Among different trace elements present in bone cells, strontium was found to exert beneficial effects on osteoblastic activity and prevent caries. At low concentrations, strontium administration was shown to reduce bone resorption and stimulate bone formation. Hence, its presence in calcium phosphates can lead to a better osteointegration (5). For this reason, the incorporation of Sr in calcium phosphate ceramics and cements has been the subject of much interest in the last decades (5-8). A partially strontium-substituted HA (6), $\alpha$-tricalcium phosphate (5), as well as biphasic ceramics (7) have been used as bone filler. In previous reports, strontium-substituted calcium phosphates have been prepared by precipitation in aqueous solution (9), hydrothermal process (10), drymechano synthesis (11), and by maturation of cement made of a mixture of calcium phosphates and an acidic solution (7). Earlier, we prepared pure and fluoride-doped HA by gel method using CaCl$_2$.2H$_2$O as precursor solution (12). Gels have proved to be very useful for the investigation of biomineralization process, because they are less rigid than the templating insoluble organic matrix for the morphology control of biominerals. Moreover, by selecting appropriate gelling medium and experimental conditions, they offer the possibility to mimic the growth of various
biominerals in environments similar to natural biomineralization (12). In this study, we report the synthesis of strontium-substituted BCP by gel method using anhydrous CaCl₂ as precursor solution and their mechanical properties. We have also performed in vitro studies to investigate the bioactivity of the strontium-doped BCP ceramics.

MATERIALS AND METHOD

The single diffusion gel method was employed to prepare the BCP using sodium metasilicate (SMS) gel. The gel was prepared by mixing SMS solution of specific gravity 1.03 g/cm³ and 0.6 M of disodium hydrogen phosphate (Na₂HPO₄) in the ratio 1:1. The pH of the solution was adjusted to 7.4 using 10% glacial acetic acid. About 10 mL of this solution was allowed to gel in a test tube over a period of 2 days. After the gelation, 1 M anhydrous CaCl₂ solution was poured on the gel without disturbing it. The experiments were carried out at room temperature for a period of three weeks. After the crystallization was complete, the samples were harvested, washed with double distilled water and dried at 60°C. For preparing BCP samples in presence of strontium (Sr), the supernatant solution was prepared by mixing 1 M of anhydrous CaCl₂ with 0.05 and 0.1 M of strontium chloride (SrCl₂·2H₂O) and the growth was carried out similar to that of pure BCP. We have collected disc-like BCP samples from the middle layers of each test tube and carried out systematic studies to understand the role of strontium on the growth and mechanical properties. In order to check the thermal stability of the BCP phase, both pure and Sr-doped samples were sintered for 5 hours at 1000°C in ambient atmosphere.

A powder X-ray diffraction pattern was recorded on a Bruker AXS D8 advanced diffractometer (Madison, WI, USA) within the 2θ range of 10° to 80° using CuKα as X-ray source (λ=1.5406Å). Surface features and compositional analyses were done by using a scanning electron microscope (SEM) attached to an INCA energy dispersive X-ray spectrometer (Oxford Instruments, Tubney Woods, Abingdon, Oxfordshire, UK). Thermogravimetry of samples was analyzed using the SDT Q600 V8.3 (TA Instruments, Bangalore, India) in the temperature range of 30°-1200°C at the heating rate of 20°C/minute in nitrogen atmosphere.

In vitro bioactivity test

To test the in vitro bioactivity, the samples were immersed in simulated body fluid (SBF). The SBF was prepared according to the procedure described by Kokubo (13). The ion concentration and pH of the SBF solution were nearly equal to that of human blood plasma. The SBF solution was prepared by mixing appropriate quantities of chemicals in the order NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂ and Na₂SO₄ into deionized water and buffering at pH 7.4 with trihydroxymethyl aminomethane and 1.0 M hydrochloric acid at 37°C to mimic the biological conditions. Samples were pressed into pellets of about 8 mm diameter and 1 mm thickness and the pellet was immersed in 15 mL of SBF solution in an airtight plastic container. The solution was renewed every 48 hours for a period of three weeks. After three weeks the samples were taken out and washed with deionized water and dried at room temperature. The morphological variation of the discs was studied using SEM.

Nanoindentation studies

To characterize the mechanical response, prepared samples were die-pressed and sintered at 600°C for 5 hours in ambient atmosphere. The indentation impressions were made by nano-based indentation system NHTXS/S: 50-0172 with a diamond Berkovich tip indenter. The force used for all the samples was 100 mN with a loading and unloading rate of 20 mN/min. A minimum of 10 indents were made on each sample. The distance between two separate indents was more than 20 μm to avoid the influence of residual stresses from adjacent impressions. From the load-depth penetration curves, the shape of the unloading curve can be used as a means of obtaining the elastic properties of the sample. The slope, S is the initial unloading stiffness, and reduced modulus Eᵣ is determined by a method that has been developed and described by Oliver and Pharr (14).

\[
E_r = \frac{A}{2} \sqrt{\frac{S}{A}}
\]

where A is the projected area of contact. The elastic modulus of the sample Eᵣ can be calculated using the relation

\[
\frac{1}{E_r} = \frac{1}{E_i} - \frac{(1-\nu_i^2)}{E_i} \frac{1}{E_s} + \frac{(1-\nu_i^2)}{E_i}
\]

where Eᵢ is elastic modulus of the indenter tip, νᵢ and νs are the Poisson’s ratio of the sample and indenter tip respectively. The hardness (H) which is defined as the mean pressure that the material will support under load is determined by the following relation

\[
H = \frac{P_{\text{max}}}{A}
\]

where P₀ is the maximum applied load.

RESULTS AND DISCUSSION

Synthesis of BCP

BCP ceramics was successfully synthesized by gel method using anhydrous CaCl₂ as the supernatant solution.
The substitution of Sr at the Ca site causes a crystal lattice expansion due to the larger atomic radius of Sr. In all three experiments, dicalcium phosphate dihydrate (DCPD, CaHPO$_4$.2H$_2$O) crystals grew between the Liesegang rings. Pure DCPD crystals have platelet morphology. The presence of Sr in the growth environment leads to crystals with irregular shapes and a bunch of crystals grew like a cluster in the midst of BCP rings, as can be seen in Figure 1.

X-ray powder diffraction (XRD) analyses

The as-prepared, pure samples and the Sr-doped BCP samples were identified as the mixture of HA and β-TCP from XRD (Figs. 2a and b) which are in good agreement with the JCPDS standards (09-0432 for HA and 09-0169 for β–TCP). In the case of Sr-doped BCP, the XRD peaks were found to be broader and less intense when compared to undoped samples. This indicates the reduced crystallinity and size of the BCP crystallites due to partial substitution of Ca by Sr (8, 15).

The substitution of large Sr$^{2+}$ (1.13 Å) into small Ca$^{2+}$ (1.00 Å) leads to denser atomic packing of the system, causing retardation of crystal growth. When the crystal has less freedom to grow, the formation of β-TCP will be blocked (16). This proposal is in good agreement with the XRD observation, which shows that the β-TCP phase minimizes while the peaks corresponding to HA become less intense at 0.1 M Sr addition.

The addition of CaCl$_2$ on the top of the gel in the test tube led to the formation of a white precipitate at the gel solution interface. Just below this, a circular white disc was observed after one day. Subsequently, 12 discs of about 3 mm thickness each grew in the gel medium over a period of three weeks. The space between successive white discs was found to increase towards the bottom of the test tube from the interface due to the lack of diffusion of Ca from top of the gel down to the bottom of the tube. These white-colored circular discs, commonly known as Liesegang rings, were identified as BCP (Fig. 1a). Earlier we prepared pure hydroxyapatite using CaCl$_2$.2H$_2$O (pH value 4.96) as precursor solution (12). The higher pH value (8.03) of anhydrous CaCl$_2$ seems to have promoted the formation of the β-TCP in addition to HA.

Strontium-doped BCP was synthesized by adopting a similar procedure. The pH value of the precursor solution prepared by mixing 0.1M SrCl$_2$.H$_2$O and anhydrous CaCl$_2$ was found to be 7.59. Here, white-colored discs appeared only after three days, which indicate that the initial nucleation and subsequent growth got reduced due to the presence of Sr in the supernatant solution (Fig. 1b). Consequently, only seven discs of about 2 mm thickness grew in the gel medium even after three weeks. In the case of 0.05 M SrCl$_2$.H$_2$O concentration, five discs of about 2 mm thickness were observed (Fig. 1c). The BCP samples from the middle layers of each test tube were chosen for further studies. Christofferson et al (8) reported significantly reduced HA formation in the presence of Sr and that the substitution of Sr at the Ca site causes a crystal lattice expansion due to the larger atomic radius of Sr.

In all three experiments, dicalcium phosphate dihydrate (DCPD, CaHPO$_4$.2H$_2$O) crystals grew between the Liesegang rings. Pure DCPD crystals have platelet morphology. The presence of Sr in the growth environment leads to crystals with irregular shapes and a bunch of crystals grew like a cluster in the midst of BCP rings, as can be seen in Figure 1.
Influence of Sr on the synthesis and surface properties of BCP

Further, peaks in the XRD pattern of the Sr-doped sample were slightly and continuously shifted to lower diffraction angles (15, 17). This is characteristic of an increase in the lattice parameters as shown in Table I. In both cases, high temperature annealing (1000°C) leads to transformation of BCP into β-TCP with a high degree of crystallinity (Figs. 3a and b).

SEM micrographs reveal a significant difference in morphology between the pure and Sr-doped BCP sample. The pure BCP consists of fibers with an anisotropic aspect approximately 6-10 μm in length and 0.5-1.0 μm in width (Figs. 4a and b), while the Sr-doped BCP consists of an aggregate of the fibers and formed platy surface (Figs. 5a and b) with reduced mean crystal size (18).

The presence of Sr changed the growth pattern and morphology significantly. EDX spectra showed incorporation of Sr²⁺ into the BCP ceramics (Figs. 6a and b). In vitro bioactivity tests of the Sr-doped sample revealed the formation of an apatite layer on the surface of the pellets. The newly formed apatite layer consisted of tiny spherical mineral particles and many of these particles formed agglomerates (Fig. 7). This is in accordance with the fact that bioactive material will form a layer of apatite between bones and implant which helps in bonding the implant to bone (13).

**Thermogravimetry (TG) analyses**

TG curves of the as-prepared samples are shown in Fig. 8. In both the samples, initial weight loss up to approximately 450°C was due to the loss of adsorbed water (up to 200°C) and lattice water (up to 450°C) (29). Above 450°C, weight loss occurred in several steps.
between 450°C and 900°C, indicating the transformation of BCP into the β-TCP phase (20). The total weight loss of pure and strontium-doped samples was found to be 10.02% and 19.23% respectively. Higher weight loss in the Sr-doped sample indicates that the BCP matrix contains more HA than the TCP.

**Nanoindentation test**

The mechanical behavior of the BCP samples was characterized in terms of hardness and elastic modulus obtained by nanoindentation. Figure 9 shows the typical load-displacement (p-h) curves obtained for pure and Sr-doped samples. The p-h curve of pure and 0.05 M Sr-doped BCP samples is associated with the displacement bursts or pop-ins, as indicated by arrow marks in Figure 9. Similar pop-ins observed in the p-h curves were reported to be associated with pile-ups around the indent mark for HA and β-TCP single crystals (21, 22). The differences in hardness and modulus of the three investigated samples are apparent from the differences in the penetration depth (h) attained at a particular load (23). The mean value of elastic modulus (E) and hardness values (H) of pure and Sr-doped BCP calculated from the p-h curve are shown in Table II. The observed decrease in E and H values for the Sr-doped BCP ceramics could be attributed to the induced morphological change due to Sr doping. However, all the p-h curves show an elastic recovery after the removal of the applied load.

**CONCLUSIONS**

Strontium-substituted calcium phosphate ceramics have potential for use in orthopedic surgeries. In the present study, gel method was used to introduce strontium into BCP ceramics. Formation of mixed HA/β-TCP phases was confirmed by powder X-ray diffraction analysis. The SEM micrographs showed that the microstructural morphology of HA/β-TCP changes from fibrous in pure samples to platy surface in Sr-doped BCP. Powder XRD and EDX analyses.
Influence of Sr on the synthesis and surface properties of BCP

confirmed Sr doping into the BCP phase. Nanoindentation studies revealed that the important mechanical properties such as hardness (H) and elastic modulus (E) of BCP decrease due to Sr doping. The in vitro bioactivity study showed that the strontium-doped sample could induce apatite formation on the surface after three weeks of soaking in SBF.

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