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Synthesization and characterization of chlorhexidine/calcium phosphate/poly(lactic-*co*-glycolic acid) bone cement

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Abstract. Calcium (Ca) and phosphorus (P) elements are important for bone growth and bone remodeling process while chlorhexidine (CHX) is mainly used as an antiseptic due to its broad antibacterial effects. In this study, CaP nanoparticles were synthesized by a wet chemical route from the precursors of calcium hydroxide (CaOH) and diammonium hydrogen phosphate, $(NH_4)_2HPO_4$. Poly(lactic-*co*-glycolic acid) PLGA polymer was incorporated into the mixture of CaP, acted as a matrix binder to form bone cement composite. Different concentrations of CHX (0.5, 1.0, 1.5 and 2.0 mM) were then added into the CaP/PLGA mixtures (CHX/CaP/PLGA bone cements). The bone cements were then characterized by ATR-FTIR, XRD and SEM analyses. The CHX was successfully incorporated into the bone cements, indicated by the appearance of aromatic amine and alkane C–H stretching vibrations which contributed by the ring and long hydrocarbon chain of CHX. The XRD spectra of bone cements showed poorly crystalline phase of apatite. A porous structure of bone cements was also observed with the dispersion of CaP nanoparticles over the PLGA. The CHX/CaP/PLGA bone cement is intended for bone resorption and bone infection applications that require further exploration on its capability to act as bone cement.

1. Introduction

Calcium phosphate (CaP) compounds are being utilised as artificial bone substitute materials due to its bioactivity, biocompatibility and osteoconductivity properties [1, 2]. The major principle of bone cement is lying on their mechanical strength to reinforce damaged bone structures and their capability to be fabricated as an injectable paste cement [3]. Despite of its excellent biocompatibility, CaP (mostly hydroxyapatite) has low degradation rate which limits bone growth onto biomaterials. Thus, incorporation of natural or synthetic polymers such as poly(lactic-*co*-glycolic acid) (PLGA) into CaP bone cement will increase its degradability [4]. In some cases, bone cement is being used to immobilise metallic implants in the bone. Appropriate degradability of bone cement is crucial to allow direct osseointegration on metallic implant surfaces without interruption on the bone-implant interface.

In orthopaedic surgery, infections are one of the largest problems and often lead to severe pain and loss of bone tissues [5]. While in dental applications, CaP may act as a reservoir for accumulation of

periodontal and carious bacteria that often lead to bone resorption and demineralization [6]. There are several strategies to reduce infections including the incorporation of antibacterial agent into bone cements. Calcium phosphate cements, either in a form of hydroxyapatite or brushite had been loaded with antibacterial agents such as gentamycin and chlorhexidine (CHX), either through microcapsule development or simply manually blended cement [7-9]. The cements can provide controlled release of antibacterial agents, through diffusion and/or cement degradation [6].

Chlorhexidine has long been used as skin antiseptic due to its broad antibacterial effects against Gram-positive or Gram-negative bacteria [10]. It has very low toxicity and strong binding to skin, mucus and other structures in the oral cavity due to cationic nature of CHX and mouthwash pH of 5.4 [11]. Previously, CHX has been clarified compatible with osteoblast cell and able to support osteoblastic maturation and mineralization [12]. Therefore, the purpose of this study was to incorporate different concentrations of CHX into CaP/PLGA to form bone cement composite. The characterization analyses were concerning the chemical functionality, crystallinity and morphology of the bone cement using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), X-ray diffractometer (XRD) and scanning electron microscopy (SEM, respectively.

2. Methodology

2.1. Sample Preparation

2.1.1. Synthesization of CaP

The CaP powders were prepared by titrating 100 mL of 0.6 M calcium hydroxide (CaOH) into 100 mL of 0.2 M diammonium phosphate ($(NH_4)_2HPO_4$) at 40°C under stirring condition. The pH was controlled at 9, by adding either ammonia (NH_4) or nitric acid (HNO_3). The CaP solvent was further stirred at 40°C for 4 hours. The homogenized CaP mixture was sit at room temperature for 24 hours to allow complete reaction and precipitation. The CaP precipitates were then filtered using a filter paper and were dried in an oven at 110°C for 5 hours. Further heat treatment at 1000°C for 4 hours was performed for the calcination purpose. Finally, the CaP was crushed using a mortar and pestle to form fine CaP powders.

2.1.2. Preparation of CHX/CaP/PLGA bone cement

The CaP/PLGA bone cement was prepared with a composition of 80 wt% CaP and 20 wt% PLGA. This composition of bone cement was set as a control. Initially, the PLGA was dissolved in dimethyl sulfoxide (DMSO). The synthesized CaP powders were then added into the PLGA solvent and mixed vigorously. The mixture was casted gently into a pellet form and the casted bone cement was stored at room temperature. While the CHX/CaP/PLGA bone cements were fabricated by incorporating 80 wt% CaP and 19 wt% PLGA and 1 wt% CHX. The CHX concentrations were varied to 0.5, 1.0, 1.5 and 2.0 mM.

2.1.3. Characterization analyses

The chemical functionalities of the bone cements were verified by ATR-FTIR (Nicolet iS5-IR Spectrometer, Thermo Scientific, USA). The obtained spectra were analyzed at a frequency interval of 500 cm^{-1} to 1000 cm^{-1}. The number of scanning was set at 36 with a resolution of 4. The crystallinity of the bone cements was investigated by XRD (D5000, Siemens, Germany). The analyses were performed at 40 kV and 40 mA to recognize the crystallographic phases and the crystal lattice of the bone cements. The data were recorded in 2θ range from 5° to 70° using CuKα radiation of 1.5406 Å. The morphology of the bone cements was then examined by SEM (Tabletop TM3000, HITACHI, Japan) at 1000× and 5000× magnifications. Each bone cement was sputter coated with an ultrathin platinum conductive film using a sputter coater (Q150R, Quorum Technologies, England) to enhance image resolution and visualization.

3. Results and discussion

3.1. ATR-FTIR analyses

Figure 1 presents the ATR-FTIR spectra of CHX/CaP/PLGA bone cements. The results showed an appearance of aromatic amine CHX on all bone cements at 1046 cm^{-1}, contributed by the ring of CHX. A strong CHX indicator was observed between 2850 cm^{-1} and 3000 cm^{-1}, which corresponded to alkene C–H stretching vibration, derived from the long hydrocarbon chain of CHX [13]. While, the peaks at 1015 cm^{-1} and 1085 cm^{-1} were derived from the vibrations of phosphate group (PO_4^{3-}), representing the composition of CaP compound [14]. The C–O bond stretching (1270 cm^{-1}) and carbonyl group, C=O (1750 cm^{-1}) were assigned to the PLGA while the broad band at 3000 cm^{-1} – 3600 cm^{-1} region was observed due to stretching vibration of hydroxyl group, OH^-.

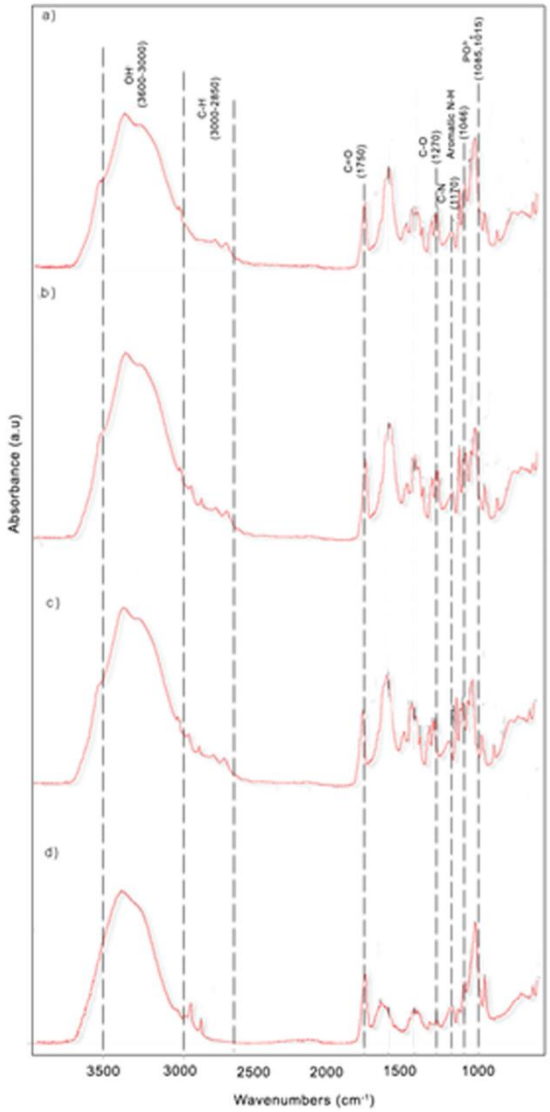

Figure 1. ATR-FTIR spectra of (a) 0.5CHX/CaP/PLGA (b) 1.0CHX/CaP/PLGA, (c) 1.5CHX/CaP/PLGA and (d) 2.0CHX/CaP/PLGA bone cements.

3.2. Surface crystallinity

Figure 2 shows the XRD spectra of CHX/CaP/PLGA bone cements. Most intense peaks were observed at 31.80° (211), 32.10° (112) and 32.85° (300), originated from hydroxyapatite and 25.90° (1010), 28.00° (214) and 31.25° (0210), originated from beta-tricalcium phosphate (β-TCP) based on the JCPDS number of 09-0432 for hydroxyapatite and 09-0169 for β-TCP. The PLGA and CHX were not identified in the XRD patterns due to its non-crystallize form [15]. It is important to point out that the patterns showed an observation of weak hydroxyapatite composition, indicating poorly crystalline phase of apatite [16].

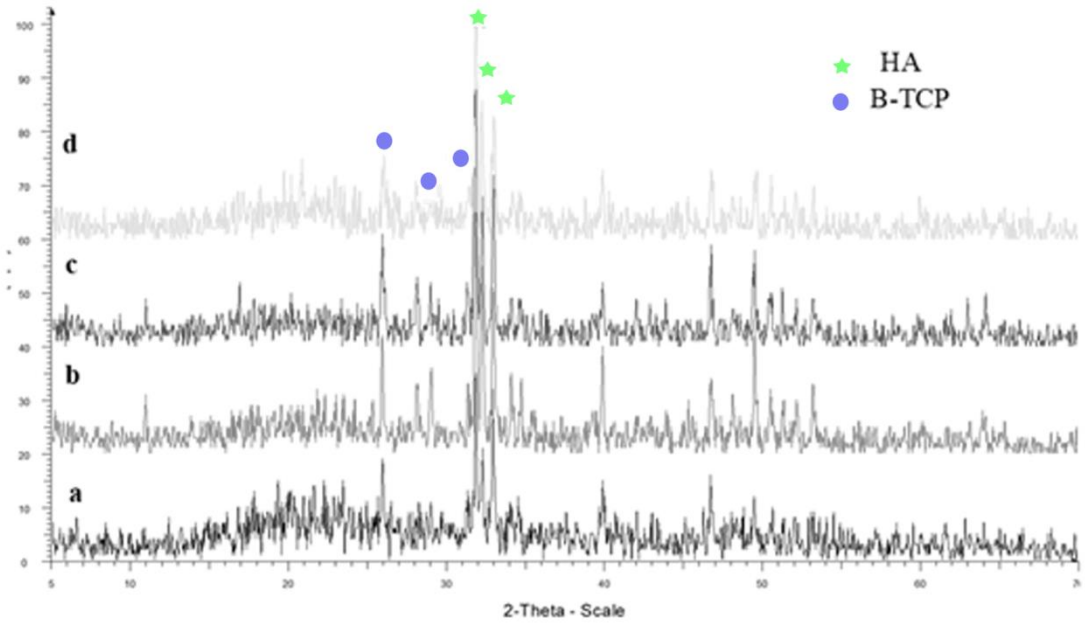

Figure 2. XRD patterns of (a) 0.5CHX/CaP/PLGA (b) 1.0CHX/CaP/PLGA, (c) 1.5CHX/CaP/PLGA and (d) 2.0CHX/CaP/PLGA bone cements.

3.3. Surface Morphology

Figure 3 visualizes the morphology of CaP and CHX/CaP/PLGA bone cements. The bone cements showed a porous structure, essential for the migration and proliferation of cells after a cement restoration [16]. The CaP particles were thoroughly dispersed over the PLGA matrix. The nanosize of CaP is important to improve the mechanical properties of materials and may aid in protein adsorption and cell adhesion as compared to micron size of CaP [17]. The incorporation of PLGA into the CaP mixture has caused the agglomeration of CaP powders within the PLGA compounds. However, the addition of CHX did not markedly change the surface morphology of the bone cement.

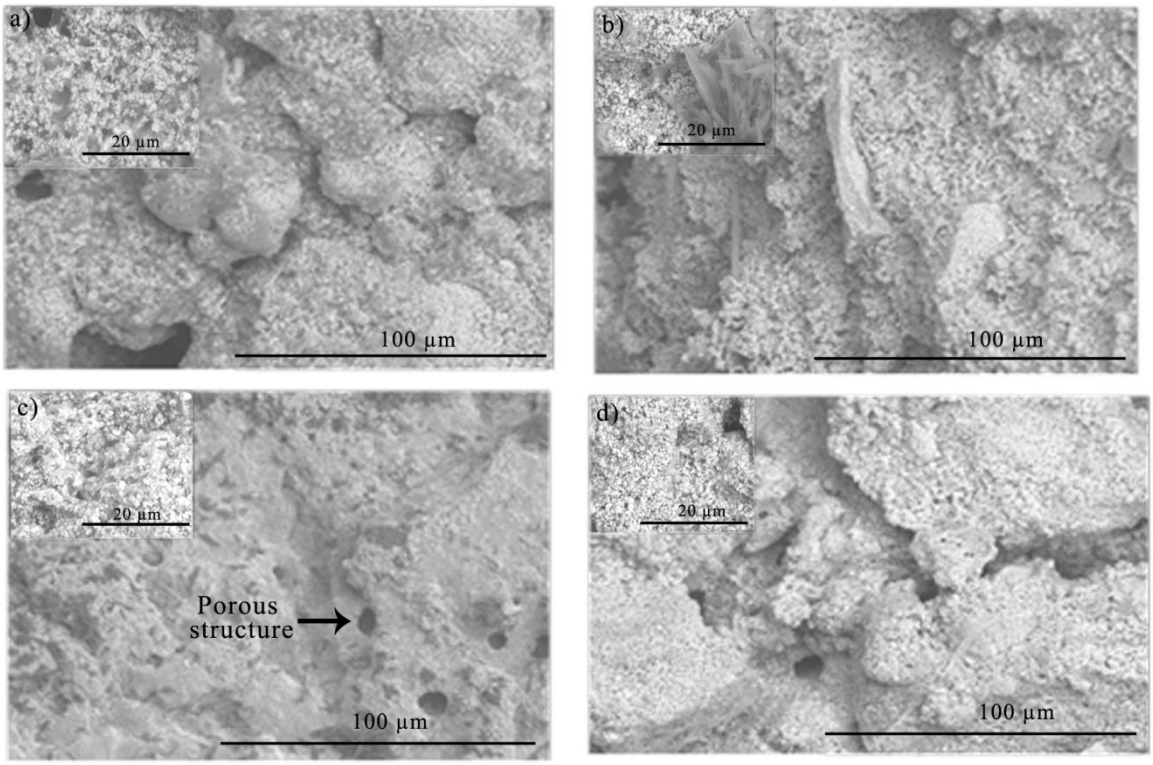

Figure 3. SEM images and EDS analysis of (a) 0.5CHX/CaP/PLGA (b) 1.0CHX/CaP/PLGA, (c) 1.5CHX/CaP/PLGA and (d) 2.0CHX/CaP/PLGA bone cements.

4. Conclusion

In summary, the CHX/CaP/PLGA bone cements were synthesized through a wet chemical route. The incorporation of PLGA and CHX into the CaP are necessary to fabricate antibacterial degradable bone cement, which is preferable for the filling of bone defect areas. Increasing the amount of CHX concentration did not significantly alter the crystallinity and morphology of the bone cements.

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