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Effects of Strontium Substitution on Bioactivity of Hydroxyapatite

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***Abstract*— Effect of strontium (Sr) substitution on bioactivity of hydroxyapatite (HA) was investigated. The substitution of Sr^{2+} for Ca^{2+} in the HA lattice increases biodegradability of Sr-substituted HA (Sr-HA) and accelerates the formation of apatite crystals on the Sr-HA surface. Moreover, the dissolution products from HA and Sr-HA samples are not cyto-toxic.**

Keywords-strontium-substituted HA; simulated body fluid (SBF); biodegradability; ion release.

I. INTRODUCTION

Strontium (Sr^{2+}) can be present in the mineral phase of bone and particularly at regions of high metabolic turnover. Accordingly, it plays a significant role in new bone formation. Stable strontium was found to show positive effects on bone metabolism. In animal studies, a low dose of strontium salts stimulate bone formation and decrease bone resorption, resulting in an increase of cancellous bone volume [1]. Strontium in the form strontium ranelate as a treatment for osteoporosis has been proven to increase the proliferation and activity of osteoblasts *in vitro*. While, in vivo, strontium ranelate has been found to increase the rate of bone formation and decrease bone resorption [2].

The growing evidence of the beneficial effect of strontium on bone justifies the increasing interest toward strontium incorporation in calcium phosphate bioceramics, especially hydroxyapatite (HA). In the HA lattice, strontium can replace calcium in the whole range of composition, inducing a linear variation of the lattice parameters. The substitution of Sr^{2+} for Ca^{2+} increased the solubility of HA [3]. Compared to stoichiometric HA, strontium-substituted HA (Sr-HA) was found to enhance alkaline phosphate activity, collagen type I production and osteocalcin presence [4]. The current study aims to investigate the effects of Strontium substitution on the bioactivity of HA including the apatite forming ability, biodegradability and cytotoxicity.

II.MATERIALS AND METHODS

Sr-HA powder containing 0, 5 and 10 mole% Sr of the total Ca content for a combined Sr+Ca/P ratio of 1.667, was produced at a temperature of 25 ºC based on a chemical precipitation method. Throughout the synthesis, the pH was kept above 10.0 by addition of NH_4OH at constant intervals. After synthesizing, the precipitate was then left to stand for 24 h. The supernatant was removed and replaced with fresh deionized water. This procedure was undertaken three times to remove any unwanted residue from the precipitation. The suspension was then filtered under vacuum. The filter cake was dried in an oven at 75ºC for 24 h.

HA and Sr-HA powder (<45µm) were compacted uniaxially in stainless steel mold at 200 MPa. The green samples were then heat treated at °C for 2 hours. To study the biodegradability, the sintered samples were exposed in 10ml of de-ionized water. pH measurement was recorded at1, 7 and 30 days. The ion release profile of each sample was measured using Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES) on a PerkinElmer Optima 3000DV (Perkin-Elmer, MA, USA). The apatite-forming ability was evaluated according to Kokubo [5]. Each of heat treated sample was immersed in a volume of Simulated body fluid for 1, 7 and 30 days. Topographical evolution of the sample surface were analysed using Scanning Electron Microscopy (SEM). MC3T3-E1 osteoblasts were used for cytoxicity study. Cells were seeded into 96 well plates at a density of 20,000 cells per well and incubated for 24 hours prior to testing. 10µl of liquid extract were added into wells containing MC-3T3-E1 Osteoblasts in culture medium and then incubated for 24 h at 37˚C/5% CO_2 . The MTT was added in an amount equal to 10% of the culture medium volume/well. The cultures were then re-incubated for a further 2 h (37˚C/5% CO2) after which, the cultures were removed from the incubator and the resultant formazan crystals were dissolved by adding an amount of MTT Solubilization Solution (10% Triton x-100 in Acidic Isopropanol (0.1 n HCl)) equal to the original culture medium volume. Once the crystals were fully dissolved, the absorbance was measured at a wavelength of 570 nm. Control media and healthy growing cell population (n=3) were used as a reference.

III. RESULTS AND DISCUSSION

Fig. 1 shows ion release profiles for HA and Sr-HA over the period of 1, 7 and 30 days. Regarding HA, Calcium (Ca) and phosphorus (P) ion release ranged from 5-7 and 3-4.5 mg/L, respectively. Ion release profiles for 5%Sr-HA are presented in fig. 1b. Release rate of Ca ranged from 6-13 mg/L, while P ion release ranged from 12.5-16 mg/L. In addition to Ca and P, strontium ions (Sr) were observed. The release rate of Sr ranged from 6-7.5 mg/L. Regarding 10%Sr-HA, Ca release rate ranged from 15-17 mg/L, while P and Sr presented incremental release which ranged from 22.5-40mg/L, and 18-30 mg/L, respectively. Moreover, it is

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observed that the ion release rate of Ca, P and Sr increased with Sr content, indicating the biodegradability of HA increases with Sr substitution. The increase in solubility with strontium content was due to a destabilization of the crystal structure by the larger strontium ion [3].

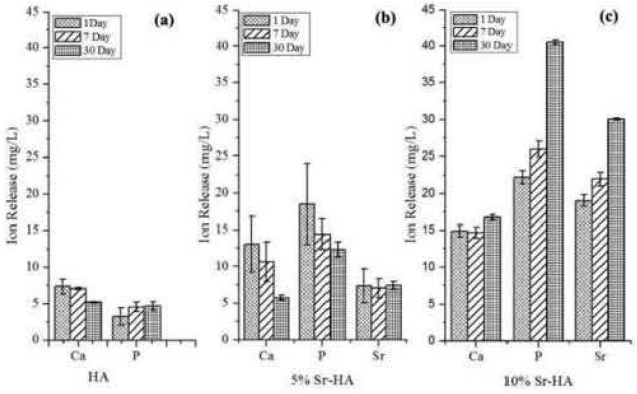

Figure 1. Ion Release profile for HA and Sr-HA.

Fig. 2 shows surface change after incubation in SBF. The surface morphology of HA, 5%Sr-HA and 10%Sr-HA remained apatite free until 7 days in SBF. After 7 and 30 days, a few crystals were found deposited on the surface of HA samples.

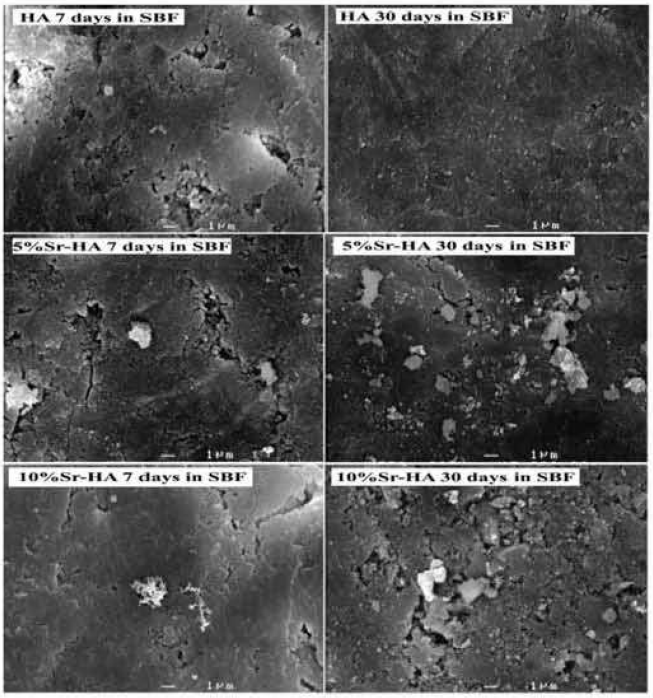

Figure 2 Change in the surface morphologies of HA and Sr-HA after incubation in simulated body fluid for 7 and 30 days.

Regarding, 5%Sr-HA and 10%Sr-HA, clusters of apatite crystals were deposited on the surface after 7 days in SBF and the amount of the clusters was found to increase with incubation time. This suggests that the Sr substitution accelerates the deposition of apatite crystals on the Sr-HA surface. Fig. 3 shows the cytocompatibility of each of the materials tested over 1, 7 and 30 days and were compared to a healthy growing population of osteoblast cells. Regarding HA samples, cell viability increased to 105% (1 and 7 days) and 113% (30 days), (p=0.26-0.36). Concerning 5%Sr-HA, cell viability increased to 113% (1day) and decreased to 107% (7 days) and 100% (30 days), (p=0.304-0.420), while 10%Sr-HA samples presented values of 92% (1 day), 100% (7days), and 97% (30 days), (p=0.102-0.156). Moreover, each materials tested, at each time period was not found to be significantly different from the control cell population, (p=0.42). This indicates the dissolution products of all materials are not cyto-toxic.

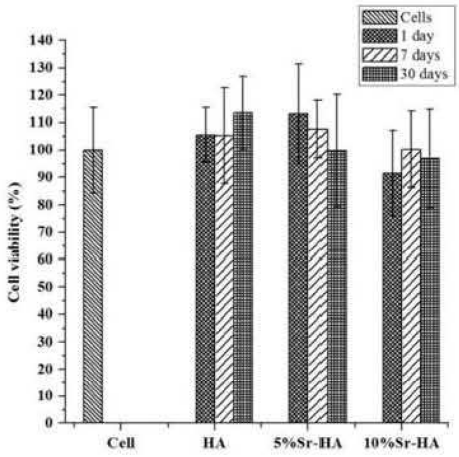

Figure 3. Cell viability analysis of HA and Sr-HA over 1, 7 and 30 days. Values are expressed as the means±standard deviation.

IV. CONCLUSIONS

Substitution of Sr^{2+} for Ca^{2+} in the HA lattice increases the solubility of HA and accelerates the deposition of apatite crystals on the HA surface, while remaining non-toxic to osteoblast cells.

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