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Synthesis and in vitro Experiment of Biomaterial Tricalcium Phosphate

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Calcium phosphate ceramics consist of materials such as hydroxyapatite, tricalcium phosphate (TCP), calcium phosphate cement (CPC), biphasic calcium phosphate, etc. CPCs have been used for filling bone defects in dentistry and orthopedics. Among these materials, β-tricalcium phosphate is suggested as an ideal candidate for bone graft in hard tissue engineering due to its high biocompatibility, bioactivity and bone bonding. The preparation, as well as the application of this powder material, has been the important topic of research in material science. In this paper, β-tricalcium phosphate (β-TCP), a component that has chemical formulation similar to bone structure, was synthesized by the precipitate method and then calcinated at 1000°C for 5 h. The physico-chemical properties of synthetic material were examined by XRD, FT-IR and SEM methods. In vitro experience was also carried by soaking β-TCP simulated body fluid powder in a different period of time. Obtained results confirmed the quality of β-TCP synthetic material and its bioactivity.

Key words: β-tricalcium phosphate; bone minerals; precipitate method; simulated body fluid; HA; physico-chemical properties

Calcium phosphates have been successfully used as bone repairing and substituting material for many applications in dentistry and orthopedics. Calcium phosphate has 12 substances but there are few materials used in biomaterial field, and one of them is tricalcium phosphate or TCP, with a chemical formula $Ca_3(PO_4)_2$ (Fernández *et al.* 1999). Due to the similar structure of the bony mineral, excellent biological properties like high biocompatibility, high biodegradation and quick biosorption, TCP has been becoming an ideal choice for clinic applications for a long time.

TCP has two allotropes forms which are α-TCP and β-TCP. β-TCP is a low-temperature phase of TCP. It is stable at room temperature and transforms into α-TCP phase at 1125°C (Welch & Gutt 1961; Carrodeguas 2010). On account of fast resorption rate, α-TCP was used limitatively in biomedical application although it has a precisely the same chemical composition like β-TCP (Bahman *et al.* 2011; Sergey 2009).

β-TCP can be synthesized via numerous techniques and methods, with a different range of reactants like wet chemical precipitate method (Kivrak & Cuneyt 1998; Rohaida *et al.* 2004; Albuquerque *et al.* 2004), hydrolysis of other calcium phosphate method (Kazuhiko *et al.* 2008), sol-gel method (Ruan *et al.* 2006) and hydrothermal method (Ain *et al.* 2008).

The primary purpose of this study was by using synthesized β-TCP by wet chemical precipitation method and then characterizing it by several physico-chemical analysis methods like XRD, FT-IR, and SEM. In vitro experiment was carried out by soaking of 50 mg of β-TCP in 100 ml simulated body fluid (SBF) solution to estimate bioactivity of this material.

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EXPERIMENTAL

Synthesize Tricalcium Phosphate Powder

Raw materials to synthesize β-TCP are tetrahydrate calcium nitrate ($Ca(NO_3)_2.4H_2O$, 99%, Merck); diammonium hydrophoshate ($(NH_4)_2)HPO_4$, 99%, Merck). Ammonia solution (NH_4OH, 25%, Merck) was used as a solvent to adjust pH of the reaction mixture. β-TCP was synthesized according to Bahman Mirhadi's research (Bahman *et al.* 2011). Briefly, 500 ml $(NH_4)_2HPO_4$ (0.2 M, pH = 4) was dropped with rate 3 ml/min into 500 ml $Ca(NO_3)_2.4H_2O$ (0.3 M, pH = 7.3). The mixture was stirred vigorously at room temperature during the process. NH_4OH 0.1 M was used to adjust pH = 8 to precipitate a white suspension. After finishing dropping, the mixture was continued to be stirred for 6 h to produce the β-TCP suspension. Then the suspension was filtered two times by distilled water to remove bad smell of the ammonia solution. After that, the white suspension was transferred into the oven and dried for 8 h at 120°C. The last step was calcination of material powder in the alumina crucible at 1000°C for 5 h to form the crystalline β-TCP powder.

In vitro Experiment in SBF Solution

In vitro analysis was to estimate bioactivity of β-TCP powder which was carried out in SBF by soaking 50 mg of material powder in 100 ml SBF. SBF was a solution with minerals composition nearly equal to those of human plasma (*Table 1*). The synthesis of SBF solution is according to Kokubo's protocol (Kokubo *et al.* 1990).

Physico-chemical Characterization

To evaluate physico-chemical properties of β-TCP powder before and after soaking in SBF solution, XRD, FT-IR and SEM analysis methods were employed. The crystalline phase of β-TCP was investigated by X-Ray diffractometer (Bruker D8 Advance). The Fourier transformed infrared spectroscopy (FT-IR) (Bruker Equinox 55) was used to identify the functional groups. Scanning electron microscopy (SEM) (Hitachi, Joel 5) was used to observe and evaluate the morphological shape and particle size of the material.

RESULTS AND DISCUSSION

Physico-chemical Characterization of Synthetic TCP Powder

Figure 1 shows XRD patterns of synthetic β-TCP and standard β-TCP (from database in website <http://icsd.fiz-karlsruhe.de>, Germany. Compared with XRD pattern of standard β-TCP, synthetic β-TCP completely had no stranger peaks. This result demonstrated the purity of obtained powder. Besides, β-TCP synthesis had sharp peaks, proved that β-TCP had good crystallization.

Figure 2 shows FT-IR spectra of synthetic β-TCP. Compared with other paper about synthetic β-TCP, FT-IR spectra of our synthetic material was almost similar. There was a range of bands at 900–1200 cm^{-1}, characterized for stretching vibration of PO_4^{3-} group of β-TCP (Behzad *et al.* 2012). Besides, there were two bands at 607 and 561cm^{-1} characterized for vibration of PO_4^{3-} group in β-TCP (14). A band at 1653 cm^{-1} was assigned to bending vibration of water.

Table 1. Ionic concentrations of SBF solution versus human plasma (10^{-3} mol/l).

Ions	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}
SBF	142.0	5.0	2.5	1.5	148.8	4.2	1.0
Plasma	142.0	5.0	2.5	1.5	103.0	27.0	1.0

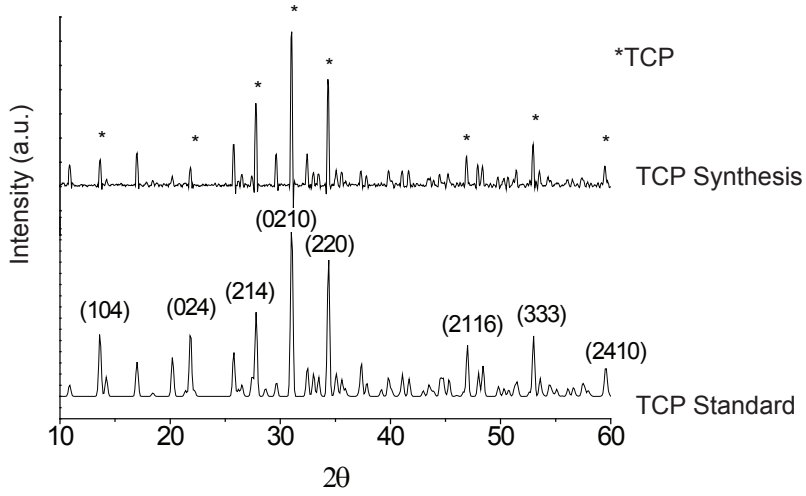

Figure 1. XRD patterns of synthetic TCP and standard TCP.

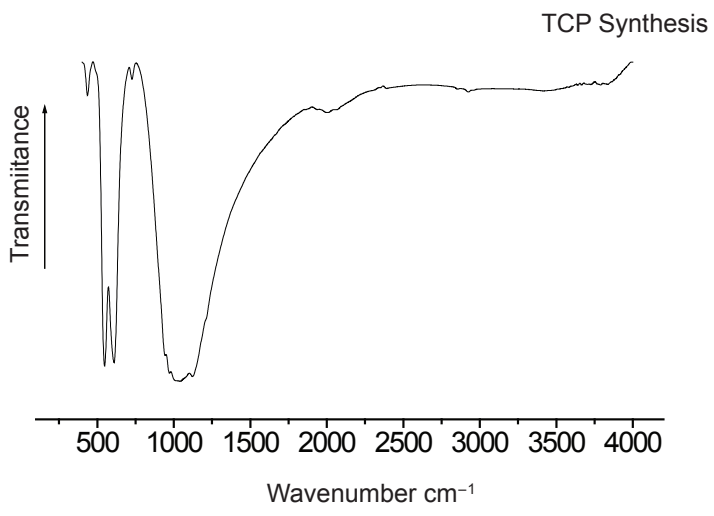

Figure 2. FT-IR spectra of synthetic TCP.

Figure 3 shows SEM micrograph of synthetic β-TCP at magnification (a) × 2000 and (b) × 5000. Synthetic β-TCP had an average diameter about 2 μm, with a cylindrical particle shape, matched with the hexagonal crystal structure of β-TCP.

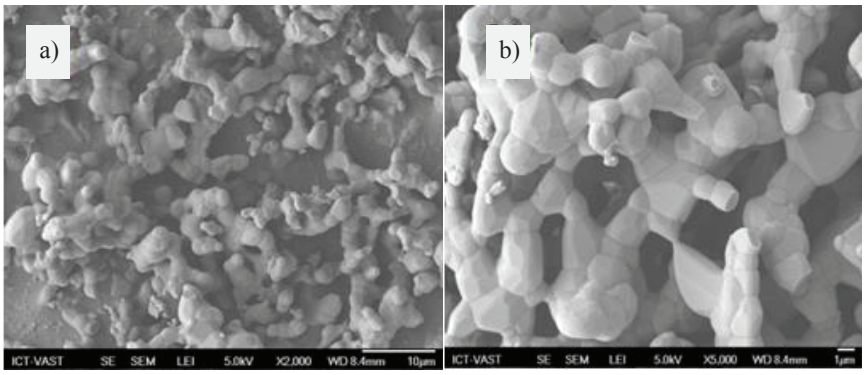

Figure 3. SEM micrograph of synthetic TCP.

Bioactivity of TCP Powder in in vitro Experiment

Figure 4 shows XRD patterns of β-TCP synthesis after 1, 5 and 10 days immersing in SBF solution. After 1 day, the peak number and peak shape of β-TCP did not change compared with initial XRD diagram. That demonstrated that β-TCP was not transformed nor decomposed to another matter when soaking in SBF solution. After 5 days of soaking in SBF, peaks of β-TCP shifted in position and changed in intensity. This demonstrated that there were chemical interactions between β-TCP material and SBF solution. These interactions would lead to the commute of β-TCP (beta-tricalcium phosphate) to HA (hydroxyapatite) versus time and continued until β-TCP completely transformed into HA (Mirta *et al.* 2012). Obtained result confirmed the bioactivity of β-TCP.

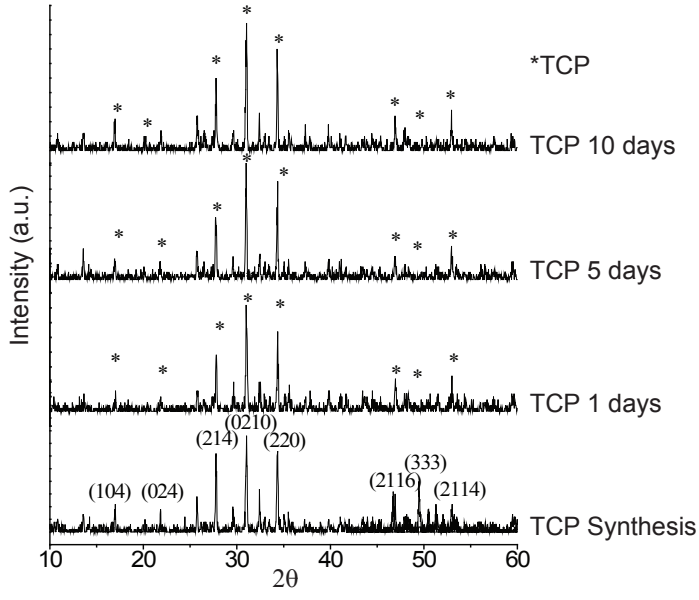

Figure 4. XRD patterns of TCP synthesis after 1, 5 and 10 days immersing in SBF solution.

Figure 5 reveals FT-IR spectra of synthetic β-TCP and β-TCP after 1, 5, ten days dipping in SBF solution. After one day in SBF solution, a band at 3433 cm^{-1} appeared, which characterized for hydrate OH^-, demonstrated that when soaking in SBF solution, β-TCP absorbed water. After five days, the band at 1042 cm^{-1} disappeared and replaced with the band at 1043 cm^{-1}, characterized PO_4^{3-} a group of HA (in the range of 1000–1100 cm^{-1}) (Mirta *et al.* 2012). That demonstrated that after 5 days in SBF solution, β-TCP had transferred one part into HA.

Figure 6 present SEM micrographs of synthetic β-TCP and β-TCP after 1, 5 and ten days in SBF solution. After one-day dipping in SBF solution, few small spots appeared on the β-TCP surface. After five days, these little spots developed into the small particle with different shape compared with the β-TCP shape. That was hydroxyapatite and was different in shape because of the different polymorphs. β-TCP had hexagonal polymorphs while HA had rhombohedral polymorphs.

CONCLUSIONS

This study presented a simple process to synthesize β-TCP powder via wet chemical precipitate method using tetrahydrate calcium nitrate and diammoni um hydrophosphate precursor. XRD patterns showed β-TCP had a good crystallization, and FT-IR favoured XRD data. In vitro experiment was carried out by dipping β-TCP powder in SBF solution. FT-IR demonstrated that there was a new apatite layer on the β-TCP surface and SEM micrograph showed that there was a particle of crystal HA appeared on β-TCP surface when dipped in SBF solution.

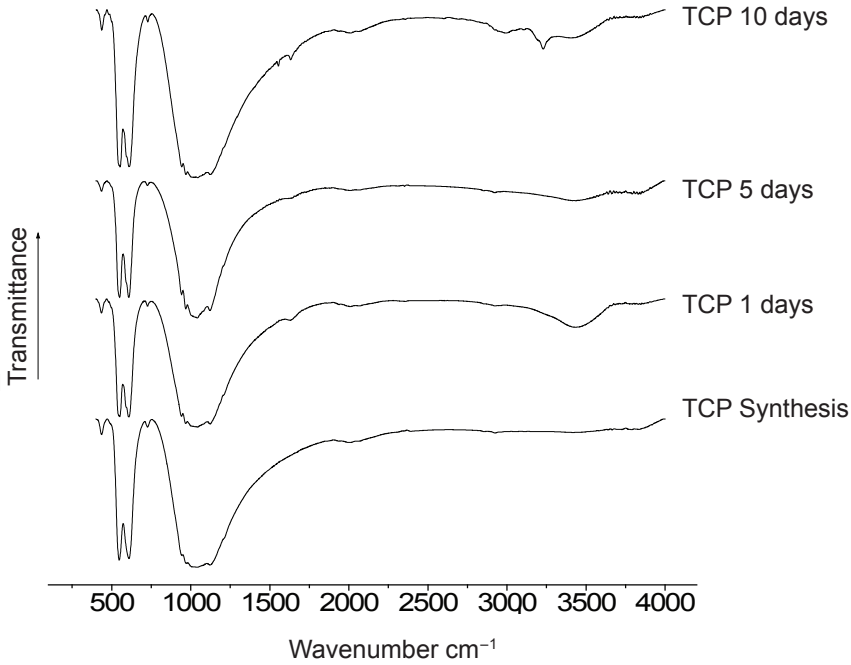

Figure 5. FT-IR spectra of synthetic TCP and TCP after 1, 5 and ten days of soaking in SBF solution.

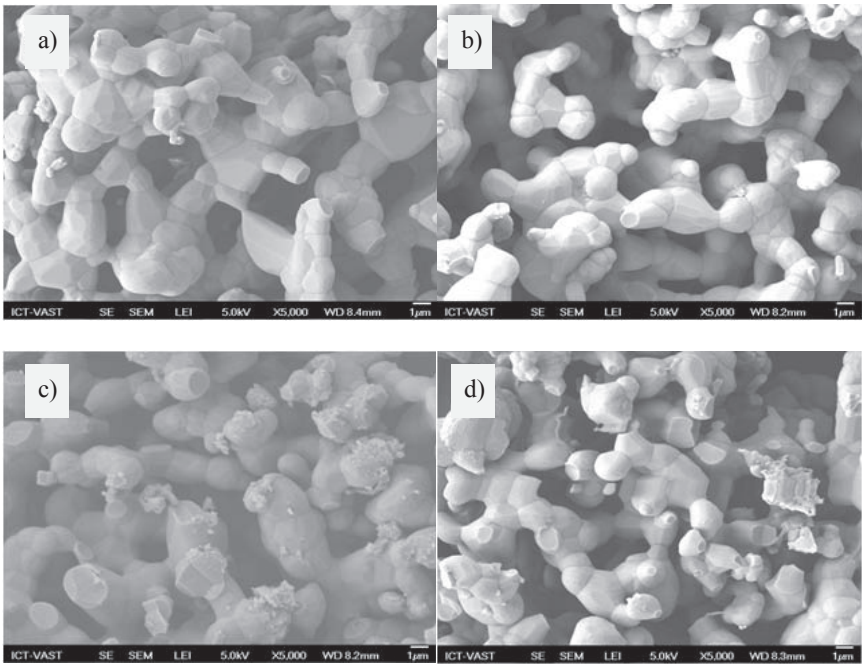

Figure 6. SEM micrographs of synthetic TCP (6 a) and TCP after 1, 5 and 10 days in SBF solution (6 b, 6 c, 6 d), respectively.

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