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# Calcium phosphate ceramic surface coating via precipitation approach

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**Abstract.** The biomimetic precipitation of calcium phosphate (CaP) coating was performed by soaking of  $\beta$ -tricalcium phosphate (TCP) ceramic scaffolds in supersaturated solution. Surface functionalization was obtained by incorporation of bovine serum albumin (BSA) as a model protein. The structure and phase composition were studied by application of XRD, FTIR, SEM, BET absorption. BSA incorporation content as a result of co-precipitation was evaluated.

## 1. Introduction

Calcium phosphates (CaPs) hold a significant place in number of biomaterials has been developed for bone tissue engineering. Being similar to mineral component of bone, the CaP based biomaterials are biocompatible, bioactive and can be processed to have osteoconductive properties [1-5]. Porous CaP scaffolds have been designed to permit vascularization and bone tissue ingrowth [2, 6, 7]. Increasing of surface area and porosity elevates cell adhesion and proliferation [8]. Functionalized scaffolds with biologically active molecules and drugs can be used for targeted drug delivery or osteoinductivity enhancement.

Dissolution and precipitation of bone mineral component occur during biomineralization process. It was already proposed that the amorphous calcium phosphate (ACP) forms under the biological conditions [9, 10]. It serves as a precursor to HAp and possess loss in long-range order and lattice imperfections [5]. It subsequently transforms to octacalcium phosphate (OCP), and eventually into HA with high degree of crystallinity [10]. OCP possess greater rate of resorbability and osteoinduction potencies [11, 12].

Based on biomineralization process the biomimetic approach was implicated to obtain the surface structure and composition similar to biological one [13]. Biomimetic precipitation of bioactive CaP coating from supersaturated ion solutions is controlled by interphase boundary processes [14]. The nucleation rate depends on degree of solution supersaturation, surface composition and condition. It was demonstrate, that ACP phase is the first-formed deposit in vitro at neutral pH and moderate supersaturation [15].

The biomimetic approach could be employed for bioactive molecules incorporation such as growth factor and drugs into the crystalline latticework [16, 17]. Salt and organic molecules ions involve in



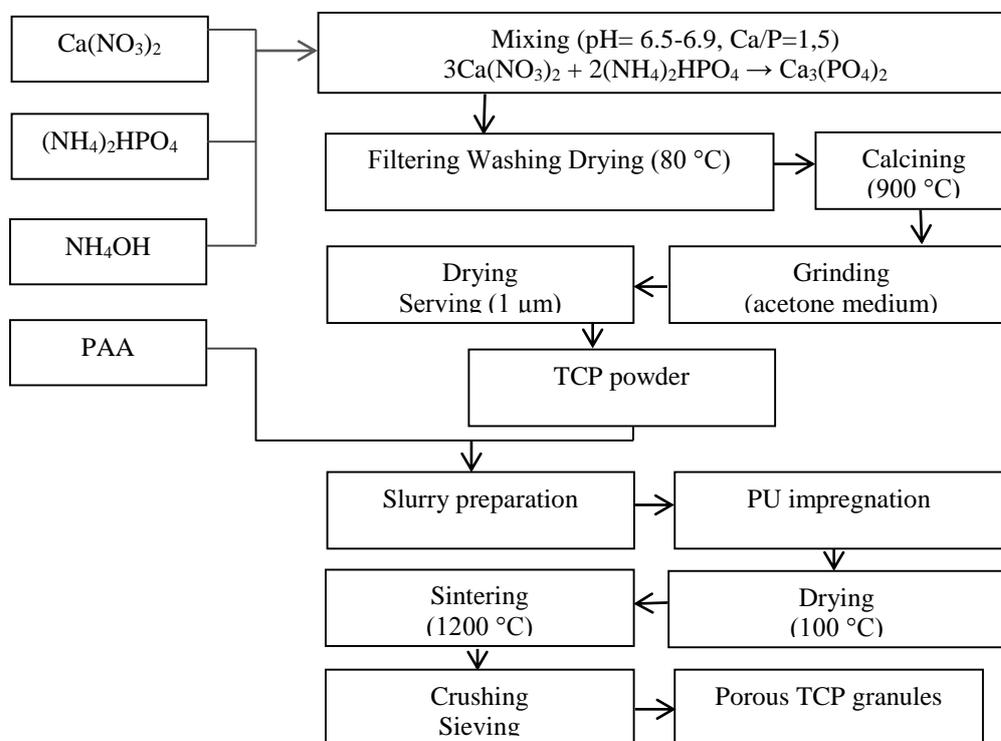
co-precipitation due to electrostatic interaction. It is proposed that drug incorporation into material structure allows its local release of therapeutic concentrations during a long time [18, 19].

This study was aimed to perform scaffolds functionalization by co-precipitation of CaPs and bioactive molecules from supersaturated solution. The tricalcium phosphate (TCP) scaffolds were used to perform biomimetic precipitation in double SBF solution. Scaffolds functionalization was appended by incorporation of bovine serum albumin (BSA) as a model protein. The composition and structure of resulted biomimetic coating at different soaking times were investigated. BSA incorporation effectiveness was evaluated on BSA content in materials.

## 2. Experimental

### 2.1. Fabrication of TCP scaffolds

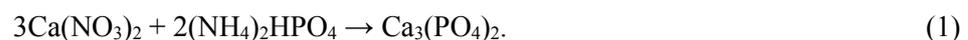
TCP porous granules of average size of 500-1000  $\mu\text{m}$  were used as ceramic scaffolds. Detail procedure and technological conditions for preparation of initial  $\beta$ -TCP powder and granules are shown in Figure 1.



**Figure 1.** Flowchart of TCP scaffolds fabrication.

#### 2.1.1. TCP powder synthesis

TCP powder were prepared by precipitation technique from aqueous solutions according to the reaction [20]:



Calcium nitrate tetrahydrate ( $\text{Ca}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$ ) and ammonium hydrophosphate ( $(\text{NH}_4)_2\text{HPO}_4$ ) were used as a source reagents. 0.5 M solution of  $(\text{NH}_4)_2\text{HPO}_4$  were mixed slowly with a 0.5 M solution of  $\text{Ca}(\text{NO}_3)_2$  in quantities according to the reaction (1) to achieve Ca/P ratio of 1.5. The pH level was kept at 6.5-6.9 by adding drop-wise of ammonia solution. After pH stabilization, the precipitate was filtered and dried at 80 °C during 24 h. Then the compact cake was crushed by using mortar and

pestle, calcinated at 900 °C and ground in a planetary mill in acetone medium. The obtained white suspension was then filtered and dried at 60 °C for 24 h. The as-dried powder was crushed and sieved through a sieve with a cell size of 100 µm. Resulted powder samples were calcinated at 1000 °C and then were examined by XRD for the main component and presence of impurities.

### 2.1.2. Scaffolds preparation

The procedure of scaffolds fabrication based on a method of porous polymeric sponge replica was used [21, 22]. The obtained TCP powder was added to polyacrylamide 10 % wt. water solution to prepare the ceramic slurry. Polyurethane (PU) reticulated foams of average pore size about 0.1-0.5 mm were used as porous matrices. The PU matrices were impregnated with TCP slurry, dried at 100 °C and completely burnt out during sintering at 1200 °C. Then the ceramics was crushed into a pieces and fraction of 500-1000 µm was isolated by sieving on a set of sieves to apply in subsequent experiments.

### 2.2. Formation of CaP coating

The CaP coating was produced by biomimetic precipitation of calcium phosphates from a double concentrated simulated body fluid (2xSBF, Table 1). The 2xSBF were prepared by successive mixing of preliminary prepared solutions of NaCl, NaHCO<sub>3</sub>, KCl, K<sub>2</sub>HPO<sub>4</sub>×3H<sub>2</sub>O, MgCl<sub>2</sub>×6H<sub>2</sub>O, CaCl<sub>2</sub>×2H<sub>2</sub>O, Na<sub>2</sub>SO<sub>4</sub> salts in distilled water. pH value of the mixed solutions was adjusted to 7.2–7.4 using TRIS buffer and HCl. Then the 50.0 mg of the materials were kept in 1.0 ml solution and incubated for 24, 72, 168 hours (1, 3, 7 days) at 37 °C and constant stirring.

**Table 1.** Inorganic composition of 2xSBF used for experiments (mMol/l)

Ion	Na <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Cl <sup>-</sup>	HPO <sub>4</sub> <sup>2-</sup>	SO <sub>4</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>
Content	284,0	10,0	3,0	5,0	295,6	2,0	1,0	8,4

### 2.3. Characterization of TCP scaffolds

Phase composition of the materials was investigated by X-ray diffraction analysis (XRD, Shimadzu) using CuKα radiation (average wavelength  $\lambda = 1.54183 \text{ \AA}$ ) on powdered samples within the 2θ range of 10–60 deg., step 2θ of 0.02 deg. and counting time 2 s/step. WinFit computer program [23] was used to evaluate the lattice parameters and average crystal size using Sherrer's equation. The surface of calcium phosphate scaffolds was investigated by scanning electron microscopy (SEM, Tescan Vega II SBU) with integrated Energy dispersive X-ray microanalyzer (EDX, INCA Energy). The IR-spectra of powdered samples were collected in the 400 – 4 000 cm<sup>-1</sup> spectral range with 4 cm<sup>-1</sup> spectral resolution on standard KBr pallets (FTIR, Avatar 330 Thermo Nicolet). The specific surface area ( $S_{BET}$ ) of ceramic scaffolds was measured by BET method (TriStar 3000, Micromeritics).

### 2.4 BSA incorporation procedure

The incorporation of bovine serum albumin (BSA, PAA) on the obtained TCP ceramics surface was performed by biomimetic precipitation from a supersaturated calcium phosphate solution (SCS) (table. 2). For this procedure 50.0 mg of the ceramic material was placed into a 1.0 ml of SCS and BSA solution at the concentration of BSA of 3.0 mg/ml (incorporation solution). The samples in closed plastic pellets were incubated at 37 °C during continual stirring for 48 h. The effectiveness of BSA incorporation was evaluated by measuring the protein concentration in incorporation solutions before and after materials soaking using BCA Protein Assay Kit (ThermoFisher Scientific, USA).

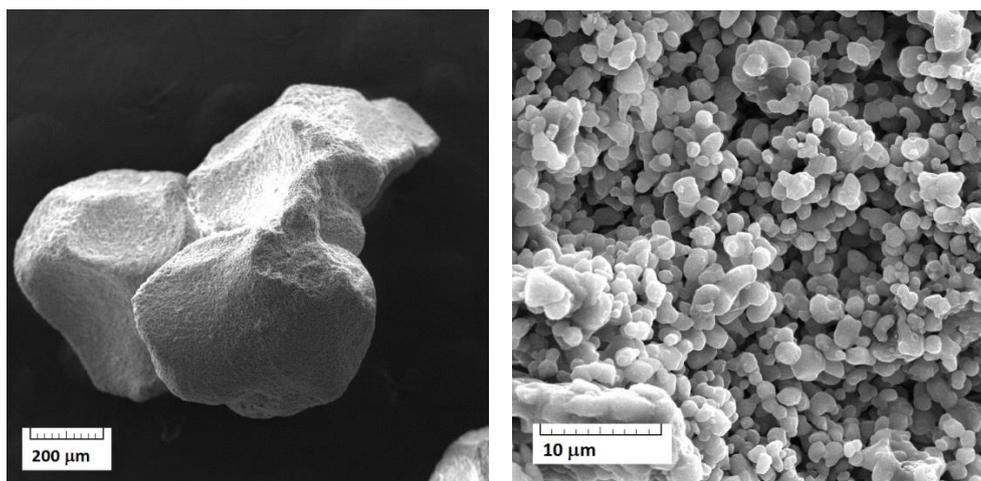
**Table 2.** Inorganic composition of SCS solution (mMol/l)

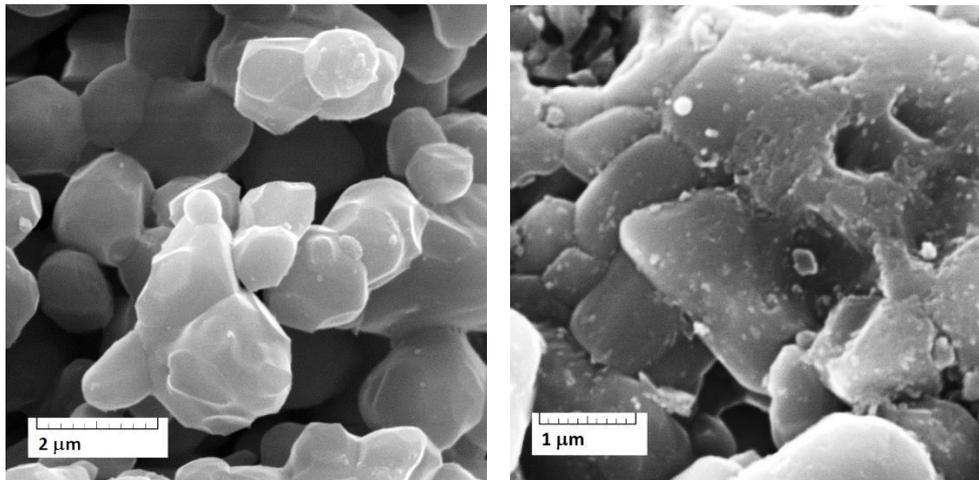
Ions	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Cl <sup>-</sup>	HPO <sub>4</sub> <sup>2-</sup>
Content	136,8	3,71	3,1	144,5	1,86

### 3. Results and discussion

The structure of initial scaffolds is shown in Figure 2. Irregular in shape granules are of size from 500 to 1000  $\mu\text{m}$  with open porosity about 40-50 %. Ceramics is composed of almost rounded shape crystals of 1-2  $\mu\text{m}$ , assembled in a porous net of the solid phase. Porosity is presented at several levels: large extended pores are of about 150-250  $\mu\text{m}$  in cross section, medium pores are up to 10-20  $\mu\text{m}$ , and small pores are up to 0.1-0.5  $\mu\text{m}$ .

Figure 3 demonstrates the surface structure of TCP scaffolds before and after soaking in 2xSBF during 7 days. On the surface of ceramics crystals there is an irregular coating of newly formed phase consisted of amorphous particles of average size from 20 to 100 nm. According to the EDX data, the new-formed layer consisted of calcium phosphate with Ca/P ratio of 1.5-1.7 enriched with sodium and potassium ions. It can be probably supposed the presence of precipitated calcium phosphate, probably ACP.

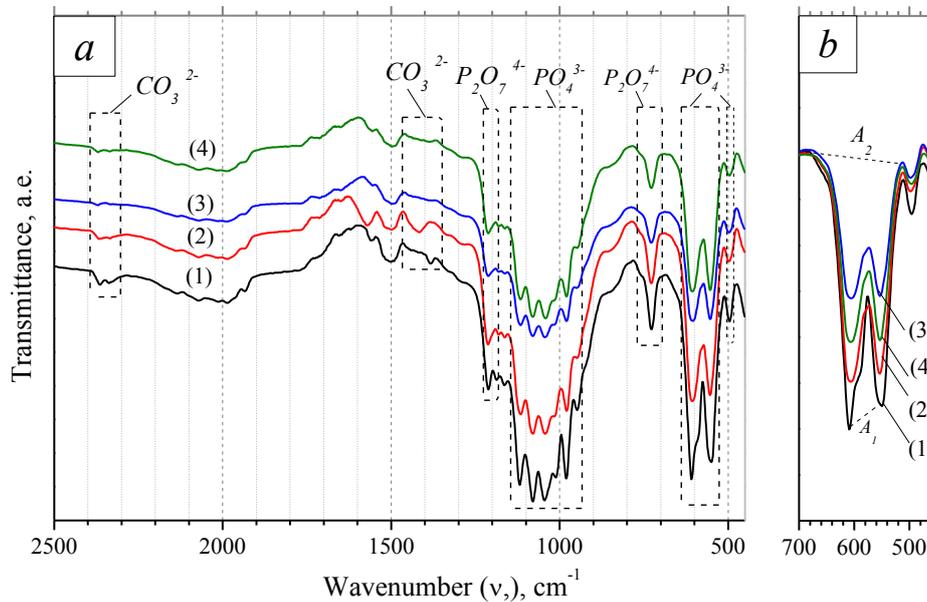
**Figure 2.** SEM-photographs of the initial TCP scaffolds (a) x50, (b) x10000.



**Figure 3.** SEM-photographs of TCP surface: (a) initial TCP and (b) TCP soaked in 2xSBF during 7 days.

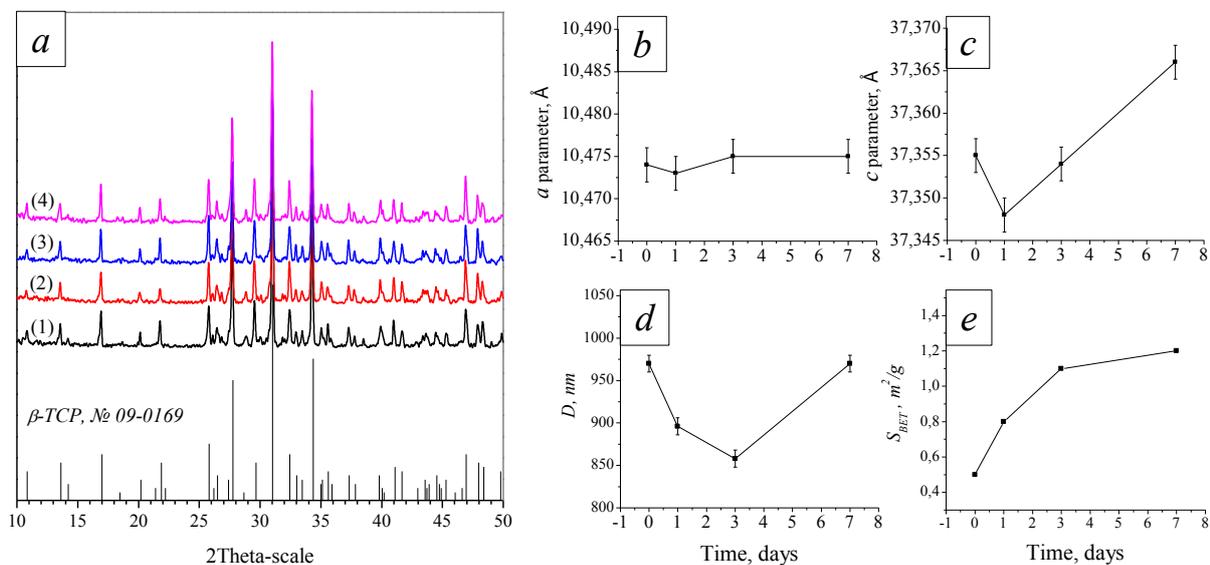
According to BET measurements, the specific surface area of scaffolds raised with soaking time increasing. Thus,  $S_{BET}$  of initial TCP was  $0.5 \text{ m}^2/\text{g}$ .  $S_{BET}$  of TCP soaked for 1, 3 and 7 were determined as 0.8, 1.1, and  $1.2 \text{ m}^2/\text{g}$ , respectively. In turn, this confirms the consistent formation of a fine-disperse crystalline layer on the ceramic surface during biomimetic precipitation and thereby enhancement of surface adsorption.

IR spectra of the materials showed a set of absorption bands typical to hexagonal ( $\beta$ ) TCP (Figure 4, a). IR lines of all samples displayed the intensive doublet at  $550\text{-}610 \text{ cm}^{-1}$  corresponded to the vibrations of  $\text{PO}_4^{3-}$ -groups, which are typical for the  $\beta$ -TCP structure. The phosphate group vibrations also showed up in bands at 948, 980, 1048 and  $1120 \text{ cm}^{-1}$  [24-26]. The bands at 727 and  $1214 \text{ cm}^{-1}$  corresponded to vibrations of  $\text{P}_2\text{O}_7^{4-}$ -groups and indicated the presence of calcium pyrophosphate  $\text{Ca}_2\text{P}_2\text{O}_7$  in small amount. However, the increasing of soaking time in 2xSBF up to 3-d day led to decreasing of intensity of the  $\text{PO}_4^{3-}$ -group lines, as well as decreasing of distances ratio  $A_1/A_2$  (Figure 4, b). This indicated a reduction of total content of the main TCP phase probably due to precipitation of amorphous calcium phosphate (ACP) on ceramics surface from salt solution. It contributed in material degree of crystallinity reduction [27]. Only the phosphate peak intensity level for the material soaked for 7 days was between the same for 3-d and 1-st day respectively, that probably showed the rising of crystal size up to 7-d day.



**Figure 4.** FTIR spectra of the initial TCP granules (1) and soaked for 24 h (2), 72 h (3); (a) – an overall view, (b) – detailing of the phosphate band ( $\nu_4$ ).

XRD analysis data of the initial scaffolds has revealed a single crystalline component  $\beta$ -TCP (Figure 5, a). All soaked in 2xSBF materials displayed an identical diffraction pattern. The coating formed during precipitation procedure was constituted of an amorphous phase that didn't produce diffraction of X-rays. The structure comparison was implemented according the X-ray data by calculating of the lattice constants and average crystallite size ( $D$ ), determined by Scherrer's equation (Figure 5, b-d). With soaking time increasing the  $a$ -axis parameter held on value about  $10.475 \pm 0.002$  Å, within the error. The  $c$ -axis parameter decreased from 10,355 to 10,348 Å within the first day of precipitation and rose strongly to the 7-th day up to 10,366 Å. Difference between lattice constants could be attributed to the prevalent growth direction of the deposited particles that have been along the  $c$ -axis. The average  $D$  size has monotonically reduced from 970 to 870 nm by the 3-d day, and then came to the initial level up to the 7-th day of precipitation. It also confirmed by the data of specific surface area of obtained materials, there was more intensive  $S_{BET}$  growth up to 3 days, by the 7th day the growth faded.



**Figure 5.** XRD and surface area data of TCP scaffolds at different soaking time.  
 (a) X-ray diffractograms of materials: (1) – initial, (2) – 1 day, (3) – 3 days, (4) – 7 days;  
 (b), (c), (d), (e) – respectively,  $a$ ,  $c$  parameters, average crystallinity size ( $D$ ) and the surface area data as the dependence of experiment time.

The BSA co-precipitation was performed on initial TCP scaffolds and TCP scaffolds with CaP coating produced by soaking in 2xSBF during 1day. The BSA incorporated content in ceramics was detected. It was found that during biomimetic co-precipitation a larger amount of BSA was incorporated on the surface of TCP scaffolds with CaP coating, 3.5  $\mu\text{g}/\text{mg}$ , whereas for the initial it was only 1.1  $\mu\text{g}/\text{mg}$ . The difference is over 3 times, that also confirms effectiveness of CaP coating formation for BSA incorporation. We propose that amorphous CaP coating enhance the interaction between ceramics surface and incorporation solution during co-precipitation.

#### 4. Conclusion

In the study the amorphous CaP coating on the surface of TCP ceramic scaffolds was obtained by biomimetic precipitation from 2xSBF. The resulted materials were investigated by SEM, EDX, XRD and BET adsorption. The amorphous CaP coating on the ceramic surface forms from the 1-st day of precipitation. It was revealed that crystallinity level of CaP coating was increased up to the 3-d day and became decrease up to the 7-th. Particle growth took place along the  $c$ -axis with constancy of  $a$  parameter during the precipitation process. The CaP coating formation on the ceramic scaffolds surface led to 3 times increasing of BSA incorporation effectiveness as a result of biomimetic co-precipitation. It could be conclude that CaP coating via biomimetic precipitation is beneficial approach to obtain the scaffolds functionalization.

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