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Magnetron co-sputtered silicon-containing hydroxyapatite thin films—an in vitro study

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Abstract

The use of silicon-substituted hydroxyapatite (Si-HA) as a biomaterial has been reported recently. In vivo testing has shown that Si-HA promotes early bonding of the bone/implant interface. In order to extend its usage to major load-bearing applications such as artificial hip replacement implants, it has been proposed that the material could be used in the form of a coating on implant surfaces. This paper reports a preliminary study of the biocompatibility of magnetron co-sputtered silicon-containing hydroxyapatite (Si-HA) coatings on a metallic substrate. Magnetron co-sputtered Si-HA films of thickness 600 nm with a Si content of approximately 0.8 wt% were produced on titanium substrates. X-ray diffraction analysis showed that the as-deposited Si-HA films were either amorphous or made up of very small crystals. The crystallinity of Si-HA films was increased after post-deposition heat treatment at 700 °C for 3 h, and the principal peaks were attributable to HA. The formation of nano-scale silicon–calcium phosphate precipitates was noted on the heat-treated films. In vitro cell culture has demonstrated that human osteoblast-like cells attached and grew well on all films, with the highest cell growth and signs of mineralisation observed on the heat-treated Si-HA films. In addition, many focal contacts were produced on the films and the cells had well-defined actin cytoskeletal organisation. This work shows that as-deposited and heat-treated Si-HA films have excellent bioactivity and are good candidates when rapid bone apposition is required. Furthermore, heat-treated Si-HA films have improved biostability compared to as-deposited films under physiological conditions.

Keywords: Magnetron co-sputtering; Silicon-substituted hydroxyapatite (Si-HA); Thin films/coatings; Human osteoblast-like (HOB) cells; Heat treatment

1. Introduction

Synthetic hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA] has been extensively used in a number of dental, orthopaedic and other medical applications, due to its similarity in chemical composition and crystal structure with bone mineral [1]. However, HA is brittle which limits its use in load-bearing applications such as artificial hip replacement implants. This limitation has stimulated research into coating HA onto metallic surfaces, to provide implants with sufficient mechanical stability that are also bioactive. Plasma spraying, a commercialised thick film deposition technique, is the most frequently used method for depositing HA coatings [2–5]. A histomorphometric study by Moroni et al., comparing the percentage of bone bonding to HA-coated and uncoated implants in dogs, revealed a significant increase in bone amount in the HA-coated implants [6]. They also showed enhancement of bone-to-pin osseointegration and interfacial strength in HA-coated pins as compared to uncoated pins in a sheep study [7]. Despite the beneficial effects of these plasma-sprayed coatings on the bone response, several studies have shown that these coatings tend to resorb, lose mechanical integrity or

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delaminate over time [8,9]. In order to enhance the mechanical strength of the underlying implants, whilst maintaining the bioactive property of the coatings, thin film deposition techniques have been investigated. Magnetron sputtering is a promising technique to provide thin coatings. Several authors have demonstrated that dense HA films of thickness between 0.1 and 10 μm can be fabricated by the magnetron sputtering technique [10–12]. An in vitro study by Hulshoff et al. using rat bone marrow cells showed that magnetron-sputtered calcium phosphate (CaP) coatings stimulated the formation of extracellular matrix (ECM) and apatite formation after 18 days of culture [13]. Heat-treated magnetron-sputtered CaP coatings were reported to induce carbonate apatite formation when implanted in rabbits [14]. An in vivo study in dogs by Ong and co-workers also demonstrated that magnetron-sputtered CaP-coated implants achieved equivalent or higher bond strengths and percentage bone contact at the bone–implant interface compared to uncoated implants or plasma-sprayed HA [15]. Furthermore, studies by Wolke et al. [16,17] using goat and rabbit models showed the formation of fibrillar collagenous matrix and carbonate apatite on thin CaP coatings produced by magnetron sputtering.

The mineral phase of bone is a multi-substituted calcium phosphate, including traces of carbonate (CO_3^{2-}), magnesium (Mg^{2+}), zinc (Zn^{2+}), silicon (Si^{4+}) and sodium (Na^{+}) [18]. There has been an increasing research interest on the effects of these various substitutions on the bioactivity of HA [19–24]. Silicon is known to be essential in the early stages of bone mineralisation and soft tissue development. Its importance has been demonstrated by two independent research groups [25–27]. Gibson et al. demonstrated that the incorporation of silicon into the HA lattice enhanced osteoblast cell activity and apatite formation in simulated body fluid (SBF), compared to phase pure HA [22]. An in vivo study by Patel et al. showed a significant increase in bone apposition at the surface of Si-HA implants, compared to phase pure HA when implanted in rabbits [23].

To take advantage of the magnetron sputtering technique and the benefits of Si-HA, a new approach to incorporate silicon into the HA has been developed. The application of the magnetron co-sputtering technique to deposit Si-HA coatings onto metallic surfaces offers a flexible approach. This technique of producing Si-HA is novel; and it has the advantages of being able to coat complex geometries, the ability to achieve dense, well-adhered thin coatings, and the possibility of the addition of controlled levels of silicon. In order to gain a complete insight into the biological performance of these co-sputtered Si-HA films, extensive in vitro and in vivo studies have to be performed. In this study, the results of a preliminary investigation using primary human osteoblast-like (HOB) cells are reported. The ability of Si-HA films to support the growth of cells was assessed by the alamarBlue™ proliferation assay. In addition, electron and confocal microscopies have been used to study the cell morphology, and the actin cytoskeleton and vinculin focal adhesions, respectively.

2. Materials and methods

2.1. Materials

Pure titanium (Ti) plates (Advent Research Materials Ltd., UK) of dimensions 10 mm × 5 mm × 0.5 mm were used as the substrates. As a pre-treatment procedure, the substrates were abraded sequentially using silicon carbide paper of grades 240; 600; 800; 1200. This procedure was followed by ultrasonic cleaning in acetone for 30 min. The substrates were then rinsed thoroughly with deionised water and dried.

Pure silicon (Si) plate (Goodfellow Cambridge Ltd., UK) and a phase pure sintered HA plate of dimensions 55 mm by 35 mm by 2 mm were used as the target materials. The fabrication of sintered HA target is reported elsewhere [28].

2.2. Magnetron co-sputtering process

Films were deposited in a custom-built sputter deposition chamber at room temperature. The chamber was evacuated to a base pressure lower than 10^{-7} Torr. High-purity argon (Ar) gas was then back-filled into the chamber, bringing the working pressure to 5×10^{-3} Torr. A constant flow of Ar was supplied into the chamber during the deposition process. Si and HA targets were held onto the two water-cooled magnetrons by means of spring clips. The Ti substrates were placed on a circular substrate support facing the targets. Prior to deposition, both targets were sputter-cleaned to remove any surface contaminants using a shutter to block the path between the targets and substrates. The film composition was controlled by the relative power supplied to each target (Si: direct current (dc); HA: radio frequency (rf)). A total sputtering duration of 4 h was used.

2.3. Post-deposition heat treatment of Si-HA thin films

The as-deposited films were heat treated in a tube furnace at 700 °C for 3 h. A constant flow of moist Ar gas was supplied during the heat treatment process by passing the Ar through a flask containing deionised water.

2.4. Characterisation of Si-HA thin films

2.4.1. Film thickness

The film thickness was determined by masking a portion of a Si substrate with a layer of aluminium foil