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Controlling surface microstructure of calcium phosphate ceramic from random to custom-design

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Abstract

Calcium phosphate ceramics have long been studied as bone graft substitutes due to their similarity with the mineral constitute of bone and teeth, excellent biocompatibility and bioactivity. Chemical composition, macrostructure and surface microstructure are believed to be important for the bone formation within calcium phosphate ceramics. Surface microstructure has shown its crucial role in the osteogenic response of calcium phosphate ceramics; however the presence of surface irregularities and random distribution of surface microstructure in traditional calcium phosphate ceramics make it difficult to explain how surface microstructure play its role in bone formation. In the present study, we evaluated the influence of various starting apatites and sintering temperatures on the surface microstructure of the resulting hydroxyapatite ceramics. In order to minimize the randomness of the surface microstructure, laser ablation was used to generate custom-designed surface microstructures. The resulting hydroxyapatite ceramics with controlled surface microstructures would be helpful to study the role of surface microstructure on bone formation and may provide useful information for further optimization of calcium phosphate ceramics for bone regeneration.

Keywords: A. Powders: chemical preparation; A. Sintering; B. Microstructure-final; E. Biomedical applications

1. Introduction

Bone defect can result from tumor resection, trauma, disease and congenital anomalies. When a bone defect is too large to heal by itself, the so called critical-sized defect, it requires bone graft materials [1,2]. To this end, several grafting materials have been considered, including autograft, allograft, xenograft and synthetics. Autograft has long been considered as the “Gold Standard” in bone repair because of its excellent osteoconductive and osteogenic properties, high biocompatibility and immunological safety. However, the necessity of a second surgical procedure may lead to undesired scarring, long healing time, morbidity and pain, limiting its use. Allograft and xenograft could be alternatives to autograft, but they have less bone regeneration potential and may cause immunological rejection. Synthetics do not have such problems and could be ideally optimized with respect to biocompatibility, osteoconductive property and resorption rate by controlling their chemical composition, macro and microporosity, etc.

Among synthetic materials, calcium phosphate ceramics have been extensively evaluated as bone grafts due to their chemical similarity to bone minerals, excellent biocompatibility, osteoconductive and bioactivity properties [3,4]. Moreover, calcium phosphate ceramics with specific physico-chemical properties could induce ectopic bone formation, thus are osteoinductive. Although this phenomenon has been shown in different animal models, such as baboon [5], monkey [6], goat [7,8], sheep [9,10], dog [11–15], rabbit [16,17], and mouse [17–19], the exact physico-chemical parameters governing the successful ectopic bone formation are not fully understood. It is believed that the osteoinductive capacity can be modulated by controlling the crystal size, crystallinity, Ca/P ratio, macroporosity and microporosity. Recently an increasing number

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of studies have illustrated that the micropores might be an important determinant of osteoinduction [6,7,20,21]. It has been suggested that micropores could increase the surface area of calcium phosphate ceramics, which facilitated ion exchange and bone-like apatite formation. This could be accompanied by the binding of endogenous bone inducing proteins to the surface from body fluids (i.e. growth factors like bone morphogenetic proteins). All of these events may, in turn, facilitate the recruitment and homing of relevant pluripotent stem cells (i.e. bone marrow mesenchymal stem cells) to form new bone in ectopic sites [22].

At the cellular level, the substratum interface functions as more than a simple definition boundary between the host and implanted devices; instead, it presents primary cues for cellular adhesion and subsequent induction of tissue integration, which play crucial roles in tissue regeneration [23,24]. As the functions of the extracellular environment have been explored for years, the surface microstructure has been proved to have complex interactions with cells [25–36]. The ability of the substrate topography to influence cell behaviors was first noted by Harrison in 1911 when he cultured cells on a spider web. It was observed that cells followed the fibers of the web in a phenomenon called physical guidance or stereotropism [37]. Later, in 1964, it was first proposed that cells react to the topography, or to their surrounding environment, in the process of “contact guidance” [38]. Thanks to the development of various micro and nanostructure fabrication techniques, a large number of research groups have devoted their attentions to this emerging area. In the last decade, the interest in basic knowledge of cell–substrate interactions has increasingly grown, and it has now been recognized that the microstructure plays a key role in causing the differences observed in cell′s behavior both in vitro and in vivo [25,26]. Studies on the interactions between various substrate microstructures and cells have included a wide variety of cell types such as endothelial cell, fibroblasts, leukocytes, chondrocytes, osteocytes, oligodendrocytes, smooth muscle cells and mesenchymal stem cells. In these studies, it has been recognized that these cells strongly react to microstructures, which modulated their adhesion, alignment, migration, morphology, proliferation, vitality and differentiation [25–36]. More specifically, it was demonstrated that the use of nanoscale surface disorder could stimulate human mesenchymal stem cells (MSCs) to produce bone mineral in vitro, in the absence of osteogenic supplements. The same study also proved that this approach has similar efficiency to others where cells were cultured in osteogenic media [35].

Concluding, the substrate topography may induce osteogenic differentiation of mesenchymal stem cells via not only specific protein adsorption but also via the specific surface topography created by the micropores on the surface of materials. In respect of this, a calcium phosphate material with proper surface microstructure should be carefully designed to distinguish the roles of protein adsorption and surface topography in instructing cells.

Careful surface structure designs have been applied on different materials with various methods [25,26]. However, a well-controlled surface microstructure or nanostructure on calcium phosphate ceramics has not been reported so far. Because of the intrinsic brittleness of calcium phosphate ceramics, it is difficult to precisely control the surface structure with conventional methods (e.g. surface roughening or plasma spraying). Laser ablation has high potential for micromachining of various materials, and this method was increasingly proved to be a promising tool for 3D micro-texturing of material′s surfaces. The advantages of laser structuring include its very high fabrication rate, high resolution, non-contact interaction, applicability for many types of substrate and reproducibility. Furthermore, lasers can be easily incorporated with computer-assisted fabrication systems for complex and customized 3D matrix structure design and manufacture [39,40].

In this study, we first synthesized hydroxyapatite ceramics with random and irregular surface microstructures by controlling the particle size and distribution of the starting apatites and sintering temperatures. We then used laser ablation method in order to get custom designed surface microstructures of the synthesized ceramics.

2. Material and methods

2.1. Starting apatites and characterization

Two apatite slurries with a Ca/P molar ratio of 1.67 were prepared with either concentrated or diluted starting chemicals. Both apatite slurries were synthesized by mixing $Ca(NO_3)_2 \cdot 4H_2O$ (Fluka) with $(NH_4)_2HPO_4$ (Fluka). In general, $Ca(NO_3)_2 \cdot 4H_2O$ solution was kept stirring at 500 rpm at 80 °C and $(NH_4)_2HPO_4$ solution was added to it. Ammonium hydroxide solution was added as well to keep the pH of the slurry above 10. After additions were terminated, stirring was kept for 2 h at 80 °C. In the case of concentrated starting chemicals, 12 mol/L ammonium hydroxide solution was added to 2 mol/L $Ca(NO_3)_2 \cdot 4H_2O$ solution at the speed of 35 mL/min, while a 2.4 mol/L $(NH_4)_2HPO_4$ solution was added at 30 mL/min. In the diluted counterparts, 3 mol/L ammonium hydroxide solution was added into $Ca(NO_3)_2 \cdot 4H_2O$ solution (0.5 mol/L) at a speed of 17.5 mL/min, then $(NH_4)_2HPO_4$ solution (0.60 mol/L) was added at the speed of 12.5 mL/min.

The two slurries were then aged at room temperature overnight, and then the supernatant was refreshed with demineralized water to remove ammonia. The slurries were then vacuum-filtered to obtain cakes. Half of the apatite cake obtained from the concentrated hydroxyapatite slurry (SCHA) were dried at 80 °C and reduced to powder using a grinder (ZM100, Retsch, Germany) with a 250 μm sieve to get apatite powder (PHA). The second half of the cake from SCHA and the one from diluted hydroxyapatite slurry (SDHA), were dried at room temperature till a water content of 20% (w/w), and stored in air-tight containers.

The chemistry of apatites from both concentrated starting chemicals and diluted chemicals were analyzed with X-ray diffraction (XRD, Miniflex, Rigaku, Japan) after sintering them at above 1000 °C for 2 h using a sintering oven (Nabertherm, Germany).