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How bone marrow-derived human mesenchymal stem cells respond to poorly crystalline apatite coated orthopedic and dental titanium implants

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Abstract

Due to the delayed and weak bone-implant integration in dental and orthopedic devices, there have been several attempts to enhance implant–bone interactions for rapid osseointegration. In this paper, the interactions of human bone marrow-derived stromal (mesenchymal) stem cells (hMSCs) with uncoated and coated titanium alloy implants with poorly crystalline apatite are studied. First the configuration and chemical composition of the apatite coatings and their deposition progress in different experimental conditions are investigated and discussed. Then, hMSCs are cultured on different substrates and cell attachment and proliferation are monitored and evaluated for different time intervals. Although the uncoated and coated substrates indicate good cell attachment, the differences in proliferation and morphology of the cells spread over the coated samples are significant. It is concluded that the coated samples improve the capability for accepting the cells in three-dimensional and slender shapes. The migration of hMSCs on both substrates are discussed. As such cell migration is directly associated to the osteoconduction, the findings confirm the hypothesis of enhancement in bone formation on the surface of biomimetically poorly crystalline apatite coated titanium implants. This *in vitro* study demonstrates that the coated samples are nontoxic and biocompatible enough for ongoing osteogenic studies in bone or dental defects in animal models *in vivo*.

Keywords: D: Apatite; Biomimetic technique; Bone marrow-derived human mesenchymal stem cells; Orthopedic and dental titanium implants

1. Introduction

Implantation of orthopedic and dental implants is affected by delayed or weak implant–bone integration and inadequate bone formation. Innovative approaches have been sought to improve the interaction between implant and bone to achieve rapid osseointegration. Among different alloys used for implantation, titanium alloys have become the most popular biomedical materials due to their biocompatibility, excellent corrosion resistance, good mechanical properties and lightness [1]. Titanium without any surface treatments is bioinert, not bioactive, and cannot bond directly to the surrounding bone tissues when implanted in the human body.

The surfaces of these implants are the sites where osseointegration occurs. Optimizing the surface characteristics of implants could promote the formation of newly formed bone and osseointegration. During the last years, many techniques have been employed to enhance the *in vivo* osseointegration of titanium-based implants such as physical machining and controlled oxidation [2,3]. Recently, biomimetic approach has been extensively used for improvement of titanium implants. An example is calcium phosphate coatings, such as hydroxyapatite, which is employed for surface modification of orthopedic and dental implants [4–6]. The addition of this highly bioactive material to the surface of oxidized metallic implants shows dramatic enhancement in bone bonding [7]. This hard tissue integration would lead to quicker patient recovery and extended life for orthopedic implants [8]. The bone-like calcium phosphate apatite coating is achieved by placing the substrates into SBF medium in body temperature and at the blood pH.

Generally, biomimetic approach has become a modern method to create bioactive surfaces in different conditions such as sodium hydroxide and/or heat-treatment [1,9], as well as hydrogen peroxide of titanium substrates treatment [10] besides various pretreatments

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[11,12]. Also, changing the SBF conditions [13,14] have been widely investigated *in vitro* and *in vivo* to optimize long-term stable interfaces between bone tissues and implants. As an important factor, crystallinity may affect the cell response. According to Shi et al. [15] crystallinity can modulate adsorption of adhesion ligands to the surface. In addition, Kim et al. [16,17] reported the production of poorly crystalline apatite thin film formed at low temperatures with similar crystallographic properties to that of natural bone. It seems that biomimetic approach might be a good way to prepare poorly crystalline coatings on the surface of metalic implants.

Hydroxyapatite is a naturally occurring mineral and the predominant mineral component of vertebrate bone and tooth enamel. Naturally-occurring bone mineral is made of nanometer sized and poorly-crystalline calcium phosphate with apatite structure [18]. The ideal stoichiometric crystalline hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, has the atomic Ca/P ratio 1.67 [19,20] but the composition of bone mineral is significantly different and can be represented by the following formula :

$$Ca_{8.3}(PO_4)_{4.3}(HPO_4,\ CO_3)_{1.7}(OH,\ CO_3)_{0.3} \quad (1)$$

Bone mineral is poorly-crystalline and non-stoichiometry due to the presence of divalent ions, such as CO_3^{2-} and HPO_4^{2-} which are substituted for the trivalent PO_4^{3-} ions. Substitution by CO_3^{2-} and HPO_4^{2-} ions produces a change of Ca/P ratios, resulting in the Ca/P ratios which may vary depending on the age and bone site [21]. It is worth to note that biomimetic approach has the ability to form such bone-like apatite. By this time, major factors which have been thought to have influence on cell behavior in case of calcium phosphate coatings were roughness, morphology, micro- and nano-structure, crystallinity, and chemical composition of the substrate [22–24]. However, the exact mechanism to explain the favorable effect of calcium phosphate coatings on bone response is still not clear. One of the features that were ignored in these studies was monitoring the cell and coating behavior during their interaction time which might help us to gain more knowledge about cell/calcium phosphate interface.

In this study, the surfaces of titanium implants were coated with thin films of poorly crystalline apatite through biomimetic approach. We monitored and analyzed the cell behavior consisting adhesion, proliferation and morphology during 15 days in comparison with uncoated implant samples. It is well-known that bone-related cells such as osteoblasts and bone marrow mesenchymal stem cells (BMSCs) play the most essential role in these biological mineralization processes [25,26]. Therefore, human mesenchymal stem cells (hMSCs) were chosen in this study. With our best knowledge hMSCs have never been examined in similar studies on poorly crystalline apatite coated titanium implants.

2. Materials and methods

2.1. Sample preparation

Commercially available Ti_6Al_4V titanium alloy (EZM Chiruline, Germany) (ASTM F136 and ISO 5832-3) samples were cut to appropriate sizes of 20 mm in diameter and 1 mm in thickness. The size of samples was checked with an electric digital caliper. Ti samples were initially polished with nos. 200–5000 grit silicon carbide (SiC) papers, and rinsed with acetone, ethanol and distilled water each for 10 min, respectively, and dried at 37 °C for 24 h.

2.2. Alkaline-treatment

The cleaned titanium samples were soaked into NaOH bath at the concentration of 5 M, and temperature of 60 °C in which they were treated for 1 day. Following the treatment, the samples were slowly washed with distilled water and dried at 40 °C in an electric oven overnight.

2.3. Heat-treatment

The Ti_6Al_4V alloy samples were alkaline treated with 5 M NaOH at 60 °C for 1 day, after which they were washed with distilled water and dried at 40 °C in an electric oven overnight. The samples were then heat-treated at 600 °C for 1 h in a Ni–Cr electrical furnace in air and then they were cooled down to ambient temperature in the furnace to avoid thermal shocks.

2.4. Preparation of SBF solution

The SBF solution was prepared by dissolving reagent-grade NaCl, KCl, $NaHCO_3$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and KH_2PO_4 into distilled water and buffered at a pH 7.25 with trishydroxymethyl aminomethane (TRIS) and 1 N HCl solution at 37 °C. Its composition is given in Table 1 and compared with the human blood plasma. Note that SBF is a solution highly supersaturated with respect to apatite [27–30].

2.5. Biomimetic apatite deposition

We carried out *in vitro* studies by soaking the samples in SBF solution at 37 °C for 14 days to investigate the formation of biomimetic apatite on the surface of samples. To keep the ionic concentrations constant, SBF solution was refreshed every 2 days. At regular intervals, the samples were taken out and rinsed with double distilled water and dried in an oven.

Table 1
Ion concentrations of simulated body fluid (SBF) and human blood plasma.

Ion	Plasma (mmol/l)	SBF (mmol/l)
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	103.0	147.8
HCO_3^-	27	4.2
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

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