

Case Report

Hydroxyapatite Particulate Nanofiber Modified Titanium: In-Vitro Bioactivity

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Received 11 November 2010; Accepted 2 December 2010

Abstract Current work demonstrates the ability of titanium based implant surfaces to promote human osteoblasts (HOBs) differentiation and matrix production, and enhance osseointegration in vitro. Titanium surface was modified by electrospinning with sol-gel-derived hydroxyapatite (HAp) and successively calcined at various temperatures. After heat-treatment, the crystal structure of the filmed titanium oxide and sol-gel-derived crystalline HAp on titanium's surface was identified using wide-angle X-ray diffraction. Surfaces of three different samples, HAp electrospun and calcined at 600, 700 and 800 °C, were investigated in terms of their ability of promotion, adherence, proliferation and differentiation of human HOB cells in vitro up to 6 days. The cells cultured on electrospun and 800 °C calcined titanium surfaces showed the best results among three samples in terms of adhesion, growth and proliferation of HOBs. This work would provide a promising alternative for titanium-based medical devices since it provides enhancement both on the surface and bulk properties.

Keywords biomaterial; titanium; osteointegration; electrospinning; hydroxyapatite

1 Introduction

Various metal and metal alloys such as 316L stainless steel, cobalt-based alloys and titanium alloys are frequently used as an implant material for bone plates, screws and hip joint prosthesis [7]. A typical 316L stainless steel is stronger and cheaper but its corrosion properties along with more susceptibility to infection brings inferiority to titanium based alloys. Cobalt alloys exhibit reasonable biodegradation properties when exposed to human tissues, but not as resistant to corrosion as titanium [6]. In relation to implant materials, titanium and titanium alloys are highly regarded as skeletal biomaterials because of their good biocompatibility and bioinertness [7]. This material allows

direct bone-to-implant contact that has also been called as “osseointegration” [3]. Unfortunately, like most metals, Ti exhibits poor bioactive properties [8,11]. The biological tissues typically interact with the outermost atomic layers of an implant, and therefore the surface properties of the implant material play a very important role in immediate reactions on the implant surface after exposure to the tissue and influence the osteoconductivity [5,9,11]. In this respect, titanium with its native bioinert surface, it is difficult to achieve good chemical bonding with bones and form new bones on its surface at the early stage after implantation. Hence, titanium and titanium alloys do not meet all the requirements of the “ideal” biomaterial [10]. So, there is the urgent need of surface modification with bioactive material [12].

Hydroxyapatites, the most ubiquitous family of bioceramics, are attractive as they can be produced in such a way that they mimic the mineral composition and/or the porous structure of bone; form a direct bond with neighboring bone with their ability to induce mesenchymal cells to differentiate towards osteoblasts [4,12]. The incorporation of Ca or P ions into the surface layer and the validity of these results have been confirmed by several different researchers. Present work reports the surface modification of Ti with HAp particulate nanofiber by using a simple electrospinning method for the first time. Such a surface modification of titanium nano-architectures by sol-gel-derived HAp nanofibers is cost-effective, enabling the realization of desired platform topologies on existing nonmodified implant materials.

2 Materials and methods

Commercially pure titanium (CP Ti, Grade 2, Ka-Hee Metal Industry Co., Korea), machined into disks ($10 \times 10 \times 2$ mm³), was used as the substrate. The samples were pickled using a mixture of hydrogen peroxide (H₂O₂) and sulfuric acid

(H₂SO₄). Calcium glycerol phosphate (Ca-GP), calcium acetate (Ca-Ac) and all the reagents were purchased from Sigma-Aldrich Co., USA. Polyvinyl alcohol (PVA, M_w = 65,000 Daltons) was purchased from Dong Yang Chem. Co., Korea. HOBs cell line CCRL-11372 was purchased from ATCC, USA. For electrospinning [1,2], a homogenous aqueous slurry of Ca-GP and CA with Ca/P ratio 1.67 was treated with 9% (w/w) PVA (aqueous) solution in the ratio of 3:7 by wt. to get a clear sol-gel, and electrospun by applying 15 kV at an electrode distance of 15 cm. The fibers were subjected to calcination at various temperatures ranging from 200 °C to 1400 °C. A titanium disk that was electrospun and not calcined is referred to as Ti-HAp, whereas the disks that were electrospun and calcined at temperatures ranging 200–1400 °C are referred to as Ti-HAp 200, Ti-HAp 400 and so on, accordingly. HOB cells were plated at a seeding density of 10⁵ cells/cm² on the sample surfaces. Cell viability was investigated 6 days after seeding the cells using a commercially available MTT (Sigma Co., USA).

3 Results and discussions

FT-IR spectra (Figure 1) of sol-gel-derived HAp on Ti disks were calcined at various temperatures. The observed PO₄³⁻ asymmetric stretching mode of vibration is characterized by a strong complex band in the 1180–1000 cm⁻¹ range and a medium intensity band at about 975 cm⁻¹ due to symmetric stretching-induced vibrations. The crystalline HAp generates characteristic OH bands at about 3400 cm⁻¹ and the small peaks at 1700–1450 cm⁻¹ indicated the existence of a Ca–O phase in the structure.

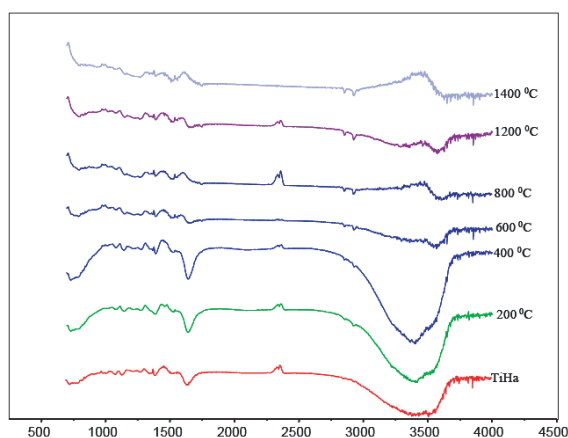


Figure 1: FT-IR spectra of Ti-HAp electrospun matrix after successive calcinations at 200–1400 °C.

X-ray spectra (Figure 2) showed that the titanium surface has been transformed into rutile when calcined at and above 800 °C in air. The broad diffraction peak at a

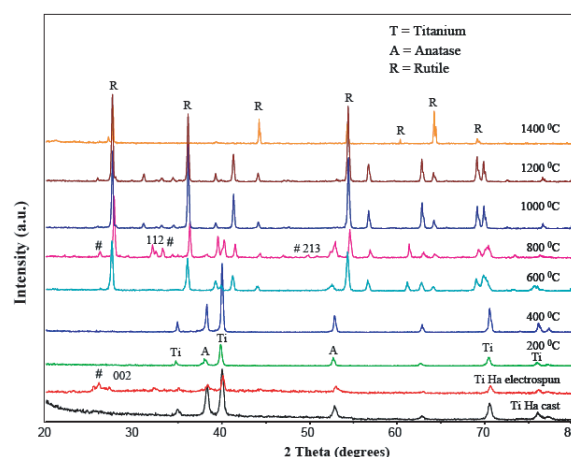


Figure 2: XRD patterns of sol-gel derived HAp, electrospun on titanium discs before and after calcinations at temperatures ranging from 200 °C to 1400 °C at the interval of 200 °C.

lower temperature (200 °C) displays that the sol-gel-derived hydroxyapatite is in an amorphous state. Such HAp was crystallized at a higher temperature and the sizes of the crystals were increased with an increasing calcination temperature. The surface morphologies of pure Ti, Ti-HAp and heat treated Ti-HAp disks were AFM (Figure 3). Further, samples calcined at 800 °C show some rod-type crystals in addition to a majority of hexagonal-type crystal. Also, samples calcined at 800 °C were found to have well-attachment of HAp on the surface of their disks. However, this was easily removed in samples calcined at and above 1200 °C. Also, the bioactive rutile TiO₂ is abundantly observed at calcination temperatures at and above 800 °C. The hydrophilicity of Ti-HAp 800 was expected to vary when compared with other samples.

Most of the cells examined were flattened, polygonally-shaped and showed an evidence of spreading, as well as numerous, highly-extended filopodia with apparent inter cellular communication (Figure 4). After 6 days of culture, the HOBs showed a polygonal morphology on titanium surfaces. These results indicate that the HOBs are able to spread faster on Ti-HAp 800 surfaces compared to other samples (controlled Ti and Ti-HAp 600) within 6 days of culture. These cells spreading behavior seems to help the cell anchor itself to the Ti-HAp crystals, particularly those that are rod-shaped. To ascertain the long term cytotoxic effects of Ti and Ti-HAp matrices, osteoblasts were cultured up to 6 days. HOBs without a sample matrix were used as our control. The LDH assay was used to compare the extent of cell membrane disruption in the presence of various samples. Neither Ti nor Ti-HAp matrices induced significance damage to the cell membrane. All the samples exhibited a similar absorbance showing no toxicity with

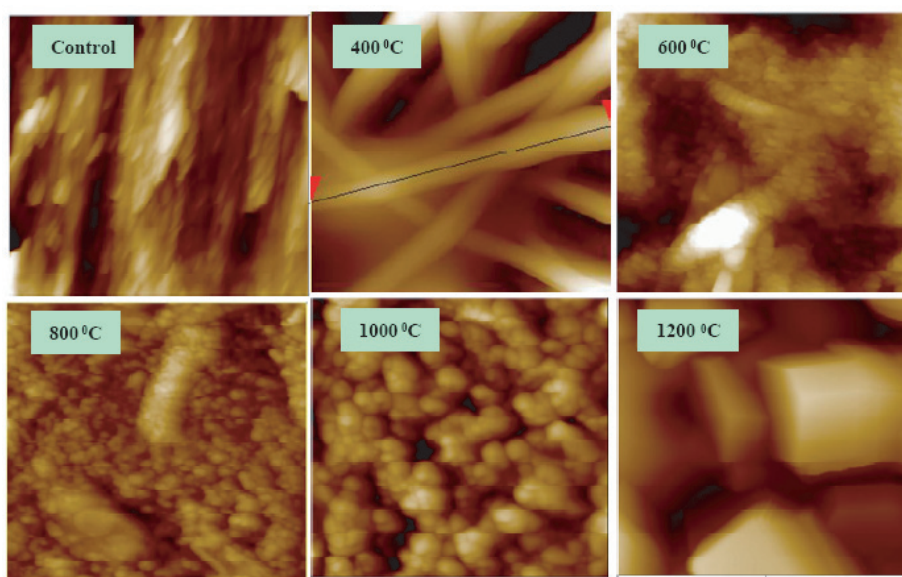


Figure 3: AFM images of titanium discs. (A) chemically etched; (B) electrospun disc with HA; (C) electrospun disc calcined at 600 °C; (D); (E) 800 °C; (F) 1000 °C; (G) 1200 °C.

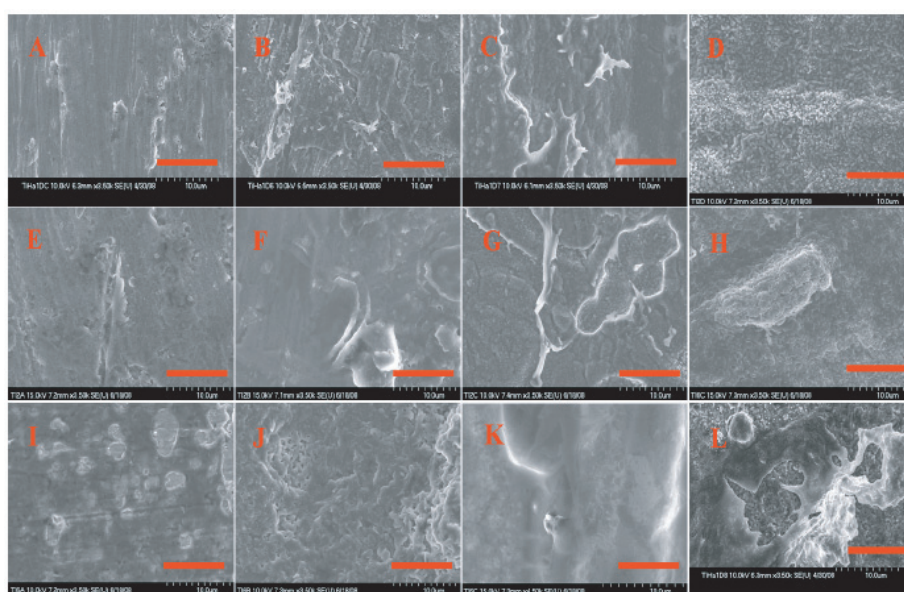


Figure 4: FE-SEM images of HOBs cultured on TiHAp surfaces in 6 well plates for control, Ti-HAp 600, Ti-HAp 700, and Ti-HAp 800 in day one (A–D), two (E–H) and three (I–L), respectively.

HOB proliferation. However, all the samples (Ti and Ti-HAp) showed slightly lower cell disruption than the control.

4 Conclusions

This study highlighted the surface modification of Ti disks with sol-gel-derived HAp via electrospinning, resulting in an hierarchal structure. The composite matrix thus obtained is found to have a superior topographical structure to former

surface modification efforts in its promotion of HOB proliferation. This finding will add a new dimension to the surface modification of implant materials to create an appropriate environment for growth and differentiation of cells.

Acknowledgment This research was supported by the Korean Research Foundation (KRF2007-211-D00032, Korean Government Project NO. 10028211) and Center for Healthcare Technology Development, Chonbuk National University, Jeonju 562-756, Korea.

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