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X-ray photoelectron spectroscopy study of the growth kinetics of biomimetically grown hydroxyapatite thin-film coatings

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ABSTRACT

Hydroxyapatite (HA) thin-film coatings grown biomimetically using simulated body fluid (SBF) are desirable for a range of applications such as improved fixation of fine- and complex-shaped orthopedic and dental implants, tissue engineering scaffolds and localized and sustained drug delivery. There is a dearth of knowledge on two key aspects of SBF-grown HA coatings: (i) the growth kinetics over short deposition periods, hours rather than weeks; and (ii) possible difference between the coatings deposited with and without periodic SBF replenishment. A study centred on these aspects is reported. X-ray photoelectron spectroscopy (XPS) has been used to study the growth kinetics of SBF-grown HA coatings for deposition periods ranging from 0.5 h to 21 days. The coatings were deposited with and without periodic replenishment of SBF. The XPS studies revealed that: (i) a continuous, stable HA coating fully covered the titanium substrate after a growth period of 13 h without SBF replenishment; (ii) thicker HA coatings about 1 μm in thickness resulted after a growth period of 21 days, both with and without SBF replenishment; and (iii) the Ca/P ratio at the surface of the HA coating was significantly lower than that in its bulk. No significant difference between HA grown with and without periodic replenishment of SBF was found. The coatings were determined to be carbonated, a characteristic desirable for improved implant fixation. The atomic force and scanning electron microscopies results suggested that heterogeneous nucleation and growth are the primary deposition mode for these coatings. Primary osteoblast cell studies demonstrated the biocompatibility of these coatings, i.e., osteoblast colony coverage of approximately 80%, similar to the control substrate (tissue culture polystyrene).

1. Introduction

Synthetic hydroxyapatite (HA) is a bioactive ceramic with a specific interfacial response that results in bone bonding [1]. HA is commonly used as an orthopedic implant coating material to stimulate bone growth around the implant, forming strong bone–HA–prosthesis interaction [2]. The shear strength of HA-coated titanium implants has been observed to be 5–8 times stronger and bone in-growth improved in comparison to non-HA-coated titanium implants [3–5]. HA coating also improves the implant stability due to improved osteoconductivity and bioactivity [3,6–11]. These improvements have been attributed to several mechanisms, including the greater ability of HA-coated implants to adsorb surrounding bioactive proteins compared to the surface oxides on metallic implants [2,12]. The adsorption of proteins onto the HA matrix may aid implant-bone integration, as both bone cell adhesion and cellular differentiation are promoted [12,13]. The continuous dissolution of the calcium-rich HA coating may provide ionic calcium saturation near the implant surface stimulating bone cells to synthesise the extracellular matrix [14] as well as reduce the action of bone resorbing cells (osteoclasts) the activity of which is inhibited by high local calcium concentrations [15,16].

High-temperature plasma spraying is the most common commercial method for coating bone implants with HA [6]. However, process-induced problems such as physical and chemical stability, solubility, phase separation and purity are generally associated with the plasma-sprayed HA coatings [17]. The line-of-sight nature of the plasma spray technique is a major limitation in coating fine- and complex-shaped implants such as orthopedic and craniofacial screws and external fixation pins [18]. Global efforts are underway to address these problems, and several alternative methods have been developed for depositing HA coatings, including sputtering, electrodeposition, spray pyrolysis and growth from biomimetic solutions such as simulated body fluid (SBF) [19–21]. These alternative techniques produce thin-film coatings (*ab initio* growth, by definition) of HA. In particular, the electrodeposition

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and biomimetic methods are non-line-of-sight in nature, employed at or near room temperature, and hence most suitable for coating complex-shaped implants. Since electrically conducting substrates is a pre-requisite for electrodeposition, this process is less versatile than the biomimetic method.

Compared to plasma-sprayed HA coatings (generally 50–100 μm in thickness), HA thin-film coatings a few μm thick are expected to exhibit superior physical (particularly adhesion) and chemical (including purity) properties and better process control. All these features make HA thin-film coatings highly desirable for orthopedic and dental implants. In tissue engineering, thin-film HA coatings grown onto the internal pore walls and struts of three-dimensional scaffolds are seen as a vehicle for localized delivery of drugs and/or biomolecules [22]. The presence of discrete, discontinuous HA depositions or nanostructured HA into the microconcavities of metallic orthopedic surfaces has been suggested as a possible approach for inducing bioactivity (bone bonding ability) in otherwise bioinert materials [23]. For drugs with high affinity for calcium phosphate materials, such as bisphosphonates, the degree of discreteness (discontinuity/coverage) of HA thin-film coatings can be used to control the amount of drug adsorbed and eventually delivered locally.

Kokubo and Takadama's SBF is the most used biomimetic solution for growing HA coatings and as a means for confirming the bioactivity of materials [21]. SBF is a synthetic, supersaturated calcium phosphate solution designed to have ion concentrations similar to human blood plasma [24,25]. At physiological conditions, HA is the most thermodynamically stable solid form of calcium phosphate [11]. HA generally forms on a substrate immersed in SBF at pH 7.4 resembling the *in vivo* conditions of the human body [21,25–27]. The well-defined surface characteristics (roughness and area) of SBF-grown HA coatings also make them an attractive choice as a vehicle for localized and/or sustained delivery of biomolecules and drugs to improve bone implant fixation. Owing to the biomimetic nature of the SBF method, the bioactivity of the loaded biomolecule/drug can be preserved. For example, we have recently published [28,29] the proof-of-concept of loading of a bisphosphonate drug (pamidronate) in its bioactive form both onto (by adsorption) and into (by co-precipitation) SBF-grown HA coatings. Such drug-loaded HA coatings with localized, sustained drug release capability are a topical area of research in fixation of bone implants, with general implications for drug delivery. The length of time required to grow a layer thick enough to adsorb biomolecules has been varied from hours to weeks, both with and without periodic replenishment of the SBF solution [10,30–33]. There has been speculation on whether there is an advantage in replenishing the SBF solution during the HA growth. For example, Stoch et al. [34] replenished the SBF solution every 4 days, whilst other investigators [31,33,35–37] chose to incubate for the length of the experiment without replenishing the SBF. The theory and practice of SBF-grown HA coatings onto a range of materials have been summarized in some recent publications [24,38,39].

In view of the above, it is clear that SBF-grown HA thin-film coatings are desirable for a range of applications such as improved fixation of fine- and complex-shaped orthopedic and dental implants, tissue engineering scaffolds and localized and sustained drug delivery. Since the work presented in this paper is primarily driven by our interest in developing drug (mainly bisphosphonates)-loaded HA coatings, some inclination towards this particular application may be noticed throughout this paper. The success of SBF-grown HA coatings in all these applications will primarily depend on their chemical purity, structure, micromorphology and overall thickness on a given substrate. Titanium is the substrate of choice for our research focus. A key aspect under-reported in the published literature is the growth kinetics of SBF-grown HA coatings over short deposition (incubation) periods, hours rather than weeks. Another unknown key aspect is the possible difference between the coatings deposited with and without periodic SBF replenishment. A study of these two aspects forms the core of this paper. We have employed X-ray photoelectron spectroscopy (XPS) to study the growth kinetics of SBF-grown HA coatings for incubation periods ranging 0.5 h to 21 days. Scanning electron microscopy (SEM), atomic force microscopy (AFM), photoacoustic infrared spectroscopy (PA-FTIR) and X-ray diffraction (XRD) are the other techniques that we have employed to complement the study. The biocompatibility of the resulting HA coatings was also tested using osteoblast cell cultures. The results presented and discussed have implications for: (i) inducing bioactivity in three-dimensional tissue engineering scaffolds [40]; (ii) near-molecular level understanding of biomineralisation [41]; and the emerging field of HA-based nanostructured organic–inorganic biomedical composite materials [42].

2. Experimental

Titanium sheets (0.25 mm thick, 99.7% Ti, Aldrich, Milwaukee, WI, USA) were cut into $5 \times 5\,mm^2$ squares and a small hole drilled on a corner of each square. The squares were cleaned by sonication in an ultrasonic cleaning bath (Soniclean Ultrasonic Cleaner, model 160T, South Australia) in both detergent and acidic solutions. The squares were sonicated in 5% Deacon 90® for 10 min before a 2 min sonication in Milli-Q water. The squares were then sonicated in 10% HNO_3 for 10 min before a final 2 min sonication in Milli-Q water. They were also sonicated in Milli-Q water between the detergent and acidic sonication steps to ensure that no additional contamination was added during these phases.

2.1. Protocol for preparing the simulated body fluid solution

SBF solution with inorganic ion concentrations simulating human blood plasma (Table 1) was prepared following the method of Kokubo et al. [25]. Reagent-grade chemicals (NaCl, $NaHCO_3$, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2$, Na_2SO_4) were dissolved in Milli-Q water. The solution was brought to physiological pH 7.4 using 1 M HCl. The SBF solution was kept at 4 °C overnight to ensure that no precipitation occurred prior to exposure to the titanium substrate. The replenishment SBF solutions used were made fresh each week.

2.2. Preparation of simulated body fluid-grown HA samples

To prepare the HA coatings, the cleaned titanium squares were suspended in the SBF solution using a nylon suture at 37 °C for periods up to 21 days. The squares were suspended vertically to ensure that only heterogeneous nucleation was responsible for the HA layer deposition rather than any precipitated HA depositing on samples (i.e., if the sample had been placed horizontally at the base of the solution). The squares were removed from the SBF solution at

Table 1
Concentration of ions in SBF as compared to that in human blood plasma.

Ion	Concentration (mM)	
	Human blood plasma	SBF
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-}	27.0	4.2
$HPO_4{}^{2-}$	1.0	1.0
$SO_4{}^{2-}$	0.5	0.5