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# Preparation and properties of plasma sprayed strontium-doped calcium polyphosphate coating for bone tissue engineering

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**Abstract**

The purpose of this study was to scale-up the usage of strontium-doped calcium polyphosphate (SCPP) to major load-bearing applications in bone substitute application. SCPP powders were used to prepare biological coatings on titanium (Ti) substrates by plasma-spraying under a series of different conditions. Microstructure and bonding strength (with titanium substrates) of the SCPP coatings were investigated by an inductively coupled plasma-mass spectrometer, X-ray diffraction analysis, a scanning electron microscope and a strength test machine. The results showed the main crystal type of coating remained β-crystal SCPP. The surface and cross-sectional morphologies of coatings showed that the SCPP coatings were fairly dense. The bonding strength between the SCPP and Ti was higher than normal plasma spraying coatings. Furthermore, the research on the interactions between ROS17/2.5 cells and various SCPP coatings-Ti substrates indicated that the coating-implants possessed good cytocompatibility with osteoblasts. Therefore, SCPP is considered to be a biological coating material with good prospect in clinical application.


*Key words:* Strontium-doped calcium polyphosphate; Plasma-spraying; Coating; Biocompatibility

## 1. Introduction

With population aging and prolonged life expectancy, bioactive porous calcium orthophosphate ceramics that could promote bone regeneration have attracted particular attention in the past few decades [1]. As a kind of calcium orthophosphate, calcium polyphosphate (CPP) is widely investigated due to its good biodegradable property, biocompatibility and mechanical strength [2,3]. On the other hand, the role of strontium (Sr) in disease therapy has been one hotspot-research since the development of the drug strontium ranelate, which has been proved to reduce the incidence of fractures in osteoporotic patients [4]. In recent years, there is growing evidence that strontium has a beneficial effect on bone formation in the field of pharmacology. Based on the chemical resemblances of $Ca^{2+}$ and $Sr^{2+}$, partial $Ca^{2+}$ in CPP could be replaced by $Sr^{2+}$ to develop Sr-doped CPP (SCPP) scaffold to obtain the advantages of both of the aforementioned materials. The structural changes, degradable behaviors, mechanical and biological properties of SCPP scaffold were undertaken in the systematic research [5–10]. The studies showed that SCPP scaffold could not only promote the physics-chemical properties of CPP but also would be a potential biomaterial with bioactivity to induce angiogenesis for bone tissue engineering. However, the clinical usage of SCPP scaffold is limited due to its poor mechanical property.

Titanium and its alloys have been used in medicine and dentistry for decades due to their outstanding mechanical property and corrosion resistance. However, a variety of biological coatings such as HA coating are developed as a surface modification technique to improve osteoconductivity of titanium (Ti) implants for clinic application. Some researches indicated that the biological fixation of Ti to bone tissue was proved faster with a calcium orthophosphate coating than without, which seemed rational to imply that not only the bone repairing process around the titanium implants was enhanced by the formation of biological apatite layer but also the mechanical property of biological apatite was enhanced by preparing as coatings.

Compared with HA, SCPP is in possession of some unique advantages (mentioned above) suitable for biological coatings. Therefore, in order to expand the usage of SCPP in bone

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substitute application, we focus our interest on SCPP as a coating for applications in which its good mechanical properties and bioactivity are exploited. Plasma spraying has been widely utilized as one method for surface modification of materials with a biological coating. Plasma treatment can be directly or indirectly applied for introducing the functional groups on inert surface. In this study, SCPP coatings on Ti alloy substrates were prepared by plasma spraying. The microstructure and mechanical properties of the coatings-substrates were characterized. The effects of the coatings-substrates on osteoblasts were also evaluated.

## 2. Materials and methods

### 2.1. Preparation of materials

SCPP powders were prepared according to the previous study [5]. Briefly, the $CaCO_3$ powders were mixed with $SrCO_3$ to prepare SCPP with the molar ratio of Ca/Sr = 98/2. The mixture was added slowly into the 85% phosphoric acid with stirring to prepare $Ca_{0.98}Sr_{0.02}(H_2PO_4)_2 \cdot H_2O$ based on the following reaction:

$$0.98CaCO_3 + 0.02SrCO_3 + 2H_3PO_4 \rightarrow Ca_{0.98}Sr_{0.02}(H_2PO_4)_2 \cdot H_2O + CO_2$$

After the reaction went on at room temperature overnight, the solutions were evaporated in vacuum. The precipitates were washed by 95% ethanol until the pH of filtrate was about 7. These precipitates were sintered to form amorphous SCPP glass according to the following reactions:

$$Ca_{0.98}Sr_{0.02}(H_2PO_4)_2 \cdot H_2O \rightarrow Ca_{0.98}Sr_{0.02}(H_2PO_4)_2 + H_2O$$

$$Ca_{0.98}Sr_{0.02}(H_2PO_4)_2 \rightarrow Ca_{0.98}Sr_{0.02}H_2P_2O_7 + H_2O$$

$$nCa_{0.98}Sr_{0.02}H_2P_2O_7 \rightarrow nCa_{0.98}Sr_{0.02}(PO_3)_2 + nH_2O$$

The amorphous SCPP was sintered to form β-SCPP and then was crushed by an agate motor, the powders with a size range smaller than 100 μm were collected to fabricate coatings.

The Ti surface was gritblasted before plasma spraying with pressurized air accelerated corundum milling to make the roughness of Ti surface to about 5 μm. With the aid of an AR2000 atmospheric plasma spraying equipment (Sulzer Metco, Switzerland), SCPP powders were sprayed on Ti alloy substrate (spraying parameters is shown in Table 1), and then the coatings-substrates went under annealing at 700 °C for 4 h.

### 2.2. Characterization of SCPP coatings

The phase composition of SCPP coatings prepared by plasma spraying was analyzed by an X-ray diffraction instrument (X'Pert Pro MPD, Philips, Netherlands). Meanwhile, the surface microstructure of SCPP coatings was observed by a scanning electron microscopy (SEM) (JSM-5900LV, bought from Japan JEOL company), and the bonding strength between Ti alloy substrates and SCPP coatings was measured in accordance with the United States ASTM C633-2001 coating material strength test method [11].

Table 1
The parameters of different plasma spraying conditions.

| | Current (A) | Voltage (V) | Arcing gas | Dusty gas | Powder feed rate ($g\ min^{-1}$) | Spraying distance (cm) |
|---|---|---|---|---|---|---|
| **Coating I** | 400 | 62 | $N_2$ | $N_2$ | 30 | 12 |
| **Coating II** | 400 | 62 | $N_2$ | $N_2$ | 25 | 10 |
| **Coating III** | 450 | 66 | $N_2$ | $N_2$ | 25 | 12 |
| **Coating IV** | 450 | 66 | $N_2$ | $N_2$ | 25 | 10 |

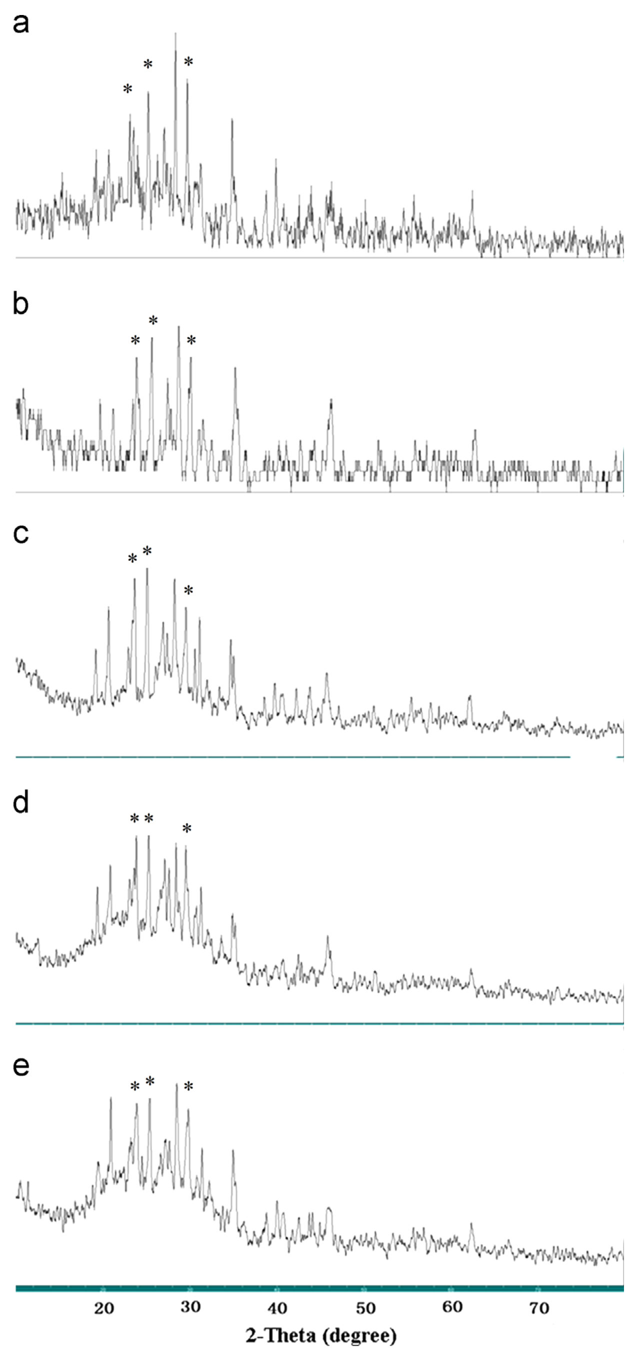


Fig. 1. XRD patterns of SCPP powders and coatings obtained under different conditions. (a) SCPP powders; (b) coating I; (c) coating II; (d) coating III; and (e) coating IV.

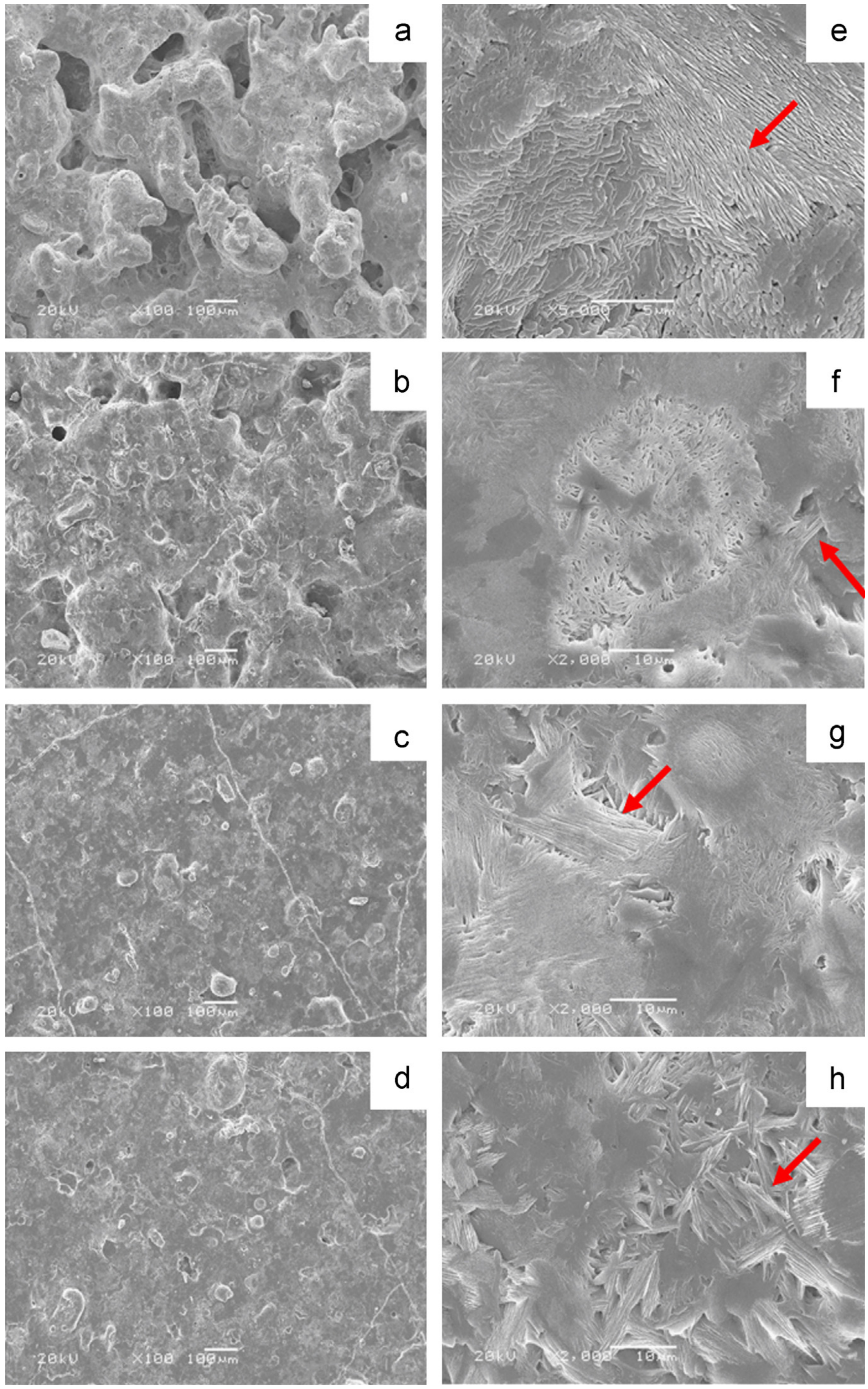


Fig. 2. The surface morphology of coatings obtained under different conditions. (a) and (e) coating I; (b) and (f) coating II; (c) and (g) coating III; and (d) and (h) coating IV; (a), (b), (c), and (d) $\times 100$; and (e), (f), (g), and (h) $\times 5000$.

### 2.3. *Cytocompatibility of the coatings-substrates*

Cell proliferation on samples (coatings-substrates and pure Ti) was analyzed to evaluate the cytocompatibility of all samples by MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) [12]. All samples were gamma irradiation-sterilized for 120 min. The immortalized osteoblastic ROS17/2.8 cell line was utilized in this study, which was purchased from West China Hospital, Sichuan University (China). ROS17/2.8 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) in humidified atmosphere of 5% $CO_2$ at 37 °C (Japan's Sanyo company MCO-18AIC). The cells were cultured to the third generation for further experiment. Each sample was dispensed into 24 well plates and then ROS17/2.8 cells were seeded into each well with 0.5 ml at a density of $5 \times 10^4$ cells/ml. The samples were incubated for

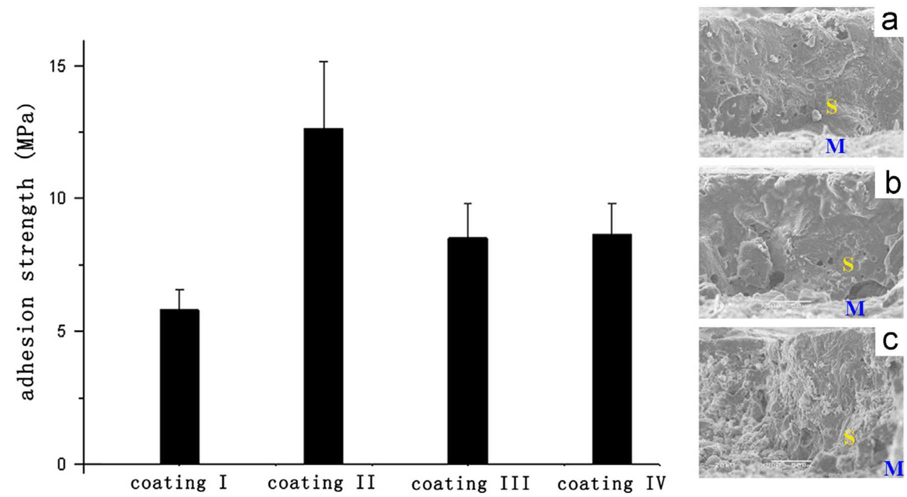


Fig. 3. Adhesion strength (left) and cross-section morphologies (right) between Ti substrates and coatings obtained under different conditions (a) coating II; (b) coating III; and (c) coating IV; S: SCPP and M: Ti matrix.

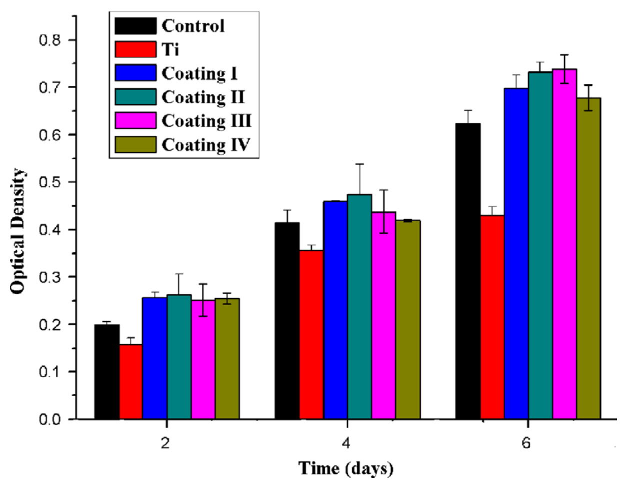


Fig. 4. OD values of ROS17/2.5 cells cultured with SCPP coating and Ti plates in different time.

different periods (2, 4 and 6 days). 50 μl/well of MTT solution (5 mg/ml in phosphate buffered saline, PBS) were added into the plate. The plate was incubated at 37 °C for another 4 h. After that, MTT solution was removed and replaced by 375 μl DMSO, and the plates were shaken for 10 min. The optical density (OD) of each well was measured by a Microplate Reader (Model550, Bio Rad Corporation) at the wavelength of 490 nm to evaluate the cell numbers which were proportional to the OD value. All measurements were performed in triplicate, and at least three independent experiments were carried out.

## 3. Results and discussion

Fig. 1 shows the XRD analysis of SCPP powders and SCPP coatings sintered under different conditions. With regard to the coatings, the typical diffraction peaks corresponding well to that of the SCPP powders appeared in the XRD spectrogram of the coatings. The curves obtained from SCPP coatings in this study were validated in agreement with the curves of β-CPP ($Ca(PO_3)_2$, JCPDS 77-1953) from the XRD pattern, especially three high characteristic peaks all appeared between 20° and 30°. It demonstrated that the main crystal type of the coatings was β-SCPP. The SCPP in coatings could retain its stable property and the intrinsic structure after high temperature plasma treatment because the treatment neither affected the crystallized phase of SCPP structure nor made the coatings decomposed. The surface morphology of coatings prepared according to different parameters was observed by a scanning electron microscope (SEM). As can be seen in Fig. 2, for all the SCPP coatings, there were some folds and aggregates on the surface of Ti, which indicated the formation of the dense coating layers on the surface of Ti. Coating I showed the toughest surface and there were many discontinuous pores in it. Furthermore, a high magnification scanning electron microscope was used to analyze the surface morphology of different SCPP coatings. And all SCPP coatings presented some cracks in different degrees due to stress. The phenomenon might be resulted from heat-expansion and cold-contraction of the coatings when they were cooled in the process of sintering.

By comparing the different SCPP coatings, it could be found that only in coating I did SCPP presents lamella structures, while the other coatings presented well distributed and tightly combined structures. The tightly combined structures are of great importance to the interface combination intensity between the coatings and the substrates. Therefore, the SCPP coatings prepared by the plasma-spraying technique have great prospect in the major load-bearing applications for bone substitute application.

The test results of interface adhesion strength and the SEM images of cross-section morphology between SCPP coatings prepared according to different parameters and Ti substrates are shown in Fig. 3. According to the data obtained, the interface adhesion strength of coating II was 12.64 Mpa, which was the highest one and higher than the normal interface adhesion strength of 10 Mpa [13]. And coating I had the minimum interface adhesion strength due to a fairly large porosity of the coatings. And the large porosity resulted in the decreasing of the interface combination intensity between the SCPP coating and the Ti substrate. This was in agreement with the SEM images of cross-section morphologies (coating I was damaged in the process of preparing test sample, thus, it is not given in this paper). For other three coating samples, there were some discontinuous pores in the coatings and the grain boundary was not obvious. At the same time, the coatings did not separate from the Ti substrates, which indicated that SCPP had good bonding with Ti substrates. Normally, during the high temperature annealing treatment, Ti would react with oxygen in the interspace between SCPP and Ti due to the weak oxidation resistance of metal Ti. [14]. However, there was no obvious oxide layer between SCPP and Ti. The reason may be that the melted coating infiltrated the surface of Ti under high-temperature plasma flame to combine the SCPP coating and Ti substrates. Moreover, it is high temperature that makes tetrad Ti ion react with oxygen ion to form a mesh structure of $[TiO_4]^-$and then adheres the SCPP coating to Ti. So SCPP powders are suitable for surface modification of Ti. Results of this study demonstrated that the SCPP coatings could adhere to Ti substrates closely and the prepared coatings-substrates were valuable for some special applications.

MTT assay is an important method to evaluate the cytocompatibility of the extractions of coatings-substrates. Proliferation of ROS17/2.5 cells cultured on various SCPP coatings-Ti substrates and the pure Ti substrate was determined by MTT assay (Fig. 4). The OD values revealed that the proliferation rate of ROS17/2.5 cells cultured on all samples increased with increasing the culture duration. It could also be seen that the OD values for the ROS17/2.5 cells cultured on various SCPP coatings-Ti substrates were higher than that of the pure Ti group and the blank control group ($p < 0.05$). It indicated that SCPP coatings exhibited the ability to enhance osteoblast cell proliferation. Therefore, SCPP coatings can improve the cytocompatibility of the metal implant. According to the results, we can get the conclusion that SCPP coatings have good bonding strength with Ti substrates and the SCPP coatings-Ti substrates have positive effects on the attachment and proliferation of ROS17/2.5 cells.

## 4. Conclusion

In this study, a novel bioceramic material (SCPP) was utilized as raw material to prepare a biological coating by plasma spraying under proper conditions. This study indicated SCPP had strong bonding strength with Ti substrate and the SCPP coatings-Ti substrates possessed good cytocompatibility. This SCPP coating is considered to be a biological coating with good prospect in clinical application. Accordingly, it is very attractive to construct the coating-substrate that incorporates SCPP into coating to improve properties of coating-substrate for special applications.

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