Preliminary study of calcium phosphate immobilized with Chinese medicine

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It is well known that calcium phosphate-based biomaterials possess excellent biocompatibility [1, 2]. However, they tend to have low osteogenic potential due to lack of osteoinductive growth factors. It often has low growth speed and limited growth depth of the bone, poor osseointegration, even loosening and functional failure of the implant [3]. It is difficult to solve massive bone defects and fractures under disease conditions completely by conventional Ca/P biomaterials. Therefore, it is necessary to develop bone grafts with osteoinductability to promote the osseointegration. There are many papers dealing with developing osteoinductive bone grafts by incorporating osteogenic cell and/or bone growth factors into Ca/P-based biomaterials. It has been demonstrated that they are potential drug delivery candidates for living cells, various growth factors and hormones [4–6].

In spite of the fact that bony defects need to be filled and reconstructed, it is still possible that they need some drugs to assist cure. Since Ca/P biomaterials are placed in the wound site, it is easy to fulfill the locally controlled release of drugs. Additionally, Ca/P carrier materials could release calcium and phosphorus ions, which are beneficial for bone repairing and reconstructing. It has been shown that Ca/P-based biomaterials are good drug delivery systems for antibiotics, polypeptides and anti-inflammatory drugs, anti-cancer drugs, aspirin and indomethacin [7–9].

Many Chinese medicines which can induce osteogenesis and greater uniformity of bone at the fracture [10], have been used to treat bone fractures in Chinese medical science. Among these, Miltiorrhiza Bunge (SMB), also so-called danshen, and Rhizoma Chuanxiong (RC) have been investigated as very effective herbs with the stimulating effect on osteogenesis [11–14]. In the present study, the Chinese medicine, SMB and RC, will be immobilized onto Ca/P powder, respectively, to develop the novel bone grafts. The characteristics of Ca/P powders immobilized with Chinese medicine are characterized.

Ca/P slurry was synthesized by a wet chemical method according to following equation [15].

$$5Ca(NO_3)_2 + 3(NH_4)_2HPO_4 + 4NH_4OH = Ca_5(OH)(PO_4)_3 + 10NH_4NO_3 + 3H_2O$$

The calcium solution was vigorously stirred at ambient and the phosphate solution was added slowly to produce the milky sediment. The pH of reactive solution was adjusted to more than 11 with NH_4OH. The sediment was aged for more than 24 hr and washed with deionized water thoroughly until no NH_4^+ remained in it.

The Ca/P sediment was stirred to get homogeneous slurry. One fourth was taken out and marked as Ca/P. The remainder was added to a suitable amount of coupling agent, polyacrylic acid (PAA), and stirred for 0.5 hr. The modified solution was divided into three parts equally. Chinese herbal parent solutions of SMB and RC were dropped into two parts of the modified solutions and stirred overnight, respectively. These samples were coded as Ca/P-PAA, Ca/P-PAA-SMB and Ca/P-PAA-RC. Subsequently, the stirred and mixed solutions were fed to the nozzle of a spray dryer (Buchi B-290, Switzerland) to prepare various Ca/P powders.

Thermo-gravimetric (TG) analysis was carried out on a Rigaku TA. The scanning temperature was from ambient up to 780 °C with a heating rate of 20 °C/min. The TG curves of various Ca/P powders showed that the weight loss started from 55 °C to near 480 °C for Ca/P, from 55 to 675 °C for Ca/P-PAA, and from 25 to 700 °C for Ca/P-PAA-RC and Ca/P-PAA-SMB. There were multi-stages of weight loss in these TG curves during heating, which indicated the evaporation of absorbed water, the loss of lattice water, and the decomposition of PAA and Chinese medicine. The weight loss of various Ca/P powders were increasing in the following series: Ca/P, Ca/P-PAA, Ca/P-PAA-RC and

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TABLE I The weight loss and mean particle size of Ca/P powders

Sample	Weight loss (%)	Mean particle size (μm)
Ca/P	15	11.922
Ca/P-PAA	20	12.166
Ca/P-PAA-RC	25	12.103
Ca/P-PAA-SMB	36	12.223

Ca/P-PAA-SMB (Table I). TG results confirmed that there were 5 wt.% of PAA, 5 wt.% of RC and 16 wt.% of SMB immobilized to Ca/P powders, respectively.

The particle size of various Ca/P powders was determined with Malven Mastersizer 2000 laser diffraction particle size analyzer. The results showed that SMB and RC have a slight effect on the particle size of Ca/P powders (Table I).

X-ray diffraction (XRD) was carried out on a Siemens D500/501 diffractometer using Cu K_α (Fig. 1). XRD confirmed that the patterns of various Ca/P powders were similar to that of natural bone. The main diffraction peaks appeared at 31.8° or so, which is the main diffraction peak of HA. There was a small amount of β-TCP appearing in XRD patterns of these Ca/P powders. Additionally, these XRD patterns possessed broad diffraction peaks with a diffuse background compared to that of Ca/P ceramics, which indicates poorer crystallinity and smaller grain size due to lack of high temperature sintering.

Fourier-transform infrared (FT-IR) spectroscopy was carried out on PE 16PC to analyze the structure of Ca/P powders immobilized with Chinese medicine (Fig. 2). FT-IR showed that the O─H adsorption band became broader and decreased resolution at 3000–3500 cm^{-1} with Chinese medicine introduced. Some new peaks were present in finger area in the IR spectra of Ca/P powders with PAA and/or Chinese herb introduced.

More recently, researches in different scientific disciplines were brought together to provide osteogenic and/or therapeutic bone grafts. It has been reported that the Chinese medicinal herbs, Gu-Sui-Bu, was grafted onto the surface of modified calcium hydrogenphosphate (CHP) as an osteoinductive bone substitute. The results of cell cultures have shown that

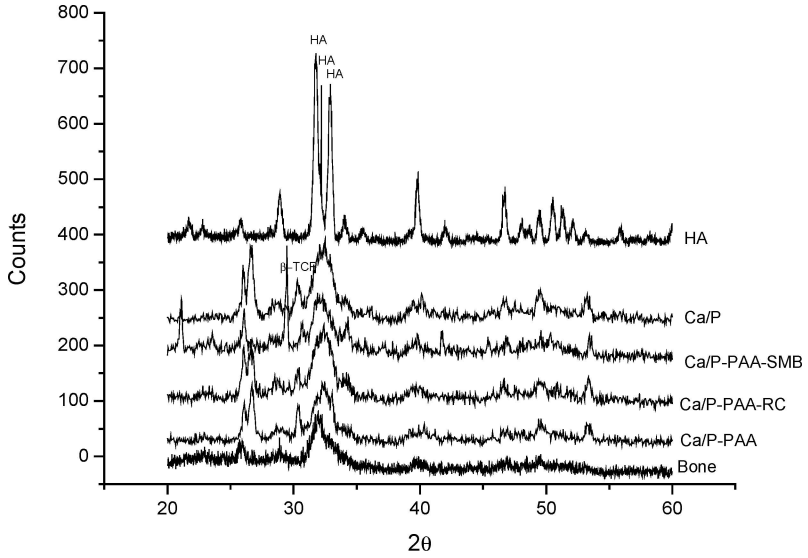

Figure 1 X-ray diffraction patterns of various Ca/P powders with or without Chinese medicine, natural bone and HA ceramics.

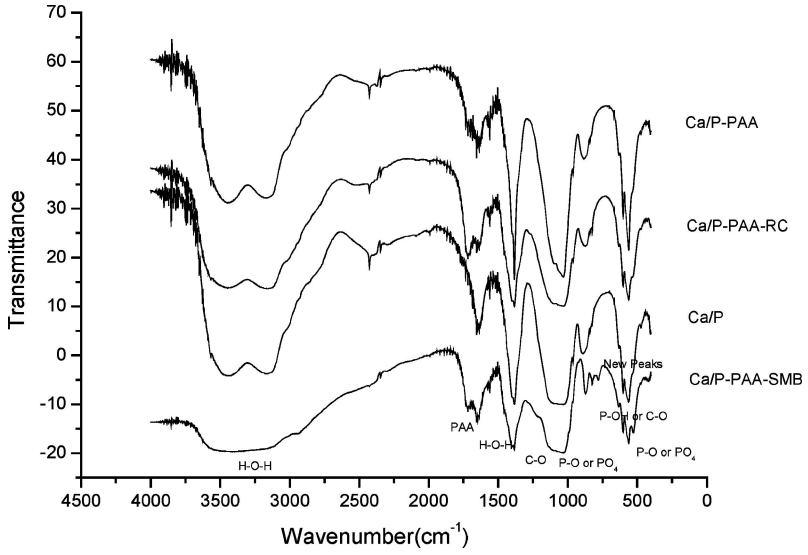

Figure 2 FT-IR spectra of various Ca/P powders with or without Chinese medicine.

the immobilized Gu-Sui-Bu still preserved its original property such as the beneficial effect on the osteoblasts [12, 13]. In Chinese medical science, SMB is reported to promote bone fracture healing by accelerating osteoblasts to excrete collagen fiber and facilitating new bone mineralization [11]. The effect is probably related to its antioxidant activity; and acetylsalvianolic acid A, a semi-synthetic analog of salvianolic acid [10]. The investigation showed that it had a stimulating effect on the phenotype of osteoblast-like cells *in vitro* [14]. RC has similar effect as SMB [11]. In the present study, SMB and RC were chosen as drugs to facilitate bone repair and Ca/P powders were chosen as drug delivery systems. Organic coupling agent, PAA, was employed to modify the surface of Ca/P powders and manipulate the surface properties of Ca/P powders. The results show that the homogeneous Ca/P slurry remained unchanged even if the stirring was stopped whereas the control Ca/P sediment precipitated immediately once the stirring was stopped. Additionally, interfacial modification will be required for enhancing the efficacy of the delivery system to fulfill the controllable release of drugs. This effect of PAA will be studied via the dissolution and release of Chinese medicine *in vitro*, which is in progress. The present results demonstrated that the Chinese medicine could be immobilized onto Ca/P powder effectively. It is possible to use Ca/P-based biomaterials as delivery systems for various drugs in this way, including Chinese medicine and Western drugs, to offer an attractive and efficient solution for the treatment of bone disease.

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