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Physicochemical and biochemical properties of iron-loaded silicon substituted hydroxyapatite (FeSiHAp)

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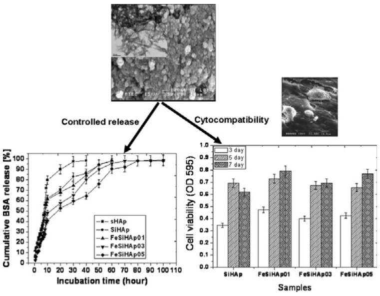
HIGHLIGHTS

- Scaffolding materials based on FeSiHAp powders exhibited the apatite structure.
- The surface charge became less negative for increasing iron loading levels.
- BSA adsorption on the FeSiHAp described by the Freundlich and Langmuir models.
- The release profile was slow from the FeSiHAp particles.
- *In vitro* study on the FeSiHAp scaffolding materials provided better cell growth on FeSiHAp01.

GRAPHICAL ABSTRACT



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ABSTRACT

We have synthesized scaffolding materials based on iron-loaded silicon substituted hydroxyapatite (SiHAp) (FeSiHAp). The compounds, $Ca_{10-x}(Fe_x)(PO_4)_5\text{-}(SiO_4)(OH)_{2-\delta}$ ($x = 0.1, 0.3, 0.5$), were synthesized using the stoichiometric amounts of the chemical constituents. Both physicochemical properties (structure, surface charge and hardness) and *in vitro* biochemical properties (Bovine serum albumin (BSA) adsorption and rat osteoblast-like UMR-106 cells response) of the products were measured. The synthesized powders still exhibited the apatite structure for iron contents up to $x = 0.5$. The surface charge became less negative, from −21.3 to −13.5 mV as the iron loading was increased. Compounds of FeSiHAp whisker showed a micro hardness in rang of 80–84 MPa for $x = 0.1$–0.5. BSA protein adsorption by FeSiHAp particles was able to fit to the energetically homogeneous behaviors in the Freundlich and Langmuir models. The release of BSA from the iron-loaded SiHAp particles was slow when compared to the release from the non iron loaded particles. Mineralization study showed the formation of apatite crystals on the FeSiHAp surface after modified simulated body fluid (m-SBF) incubation. *In vitro* cell growth on the FeSiHAp surface, the density of cells, the spread and production of calcium nodules on the substrate were seen to be higher for iron content $x = 0.1$ (FeSiHAp01). MTT tests on the FeSiHAp indicated that the UMR-106 cells were viable and there was a confluence of cells on the substrate.

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1. Introduction

The clinical demand for engineered bone tissue and orthopedic materials has been growing in recent years. Trauma and degenerative diseases due to daily activity and the aging of the population, respectively, are the main causes of bone injuries and fractures. Due to its compositional similarity to mineral bone, hydroxyapatite (HAp), $Ca_{10}(PO_4)_6(OH)_2$, has been investigated for its possible use to promote bone ingrowths when used in orthopedics [1–5] applications. However, synthetic HAp has a much lower osteogenic capacity than living bone tissue. After implantation, synthetic HAp may take days or even weeks before the mineralization of the new bone is completed. This is too long for the orthopedic and reconstructive surgery. This has lead to a growing interest in developing new generations of HAp based biomaterials, which could stimulate a faster rate of growth and lead to more a rapid healing than what occurs when the biomaterials currently available are used. Much effort has recently being devoted to developing HAp based biomaterials with tailored adhesion and adsorption properties by surface modification. For this purpose, different surface modification treatments of HAp ceramics, such as electrostatic charge storage within electrically polarized HAp [6–9], the addition of osteogenic factors (e.g., bone morphogenetic proteins) [10–12] or the use of biphasic calcium phosphate [13–15] have been employed to improve the osteoconductive properties of synthetic HAp.

Several studies have investigated the ion dopant cations such as Sr^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Na^+, K^+, Fe^{3+} or anions such as F^-, Cl^-, CO_3^{2-}, SiO_4^{2-} substituted into the HAp structure. They show that the structural stability and cellular biocompatibility properties [16–26] could be improved on. These studies were attempted to correlate the surface charge, the wetting ability, and the surface energy of polarized HAp in order to unveil the influence of these parameters on early stage mineralization and bone cell material interactions.

When the calcium in the apatite structure is replaced by a metal ion M, a vacancy (denoted by δ) may be formed at the site where the hydroxyl radical would normally be found, leading to the formation of $Ca_{10-x}(M_x)(PO_4)_6(OH)_{2-\delta}$ [17]. When the metalloid ions (X) are substituted at the phosphate group sites, $Ca_{10}(XO_4)_x(PO_4)_{6-x}$–$(OH)_{2-\delta}$ would be formed [17]. These substitutions would in turn result in changes in the chemical and physical properties of the modified hydroxyapatite. It has been observed that the crystallinity and surface charge of HAp change in a regular fashion when the degree of substitution is increased [16,19,22].

In present work, some of the calcium ions have been replaced by Fe^{3+} and some of the phosphates by the silicate groups (SiO_4^{2-}). Iron is an essential element in bones and teeth and has been shown to influence the crystallinity and solubility of apatite as well as controls the human metabolism [24–26]. Panseril S et al. [23] reported that the Fe-doped hydroxyapatite showed biocompatibility (osteoblasts test). Silicon is an important element that can influence bone formation and calcification [17,22]. Also, silicon has been reported to stimulate cellular activities, such as the proliferation and differentiation of osteoblast-like cell, and the mineralization of biological apatite [17,22].

The interaction of biomolecules with synthetic materials has received much interest in biomaterial fields. Once biomaterials are implanted, the immunology molecules and cells in the host tissue will interact with the surface of materials. These interactions can produce adverse effects and play a major role in determining the success or failure of the implantation (known as rejection). For this reason, one should look at the changes in the crystal properties such as the surface charge of the materials which occur when the iron-loaded silicon substituted hydroxyapatites interact with the host tissue. An understanding of the protein–material interactions should be sought.

We report here a method for synthesizing iron/silicon-substituted HAp, Ca_{10-x}–$(Fe_x)(PO_4)_5(SiO_4)(OH)_{2-\delta}$ ($x = 0.1$, 0.3 and 0.5) using the aqueous precipitation method. The structure, morphology, surface charge, micro hardness and chemical stability of these newly synthesized compounds were investigated. BSA was used as a model protein to study the adsorption and sustained release from the FeSiHAp particles. In addition, biocompatible *in vitro* m-SBF and bone cell response to SiHAp and FeSiHAp surface were also investigated.

2. Experimental part

2.1. Materials

The materials of iron (Fe)-loaded silicon (Si) substituted hydroxyapatite (HAp) were obtained by an aqueous precipitation method. The starting reagents, $Ca(NO_3)_2\cdot 4H_2O$, $(NH_4)_2HPO_4$, $Fe(NO_3)_3\cdot 9H_2O$, and $(NH_4)OH$, were obtained from UNIVAR (Australia). Tetraethoxysilane (TEOS, 99%, Sigma, USA) was chosen as the silica precursor. The concentration of the reactants was fixed at $(Ca + Fe)/(P + Si) = 1.67$. This leads to the formation of $Ca_{10-x}(Fe_x)(PO_4)_5(SiO_4)(OH)_{2-\delta}$, with $x = 0.1$, 0.3 and 0.5.

2.2. Preparation of ion iron (Fe)-loaded silicon (Si) substituted hydroxyapatite (SiHAp) (FeSiHAp)

For synthesizing the iron-loaded silicon substituted hydroxyapatite, the wet chemistry method was used. First, separated cation and anion solution was prepared. In the cation solution preparation, stoichiometric amounts of each dopant (Fe and Ca) were dissolved in 50 ml of de-ionized water. Solutions of $Ca(NO_3)_2\cdot 4H_2O$ and $Fe(NO_3)_3\cdot 9H_2O$ were then mixed together in a breaker under the action of a magnetic stirrer. Other mixtures of the anionic solutions (P and Si) were obtained by mixing solutions of $(NH_4)_2HPO_4$ containing appropriate amounts of P with solutions of TEOS containing appropriate amounts of Si. Both of the solutions were prepared by mixing the required amount of $(NH_4)_2HPO_4$ and TEOS in 50 ml of de-ionized water and in 50 ml of $(NH_4)OH$, respectively. The mixing of the two solutions was again done in a breaker using a magnetic stirrer. Next, the cation and anion solution were mixed together by slowly dropping the cation solution into the anion solution. The pH of the mixture was maintained at 9 by dropping $(NH_4)OH$ into the mixed solution. This was all done under constant stirring. After several minutes, formations of each concentration of iron-loaded silicon substituted hydroxyapatite began to co-precipitate. These then dropped to the bottom of the breaker (the iron-loaded SiHAp having $x = 0.1$, 0.3, and 0.5 are denoted as FeSiHAp01, FeSiHAp03 and FeSiHAp05, respectively). The powders were then removed from the supernatant solutions. The resulting powders were filtered and washed with de-ionized water until the pH was about 7. These were then freeze dried. Before the physico-chemical and biological measurements, samples of each powder were heated to 500 °C for 2 h in ambient air atmosphere. Pure synthesized hydroxyapatite and silicon-substituted hydroxyapatite were synthesized using the same method and are called sHAp and SiHAp, respectively.

2.3. Physico-chemical characterization of synthesis materials

The crystal structures of the powders were determined by powder X-ray diffraction (XRD) (Bruker diffractometer, Model D8 Advance) using the Cu K_α radiation and operating at 40 kV with 40 mA current. The XRD patterns were scanned from $2\theta = 20°–60°$ at a scanning speed of 1 s per step with an increment of 0.037° per step. For the FT-IR absorption measurements, the powders were