The influence of immersion in buffer systems simulating body fluids on properties and morphology of octacalcium phosphate granules

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Abstract. Octacalcium phosphate is the precursor of biological apatite, that is why it is of special interest in bone regeneration due to its biocompatibility and biodegradability. This study was performed to examine the in vitro stability of octacalcium phosphate granules in physiological solutions. Powder X-ray diffraction (XRD), infrared spectroscopy (IR), plasma atomic emission spectroscopy (AES) and scanning electron microscopy (SEM) data were obtained before and after granules immersion. The variation of Ca\(^{2+}\) and HPO\(_4^{2-}\) concentration in the soaking solutions suggests that the material transformation occurs through octacalcium phosphate dissolution followed by hydroxyapatite precipitation.

1. Introduction

One of the most promising materials for bone repairing is a direct precursor phase of biological apatite – octacalcium phosphate (OCP, Ca\(_8\)H\(_2\)(PO\(_4\))\(_6\)•5H\(_2\)O), due to its high osteogenic capability and rate of resorption, close to speed of new bone tissue formation. However, the main direction in tissue development is creation bio-engineering structures with drugs or growth factors addition for directed bone healing, disease treatment and improvement implant’s osteoinduction and osteoconduction. Biomimetic deposition method suits the physiological temperature and pH requirements for infiltration biological agents [1].

Although this method was frequently used with hydroxyapatite (HA) [2, 3] and many other materials [4], there is no previous report of using it on OCP samples.

The synthesis of pure OCP must be carried out under strict controlled conditions to prevent hydrolyzation OCP to HA [5]. Temperature, pH, solid-to-liquid ratio, Ca/P ratio, ion composition and concentration effect on OCP [6].

In this work, modified technique of chemical transformation was used to prepare OCP granules by precipitation from solutions of calcium acetate (0.04 mol/L) and sodium hydrogen phosphate (0.04 mol/L). The pH value and solution temperature varied in the physiological ranges [7].

Then a certain amount of material was immersed into Supersaturated Calcification Solution (SCS) and Dulbecco’s Phosphate Buffered Saline (DPBS) solutions, imitating extracellular body liquids. The choice of solutions was based on physiological pH-value (~ 6.9 - 7.4), temperature (37 - 40 °C), buffers system composition and ions concentration. According to previous research results, buffer solutions...
should have physiological pH and Ca\(^{2+}\) (≈ 1.35 mM) concentration have to be lower than (PO)\(^{4-}\) (≈ 2.0 mM) for launch of the OCP transformation to HA [8].

In the course of the work, the process of deposition of the surface layer of calcium phosphates on the ceramic substrate of OCP was studied, and the kinetics of degradation and recrystallization of the surface layer of the material was evaluated. The microstructure and composition of precipitated phosphates were studied depending on the process conditions, including the ions composition and concentration of buffer solution, similar to extracellular body fluids and exposure time in a simulated physiological environment (44 days maximum).

2. Materials and methods

OCP granules were obtained by chemical transformation of tricalcium phosphate granules (α-TCP) in a solution based on sodium acetate, ranging in size from 1000 to 2000 μm [7]. As buffer solutions, supersaturated calcification solution (SCS) and Dulbecco’s Phosphate Buffered Saline (DPBS) were used.

Buffer fluids were prepared by dissolving appropriate quantities of the chemicals in deionized water. Reagents were added one by one after completely dissolved previous reagent in 800 ml of distilled water. Table 1 shows chemical composition and pH values of DPBS and SCS solutions.

The kinetics of degradation was evaluated according to Biological evaluation of medical devices - Part 14: Identification and quantification of degradation products from ceramics at a physiological temperature of 37 ° C, for 44 days (taking out samples at 1, 3, 7, 14, 28 and 44 days) [9]. Samples were taken, the granules were washed with distilled water and dried for a day, after which they were sent for studies, including scanning electron microscopy (SEM), chemical analysis, X-ray phase analysis (XRD) and infrared spectroscopy (IR).

| Solution  | Ion  | Na\(^{+}\) | K\(^{+}\) | Mg\(^{2+}\) | Ca\(^{2+}\) | Cl\(^{-}\) | HPO\(_4^{2-}\) | pH |
|-----------|------|-----------|---------|------------|-----------|--------|-----------|----|
| SCS       |      | 136.8     | 3.71    | –          | 3.1       | 144.5  | 1.86      | 6.9|
| Concentration (mM) | | | | | | | | |
| DPBS      |      | 152.99    | 4.2     | 0.5        | 0.9       | 139.6  | 8.1       | 7.2|

3. Results and discussion

OCP granules were obtained from α-TCP granules by chemical transformation in the sodium acetate solution [7]. This method was used to transform granules of α-TCP into OCP at temperature not exceeding 40 °C. For this study, we used the samples with dimensions from 1000 to 2000 μm. Fig. 1 shows the X-ray spectra of the OCP used, showing only OCP and HA phase diffraction lines. The presence of HA as an impurity in OCP associated with the method of obtaining and properties of the material.
IR-spectroscopy results presented in figure 1 revealed specific infrared spectra of octacalcium phosphate. The spectrum of sample exhibits a strong bands of the $\nu_1$ mode of HPO$_4^{2-}$ groups at 1023 cm$^{-1}$ and the $\nu_4$ mode of the PO$_4^{3-}$ groups at 601 cm$^{-1}$. A typical feature of OCP observed in the spectrum of this sample is the presence of intense bands at 1138–1023 cm$^{-1}$ corresponding to the $\nu_3$ mode of HPO$_4^{2-}$ and PO$_4^{3-}$ and medium intensity bands at 601-560 cm$^{-1}$, which is the $\nu_4$ mode of PO$_4^{3-}$. The band at 877 cm$^{-1}$ was shared by HPO$_4^{2-}$ and CO$_3^{2-}$ due to CO$_2$ from the atmosphere.

The crystal morphology of OCP granules presented in figure 2 (a). Crystals of OCP samples is composed of needles, which are less than 1 μm thick and up to 10 μm long.

![Figure 1. XRD patterns (a) and FT-IR spectra (b) of prepared OCP](image1)

![Figure 2. SEM images of the surface morphology OCP: a) before the experiment, b) after 44 days in SCS, c) after 44 days in DPBS.](image2)

The powder X-ray diffraction patterns of the samples from SCS and DPBS solutions for each immersion duration from control granules before the immersion till 44 day of the exposure are reported in figure 3. The phase ratio shifts towards growing HA phase compared to OCP with increasing exposure time in SCS buffer system. This transformation is possible in supersaturated solution, when Ca$^{2+}$ and (PO$_4$)$^{3-}$ ions concentration is sufficient for sedimentation of HA on the surface layer. Ions concentration
of calcium and phosphor decrease in the first 3 days and then Ca\(^{2+}\) ions start to increase due to high solubility of the OCP as shown in figure 4. As presented in figure 5, the loss of mass is visible only at the first day and then became stable, because of degradation and recrystallization processes on the surface layer of OCP. SEM image of OCP granule in SCS (figure 2 b) show the heterogeneous morphology of material after 44 days in solution. Ion exchange with the rest material is stabilized by the 3rd day and tends to equilibrium.

In the DPBS solution, the dependence of the mass loss is close to linear, which may be due to the absence of calcium and magnesium ions and, accordingly, an excess of phosphorus ions (figure 7). The HA phase in DPBS does not increase (figure 3), that can be related to the presence of magnesium ions.
and the lack of phosphor ions, which contribute to HA precipitation. That is why the ion concentrations of Ca$^{2+}$ and (PO$_4$)$_{3}^{-}$ ions remain unchanged during the experiment (Fig.6).

4. Conclusions
In the study, changes in the microstructures and properties of octacalcium phosphate granules are reported, when exposed in buffer solutions of various ionic composition, simulating the extracellular body fluids. The ionic concentration of the buffer solution relations with changes in the initial characteristics of OCP material are shown. The influence of time on the kinetics of degradation, phase composition and morphological characteristics of calcium phosphate phases, including the microstructure and properties of material was studied. The results show that properties and degradation of octacalcium phosphate depend on many different factors and by using solutions with special ions concentration the surface transformation could be controlled.

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