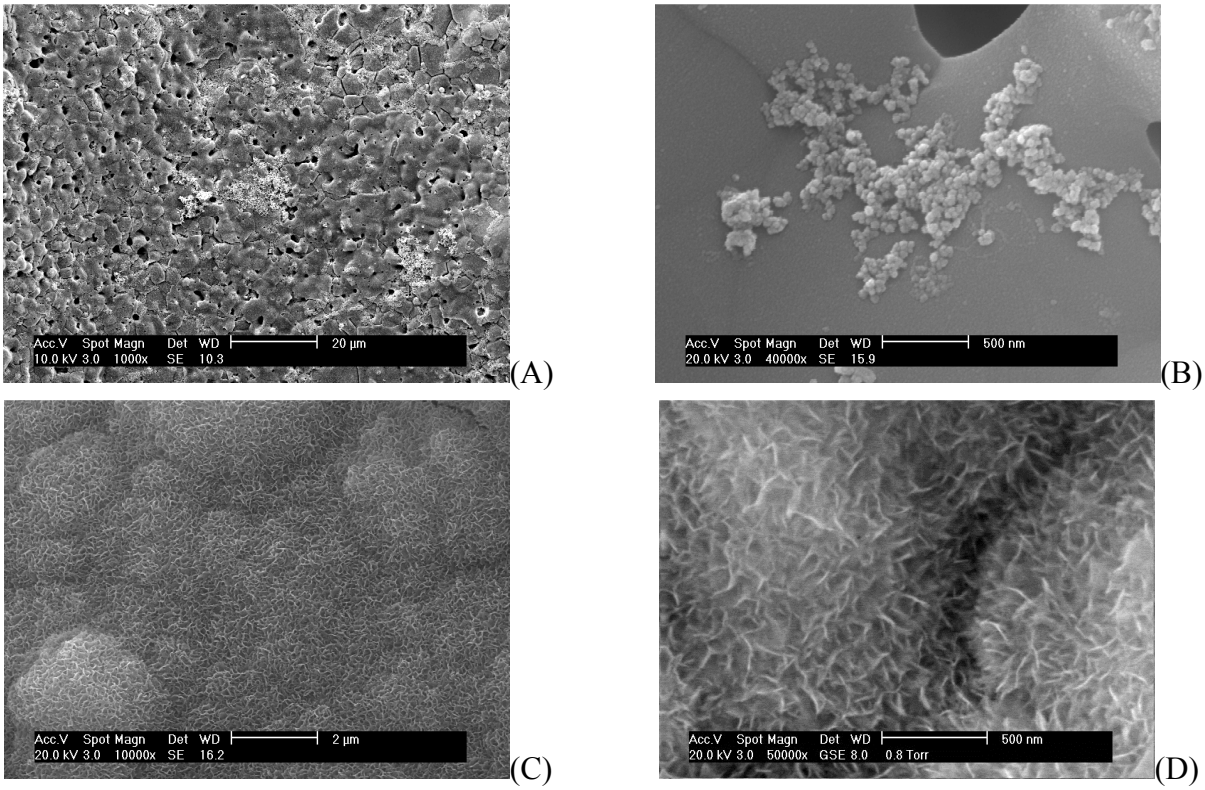

Fig.4 SEM photographs of porous β-TCP scaffolds surfaces after soaking in 1.5SBF for 14 days with (C and D) or without (A and B) pretreatment in NaOH solution

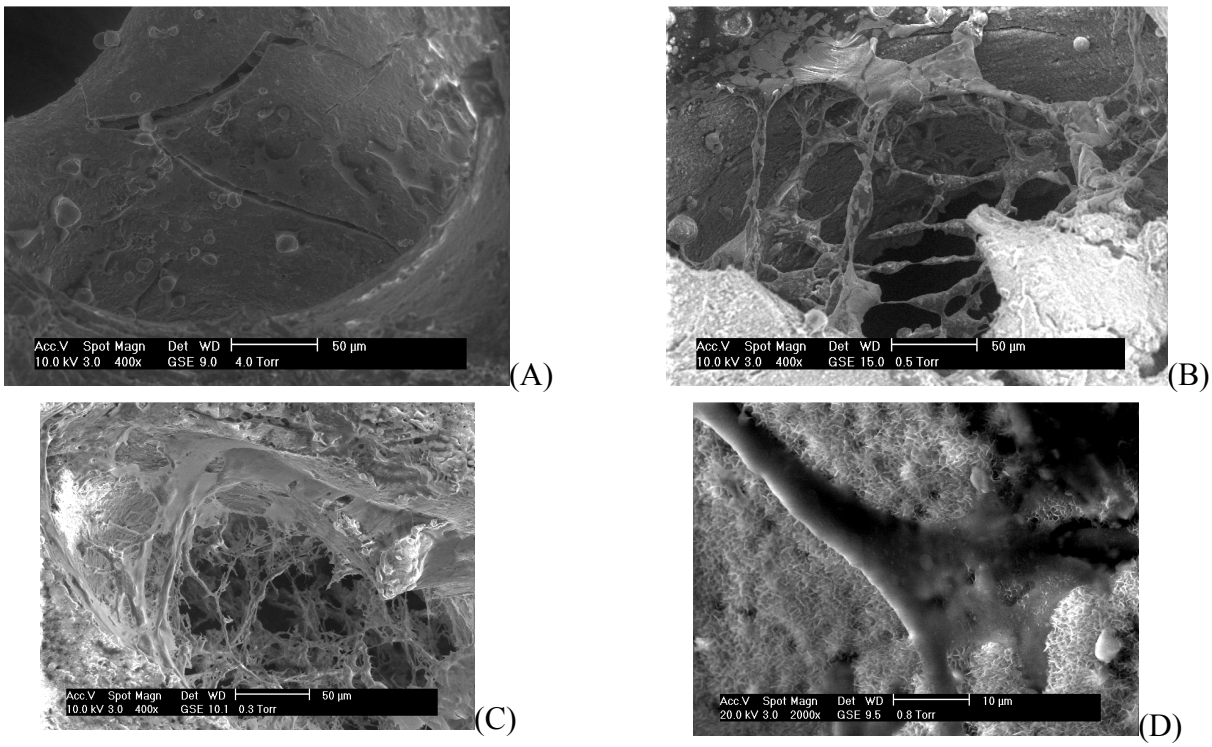

Fig.5 Cell cultured on β-TCP scaffolds for 7days (A): Without bone-like apatite layer; (B) With bone-like apatite layer but without pretreatment; (C) and (D): With bone-like apatite layer forming after pretreatment

Cell Culture and Scaffolds Bioactivity. The MC3T3-E1 cells were cultured on the porous scaffolds with or without bone-like structure to estimate their bioactivity. ESEM photographs in Fig.5 show the morphologies of cell attachment and spreading on three materials as-fabricated in this paper for 7 days. Firstly, the cell amounts attached on interior surfaces of material (C) were larger than they of material (A) and (B). And the cells could spread well on both material (B) and (C) in fig. 5, the pseudo-feet of the osteoblasts extended on pore walls (fig.5 (D)) with treatment in SBF solution. Hence, the scaffolds with bone-like apatite layer exhibit better cytocompatibility, thereof, the material shown in Fig.5 (C) is best.

After MC3T3-E1 cells were cultured for 1, 4, 7 and 10 days, MTT assay was performed and the results were shown in Fig. 6. The absorbance on 570 nm indicates the amount of live cells on the estimated samples. With the cultural time prolonged, the absorbance of the three materials was increased, which indicated that the cells could attach and proliferate on surfaces of scaffolds well. Furthermore, the absorbance of material C in fig. 6, which was β-TCP scaffolds with pretreatment by NaOH and immersion in 1.5SBF, was obvious largest. The similar disciplinarian was found at ALP activity assay shown in Fig. 7. The alkaline phosphatase activity reflects the activity of osteoblasts differentiation. Hence, the scaffolds with bone-like apatite layer exhibit better differentiation activity.

Therefore, the porous β-TCP scaffolds with bone-like apatite layer on surfaces of pore walls possess higher bioactivity than that without it, and the bioactivity of tissue engineering scaffolds could be improved by depositing the bone-like apatite layers.

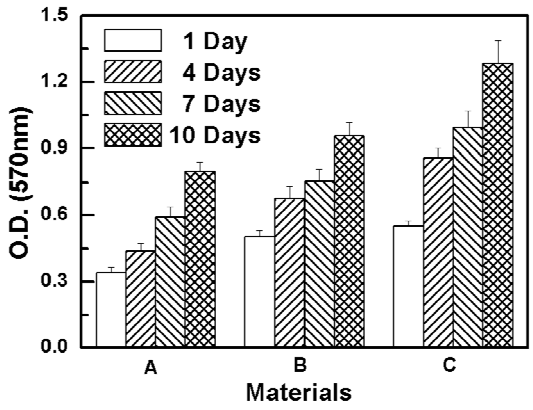

Fig. 6 MTT assay of materials after cell culture for 1, 4, 7 and 10 days. A: sintered β-TCP; B: β-TCP immersion in 1.5SBF without pretreatment; C: β-TCP immersion in 1.5SBF with NaOH pretreatment

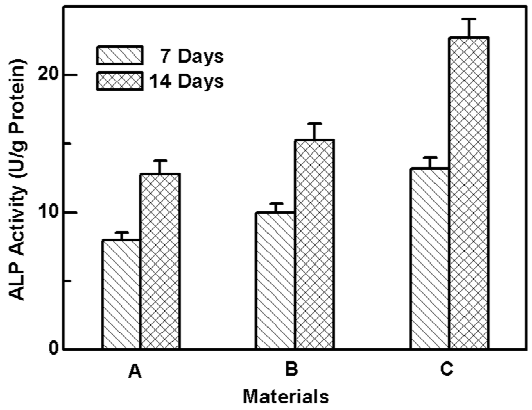

Fig. 7 ALP Activity of Materials after cell culture for 7 and 14 days. A: sintered β-TCP; B: β-TCP immersion in 1.5SBF without pretreatment; C: β-TCP immersion in 1.5SBF with NaOH pretreatment

Conclusions

The bone-like apatite layers were formed on the surfaces of scaffolds and pore walls. Furthermore, the forming ability of the layers was improved after pretreatment, because the apatite nucleation activity on surfaces of porous β-TCP scaffolds was improved by pretreatment in NaOH solution. The bioactivity, which estimated by cell culture, MTT and ALP activity assays, was obviously improved by depositing the bone-like apatite layer on their surfaces.

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