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Effect of polydopamine on the biomimetic mineralization of mussel-inspired calcium phosphate cement in vitro

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ABSTRACT

Inspired by the excellent adhesive property of mussel adhesive protein, we added polydopamine (PDA) to calcium phosphate cement (PDA–CPC) to enhance its compressive strength previously. The mineralization and mechanism on PDA–CPC were investigated by soaking it in simulated body fluid in this study. The results indicated that PDA promoted the conversion of dicalcium phosphate dihydrate and α-tricalcium phosphate to hydroxyapatite (HA) in the early stage but inhibited this conversion subsequently. PDA promoted the rapid mineralization on PDA–CPC to form a layer of nanoscale calcium phosphate (CaP) whereas there was no CaP formation on the control-CPC after 1 d of soaking. This layer of nanoscale CaP was similar to that of natural bone, which was always observed during soaking. X-ray photoelectron spectroscopy showed that the peak of C═O of PDA existed in the newly formed CaP on PDA–CPC, indicating the co-precipitation of CaP with PDA. Furthermore, the newly formed CaP on PDA–CPC was HA confirmed by transmission electron microscopy, which the newly formed HA was in association with PDA. Therefore, PDA increased the capacity of mineralization of CPC and induced the formation of nanoscale bone-like apatite on PDA–CPC. Thus, this provides the feasible route for surface modification on CPC.

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1. Introduction

Calcium phosphate cement (CPC) has been an alternative to autologous and allogenic bone grafting for bone defects due to its similar composition to those of natural bone [1]. The inadequate mechanical properties of CPC result in its inefficient application in load-bearing bone defects unfortunately [2]. Studies focus on improving the mechanical property of CPC by admixing additives with powder or liquid of CPC, i.e., polymer fibers [3], cellulose [4], carbon nanotubes [5], strontium [6], and magnesium [7]. The additives are usually randomly distributed in the CPC, resulting in composites with relatively isotropic properties or limited CPC mechanical strength improvement [8].

Many studies inspired by the adhesion of mussels to ships or rocks under wet conditions have reported that the adhesive proteins secreted by mussels mainly contain dihydroxyphenylalanine (DOPA) and lysine. Similarly, dopamine (DA) contains the same catechol functional group as that of the side chain of DOPA residues and the same amine functional group of lysine residues [9], which proves that DA is a strong adhesive with a wide range of inorganic and organic materials due to its self-polymerization to form polydoapmine (PDA) films [10]. The extraordinary adhesive property of PDA is due to its abundant catechol moieties [11], which form covalent or strong non-covalent interactions (hydrogen bonds or stacking interactions) with substrates [12]. PDA has been applied in surface modification, typically in biomaterials, because it is less time-consuming than other chemical techniques and does not require organic solvents [13]. PDA coating significantly promotes the adhesion and proliferation of osteoblasts (MC3T3-E1) [14] and human umbilical vein endothelial cells, whereas it remarkably decreases those of human umbilical artery smooth muscle cells [15]. Hong et al. [9] reported that PDA is nontoxic, and can reduce the in vivo toxicity of poly-L-lactic and cadmium selenide quantum dots in contact with tissue or blood. These show that PDA is biocompatible and can be used in biomaterials. PDA has been recently used as the intermediate layer to immobilize silver [16,17], or HA nanoparticles and RGD [18], or heparin [19], or growth factors [20,21] on a biomedical metal.

PDA obtained through the oxidation of DA in Tris–HCl buffer solution (pH = 8.5) was added into CPC (PDA–CPC) in our previous study [22], which significantly increased the compressive strength of PDA–CPC. PDA promoted the dicalcium phosphate dihydrate (DCPD) conversion into HA after setting for 24 h. In addition, it is reported that PDA has the capacity to concentrate Ca^{2+} using its catechol moieties, which results in the local supersaturation of Ca^{2+} and the formation of HA crystals on the substrate [23,24]. Several biomaterials (i.e., titanium, polyester fibers and carbon nanotubes) are easily covered by apatite layers with the aid of PDA after soaking in simulated body fluid (SBF)

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[11,23]. In addition, PDA coating has also been used in dentistry to promote dentin remineralization, such that all dentin tubules are filled by densely packed hydroxyapatite crystals [25]. Nonetheless, the effect of PDA on PDA–CPC mineralization in terms of its Ca^{2+} concentration has not been explored.

In this study, PDA–CPC was prepared according to our previous study, and was then soaked in SBF to study its in vitro mineralization. The morphology, chemical composition, crystal structure, and mineralization mechanism of PDA–CPC were investigated.

2. Materials and methods

2.1. Calcium phosphate cement

CPC, which consists of 58 wt.% α-tricalcium phosphate (α-TCP), 25 wt.% DCPD, 8.5 wt.% HA, and 8.5 wt.% analytical grade $CaCO_3$, was used in this study, with minor modifications [26]. HA and α-TCP were purchased from the National Engineering Research Center in Biomaterials, Sichuan University, Chengdu, China. $CaCO_3$ was purchased from Kelong Chemical Inc., Chengdu, China. DCPD was synthesized in the laboratory [27]. All starting powders were mixed and dried overnight at 60 °C. The cement solution was prepared by mixing Tris–HCl buffer (10 mM, pH 8.5) with 40 mg/mL DA (Sigma-Aldrich, Germany) [22]. The cement solution was exposed to air for 2 d to oxidize and crosslink. The cement solution without DA was used as control. The experimental CPC and the control CPC are referred to as PDA–CPC and the control-CPC, respectively.

CPC was prepared by the mixing cement solution with starting powder at the ratio of 0.3 mL:1 g. After mixing, the pastes were placed in cylindrical molds to form specimens with dimensions of 15 mm in diameter and 5 mm in height. The disk specimens were removed from the mold to an atmosphere of 100% relative humidity at 37 °C for 24 h.

2.2. Biomimetic mineralization of CPC

All CPC specimens were cleaned using ultrasound for 30 min and suspended in SBF [28] in a shaking (100 rpm) water bath at 37 °C for 1 d, 3 d, 7 d, 10 d, and 14 d. CPCs were rinsed with distilled water and dried in a desiccator without heating.

2.3. Characterization of biomimetic mineralization of CPC

2.3.1. X-ray diffraction

X-ray diffraction analysis (XRD; X'Pert Pro, Philips, The Netherlands) was performed to identify the crystalline phases on the surfaces of all the CPC specimens after soaking in SBF. The diffraction patterns were determined with a scanning angle 2θ ranging from 4° to 50° in step-scan intervals of 0.02°, with Cu Kα radiation at 40 kV and 40 mA.

2.3.2. Attenuated total reflectance-Fourier transform infrared spectroscopy

Attenuated total reflectance (ATR) Fourier-transform infrared spectroscopy (FTIR; 5700, Nicolet, USA) was used to identify the functional groups on the surfaces of all the CPC specimens. The spectra were collected over 4000 cm^{-1} to 400 cm^{-1}, and a reflection attachment (Spectra-Tech, FT80 RAS) at the incident angle of 80° was used.

2.3.3. Scanning electron microscopy

Scanning electron microscopy (SEM; Quanta 200, FEI, The Netherlands) and energy-dispersive spectroscopy (EDS; 7760/68ME, EDAX, USA) were used to observe the morphology and identify of the Ca/P ratios of the deposited phases on the CPC surface. The samples were sputter-coated with gold prior to examination.

2.3.4. X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS; XSAM800, Kratos, UK) was performed to evaluate the relative contents of different elements on the CPC surface, using Al Kα radiation (1486.6 eV) as excitation source, and using voltage and current values of 12 kV and 11 mA, respectively.

2.3.5. Transmission electron microscopy

Transmission electron microscopy (TEM; JEM-2100F, Jeol, Japan) and EDS (832, Oxford, UK) were used to characterize the calcium phosphate precipitation on the surface of PDA–CPC soaked in SBF for 14 d. PDA–CPC was embedded in ethoxyline resin, and was cut into 100 nm sections using a microtome (UC7, Leica, Germany). The sections were analyzed through selected area electron diffraction (SAED) and high-resolution TEM (HRTEM).

3. Results and discussion

In our previous study, the mechanical strength of PDA–CPC was significantly improved compared to that of the control CPC. Further study showed that PDA promoted the conversion of the more soluble DCPD to HA during setting [22]. In addition, the catechol in PDA concentrated calcium ions and affected the mineralization of calcium-based materials [23,29]. Therefore, the effect of PDA on the subsequent CPC conversion and mineralization in SBF in vitro was studied in this study.

Fig. 1 shows the XRD patterns of PDA–CPC and the control-CPC surfaces before and after soaking in SBF for 1 d, 3 d, 5 d, 7 d, 10 d, and 14 d. Fig. 1a and b shows that the main phases were DCPD, $CaCO_3$, α-TCP, and HA before soaking. The relative intensities of the diffraction peaks of DCPD in PDA–CPC were obviously reduced compared to that of the control-CPC. This was because of the accelerated conversion of DCPD

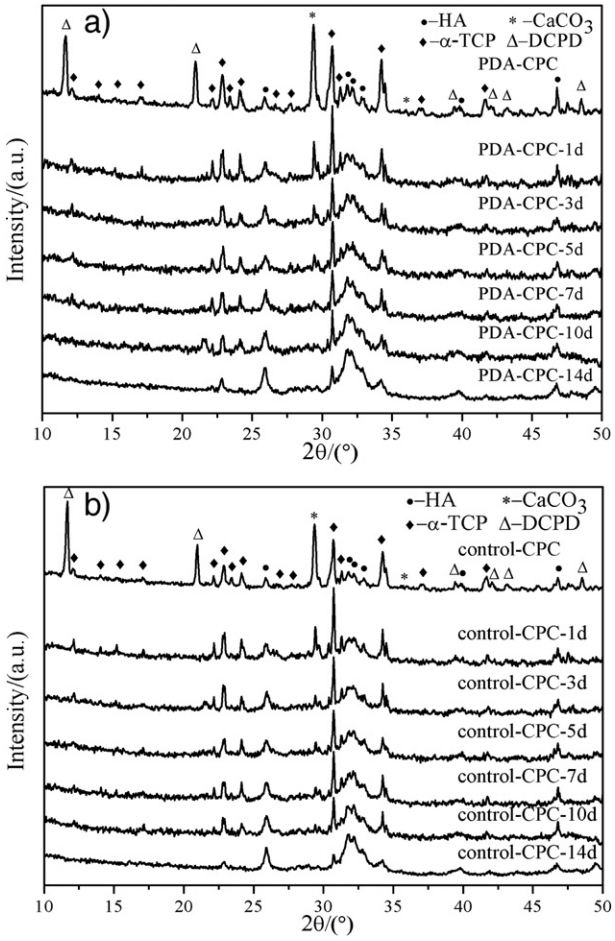

Fig. 1. XRD patterns of PDA–CPC (a) and the control-CPC (b) surfaces before and after soaking in SBF for 1 d, 3 d, 5 d, 7 d, 10 d, and 14 d. PDA–CPC and the control-CPC samples were suspended in SBF to avoid the deposition of CaP sediment on their surfaces.

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