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The Hydration Behavior of Ca–P–Si System via Mechanochemical Treatment

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In the present study, the Si-substituted amorphous calcium phosphate (Si-ACP) was prepared via a dry-mechanochemical method, and the hydration behavior of Si-ACP was investigated by X-ray diffraction, X-ray photoelectron spectroscopy, scanning electronic microscopy, and the determination of the setting time and compressive strength. The results showed that the mechanochemical treatment had a great effect on the resolution property of Si-ACP, which induced the different hydration behavior. With the presence of Si, the hydration product of Si-ACP showed better results of cell proliferation and metabolic activity, which were characterized by the methyl thiazolyl tetrazolium assay (MTT) with mesenchymal stem cells. This work was expected to have a guiding effect on the study of calcium phosphate cement.

I. Introduction

In 1986, calcium phosphate bone cement was presented by Brown and Chow,[1] and the hard-solid reaction of several different kinds of calcium phosphates had been the focus of research. With its self-setting performance, easy plasticity, and good biocompatibility having been verified in clinical practices, this material has gradually received greater attention.[2–4] In 1969, SiO_2-based bio-glass was proved to have good bioactivity by Hench and Anderson[5]; from then on, much research on silicon (Si)-containing materials had been carried out.[6–8] It was known that bone mineral had a structural similarity with hydroxyapatite (HA; $Ca_5(PO_4)_3OH$), and various substitutions existed in bone mineral, such as Na, Mg, K, Sr, Zn, Ba, Cu, Al, Fe, F, Cl, and Si,[9] which influence the solubility, surface chemistry, and morphology of a material. Some authors found that Si had an important effect on the biological performance *in vivo*.[10] And Si-HA- and Si-TCP-based materials were approved to enhance bone apposition, bone in-growth, and cell-mediated degradation compared with stoichiometric HA.[11–14] In addition, some papers had reported that the Si was required in bone, cartilage, and connective tissue formation as well as several other important metabolic processes.[15] And hydrated soluble Si could enhance the proliferation of bone cells (osteoblasts) and the active cellular production of transforming growth factors.[16–18] Some authors synthesized the amorphous calcium phosphate (ACP) powder by mechanical activation of high-energy ball milling.[4] This new technique was more ascendant compared with the traditional precipitation technique: firstly, the stronger mechanical effect induced less liquid during the setting process of cement; secondly the synthesis route is more reproducible; and the mechanically activated calcium phosphate powders appear to be more active than precipitated ACPs.[19]

Herein, the higher mechanical effect was used to activate the Ca–P–Si system; and the Si element was introduced in CPC to obtain different hydration morphology. This work was expected to open up new perspectives in terms of incorporation of foreign ions in the hydration product.

II. Materials and Methods

(1) Materials

ACP was obtained by the dry-mechanochemical method with dicalcium phosphate dihydrate (DCPD), calcium hydroxide ($Ca(OH)_2$) (Shanghai No.4 Reagent & H.V. Chemicals, Shanghai, China) (Table I). The mixed powder was ground using a planet mill at a rotation speed of 500 rpm. Yttria-stabilized tetragonal zirconia balls with a diameter ranging from 5 to 15 mm at a ball-to-powder mass ratio of 10:1 and polyamide vessels were used to reduce the contamination. The SiO_2 was prepared by hydrolyzing tetraethyl orthosilicate in NaOH solution. The as-prepared ACPs (ACP-1, ACP-2, and ACP-3 in Table I) were used for the hydration reaction with DCPD (1:1), and the corresponding ACP-DCPD cements were marked as CPC-0, CPC-1, and CPC-2.

(2) Phase and Microstructure Characterization

The as-prepared raw ACP and hydrated samples were characterized by X-ray diffraction (XRD) (X'Pert PRO, PANalytical Co., Almelo, the Netherlands); scanning electronic microscopy (SEM) (H-800, Hitachi, Tokyo, Japan); FTIR (Avatar 360, Nicolet Co., Madison); X-ray photoelectron spectroscopy (XPS) (Axis Ultra DLD, Kratos Analytical, Manchester, U.K.); and the setting time and compressive strength test.[20]

(3) Metabolic Activity Assay

Cell metabolic activity was assessed by the methyl thiazolyl tetrazolium bromide assay (MTT reagent, Sigma-Aldrich Corp., St. Louis, MO).[21]

(4) Cell Adhesion Assay

Logarithmic growth phase mesenchymal stem cells (MSCs) were harvested from two to four passage culture cells and seeded on L-DMEM 24 h infiltrated scaffold with a cell density of 3×10^3 and placed in an incubator under the condition 37°C, 5% CO_2, and saturated humidity. One hundred microliter L-DMEM was added to each sample 1 h later. Three parallel samples were tested at each time point. MSCs on each sample were harvested by 0.1% EDTA and 0.25% trypsin and counted using a CBC board.

(5) Statistical Analysis

Quantitative data are presented as mean ± standard deviation and statistical analysis was performed using a one-way analysis

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Table I. Material Components of Amorphous Calcium Phosphate (ACP)

	ACP-0	ACP-1	ACP-2
Components (DCPD/$Ca(OH)_2$)(Ca/P:1.50)	+SiO_2(0 wt%)	+SiO_2(5 wt%)	+SiO_2(5 wt%)
Mechanochemical treatment (time in hours)	5	5	8

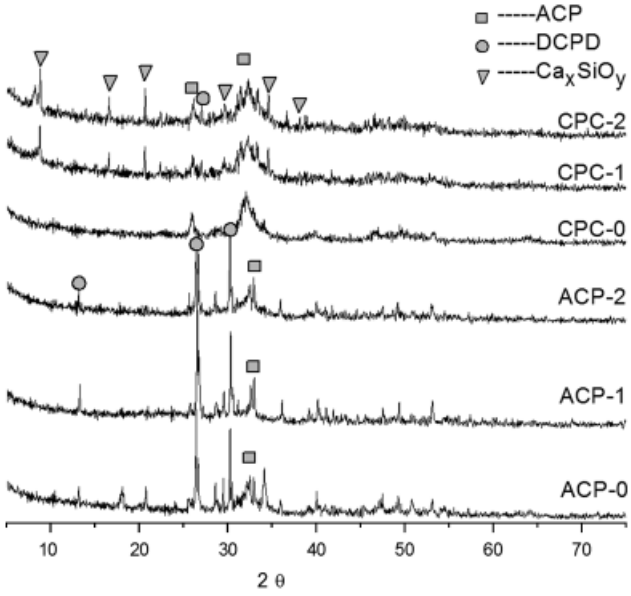

Fig. 1. X-ray diffraction patterns of as-prepared amorphous calcium phosphates and the corresponding hydration products.

of variance (one-way ANOVA). A comparison between the two means was made using the Tukey's test, with statistical significance set at $P<0.05$.

III. Results

Figure 1 showed the XRD patterns of ACP and the corresponding hydration products (the poorly crystallized HA); in addition, the XRD pattern of Ca_xSiO_y appeared in CPC-1 and CPC-2. From these figures, it can be understood that the setting reaction has occurred between ACP and DCPD. Via the mechanochemical treatment, Si in ACP was activated, and Ca_xSiO_y was formed during the setting reaction of CPC, which also can be seen from the FTIR analysis (Fig. 2); the intensity of Si–O–Si peaks in CPC was increased by the mechanochemical treatment.

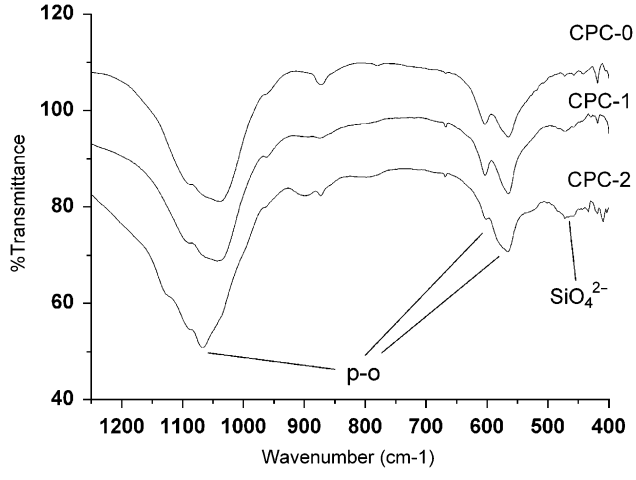

Fig. 2. IR spectrums of CPC-0, CPC-1, and CPC-2.

Table II. Binding Energy Values of Main X-Ray Photoelectron Spectroscopy Peaks for CPC-0

Name	Pos	FWHM	Area
O1*s*1	532.29	2.824	16653.1
O1*s*2	535.62	2.846	2336.1
O1*s*3	528.85	1.879	743.1
Ca2*p*	358.41	7.534	417.0

From the XPS analysis, it was observed that the O, P, and Si peaks were detected by XPS analysis and the binding energy of O peaks (532.29, 535.62, and 528.85) in CPC-0 (Table II) were changed compared with that in CPC-1 and CPC-2 (Tables III and IV). The P2*p* peak was found in CPC-1 and CPC-2, which means that the bonding energy of P–O was increased. The presence of Si2p peak in Table III was due to the adding of SiO_2 in CPC, and its peak belongs to Si–OH. From these figures, it could be concurred that the chemical state of Si in SiO_2 was changed by mechanochemistry, and the bond between Si and O was broken partly. In order to maintain stability in a chemical state, an other radical must be introduced, such as –OH and SiO_4^{2-}. From Table IV, it could be observed that the O, P, and Si peaks were detected by XPS analysis, and the bonding energy for O1*s*1 and O1*s*2 decreased compared with that shown in Table III. And the Si2*p*2 (103.57 eV) shown in Table IV indicates the presence of SiO_4^{2-}.[22]

Figure 3 summarized the setting time and compressive strength of CPC after setting for 24 h. Both the initial and final setting times of cement decreased from CPC-0 to CPC-2; but the corresponding compressive strength of cements showed the opposite trend, respectively (one-way ANOVA; $P<0.05$). Figure 4 shows the different microstructure of cements: for CPC-0, a little crystallized product was observed from the SEM photo; for CPC-1 and CPC-2, the microcrystals were observed on the surface of the material.

The cell viability on the as-prepared cement was examined by means of MTT assay (Fig. 5); data on all substrates showed an increase with culture time, being a similar pattern to that of the control. Data clearly revealed the higher levels in CPC-1 than those in CPC-0 and CPC-2 ($P<0.05$), which is probably resulted from the change of Si–OH to SiO_4^{2-}. Figure 6 shows the cell adhesion assay; an increase in the cell number was observed over the entire culture period, and MSCs on CPC-1 exhibited higher cell numbers at all time points compared with CPC-0.

Table III. Binding Energy Values of Main X-Ray Photoelectron Spectroscopy Peaks for CPC-1

Name	Pos	FWHM	Area
O1*s*1	533.31	1.423	14986.4
O1*s*2	531.96	1.157	15307.4
O1*s*3	534.85	1.611	871.7
P2*p*	135.12	1.663	395.1
Ca2*p*	348.22	3.194	486.3
Si2*p*	101.89	1.422	1040.4

Table IV. Binding Energy Values of Main X-Ray Photoelectron Spectroscopy Peaks for CPC-2

Name	Pos	FWHM	Area
O1*s*1	533.23	2.034	34025.1
O1*s*2	531.9	1.288	131521.9
P2*p*	135.10	1.738	887.2
Ca2*p*	348.17	3.336	106.5
Si2*p*1	103.57	1.680	704.8
Si2*p*2	101.83	1.411	1145.3

IV. Discussions

For CPC-0, the bonding energies of Ca and P were not evident (Table II), which was due to its amorphous phase state. The decrement of bonding energy for Ca in CPC-1 and CPC-2 was due to adding Si in the cement. Some authors believe that the local charge neutrality was mandated in order to avoid a large loss in energy, which required some other defect to be associated with the PO_4^{3-} to compensate for the charge deficit.[12,23] But there were many possible mechanisms for charge compensation during the preparation of the materials, for example, O, H, and OH vacancies. Furthermore, some authors have reported that the reaction $3Ca(OH)_2+2SiO_2 = Ca_3[SiO_3(OH)]_2 \cdot 2H_2O$ will occur by mechanochemistry.[24] From these figures, we knew that the Si–OH and SiO_4^{2-} were formed via mechanochemistry, and then the different hydration products were obtained. Some reports[22] pointed out that the rate of HA formation on the surface of $CaSiO_4$ ceramics was faster than those of the other biocompatible glass and glass–ceramics in SBF solution. Hence, it was easy for calcium phosphate to crystallize with Si–OH as the nucleating site (Fig. 4, CPC-1). Because of the existence of Si–OH in the material, amount of microcrystals formed with the Si–OH as crystal sites in CPC-1; for CPC-2, with a more mechanochemical treatment, the gel-like Ca_xSiO_y appeared as the hydration product. The different setting time and compressive strength were induced by the different hydration microstructure (Fig. 3).

Furthermore, through *a priori* modification of the nanostructure, both chemistry and topography (texture, roughness) of a biomaterial surface could be modified by the mechanochemical route. It has been shown that nanoparticle (NP)-modified surfaces could dramatically enhance the differentiation of the cell. NP-modified surfaces, therefore, present a new paradigm for probing the effects of surface-bound information on cell shape and function.[25] In this paper, we analyzed the effects of hydration morphology of CPC on cell morphology through quantification of cell spreading and activity. An increase in the cell number was observed over the entire culture period (Figs. 5 and 6). These data confirmed that the hydration morphology of CPC as conducive to cell adhesion and proliferation.

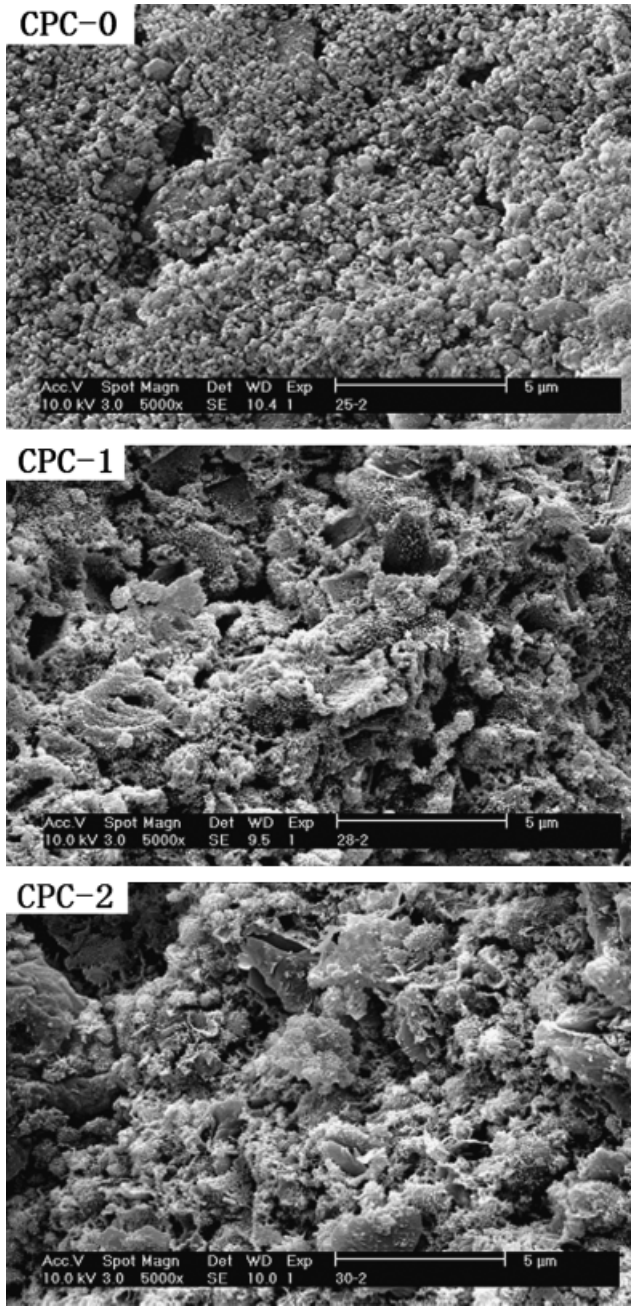

Fig. 4. Scanning electronic microscopy photographs of CPCs.

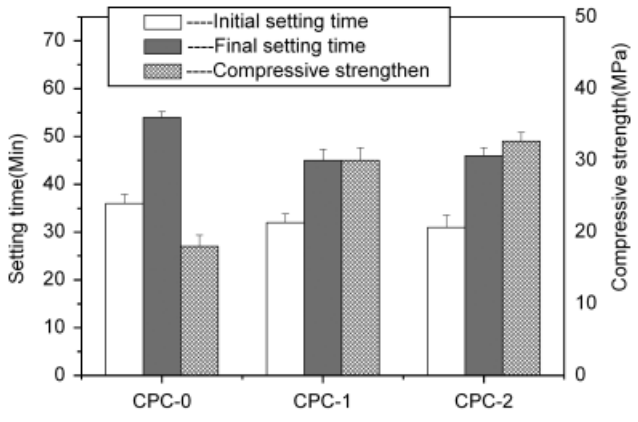

Fig. 3. Setting time and compressive strength for CPCs.

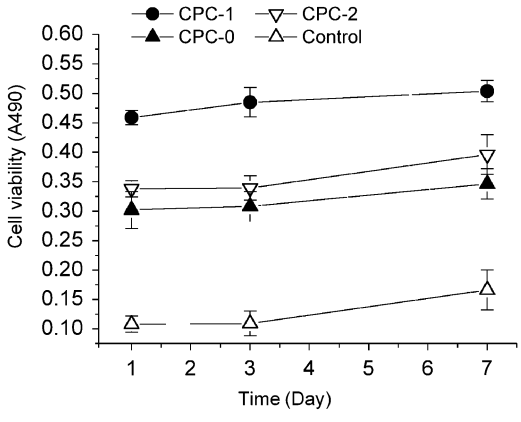

Fig. 5. The relationship between cell viability of CPC and culture time.

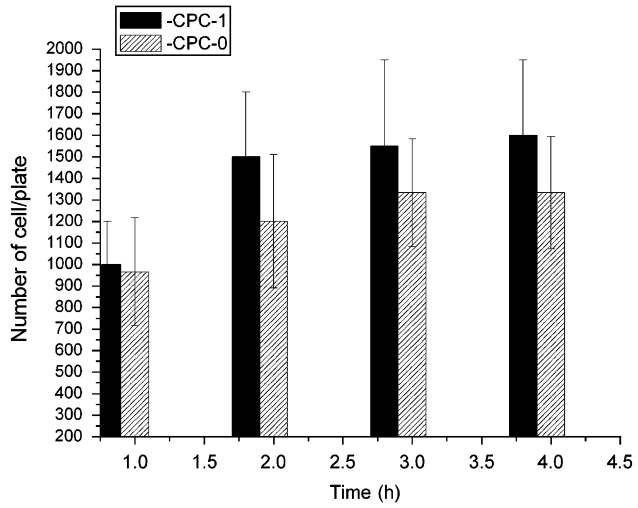

Fig. 6. The relationship between cell adhesion data of CPC and culture time.

V. Conclusions

In recent years, mechanochemistry has been the subject of increasing attention. The ACP based on Ca–P–Si system was prepared by the mechanochemical method in this paper, and the as-prepared Si-ACPs and the corresponding hydration products were characterized. These results showed that different chemical states for Si were formed after mechanochemical treatment, and then different hydration products were obtained; with the Si substitution, different hydration morphology of CPC was achieved, and the cell showed different growth activity on CPC. Summarily, although the present results indicated that this novel Ca–P–Si system was a prospective biomaterial, further investigation should be carried out to determine *in vivo* behaviors.

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