MODIFICATION OF HYDROXYAPATITE BY ANIONIC SUBSTITUTION

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Introduction

The healing of bone defects requires materials that enhance regeneration of bone tissue. Hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) is often used as filler of bone defects due to its biocompatibility, bioactivity and osteoconductivity. Application of HAP substituted with anions such as F^-, Cl^- or CO_3^{2-} instead of pure HAP could bring additional benefits such as enhanced cell proliferation and differentiation [1].

Fluorine is an essential trace element in bone tissue, which can promote the crystallization of calcium phosphate and accelerate the mineralization process [2]. However, a high concentration of fluoride was suggested to reduce osteoconductivity and also to cause adverse effects, such as osteomalacia. Therefore, it is necessary to control the release of fluoride ions into the environment.

Aim of the study was to determine and compare some biological and physicochemical properties of anion-substituted apatite and pure HAP such as cytotoxicity, ion reactivity and level of fluoride releasing from granules.

Materials and Methods

Preparation of HAP and FAP

HAP and fluoride-doped apatite (FAP) granules were synthesized using sol-gel method. Granules of a size 0,2-0,3 mm were used in the study. 800 °C was chosen for calcination treatment of apatite precursor.

Ion reactivity assessment

Before the experiment, FAP/HAP granules and SBF solution were prepared and sterilized. 0,5 g of every granules were soaked in 10 ml of SBF for 28 days at 37°C. Every 2-3 days fluid was exchanged and examined for calcium and phosphates concentration.

Fluoride ion release test

Fluoride release was determined in PBS solution for 15 days. 1 g of each type of granules were soaked in 10 ml of PBS and kept at 37°C. Fluoride content was measured in defined time points (0; 1; 2; 4; 8; 12; 24; 48; 72; 96; 120; 240; 360 hours) with the use of fluoride ion selective electrode.

Mercury intrusion porosimetry

Pore size distribution and other physical parameters of the granules were evaluated using Autopore IV 9500 (Micrometrics Inc) mercury porosimeter.

Cell culture experiment

Cytotoxicity of the biomaterials was evaluated indirectly by means of fluid extracts obtained by immersing the test materials in a complete culture medium supplemented with 2% FBS under standard conditions: 24 h, at 37ºC in a humidified atmosphere of 5% carbon dioxide and 95% air. After 24 h exposure to the extracts, viability of cells was determined using MTT test.

Results and Discussion

FTIR spectra show effect of fluoride substitution on hydroxyapatite structure (FIG. 1).

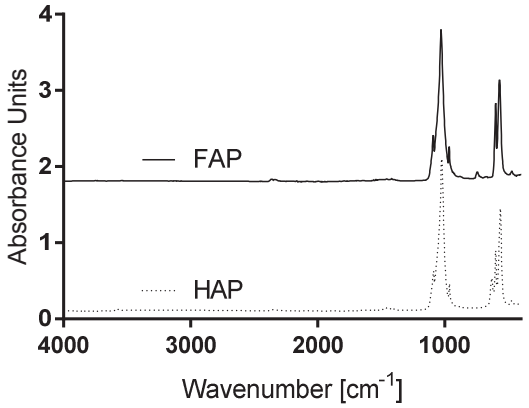

FIG. 1. FTIR spectra of hydroxyapatite and apatite modified by fluoride substitution.

Mercury porosimetry measurements revealed higher bulk density and lower porosity of FAP than HAP granules.

Ion reactivity assessment showed that both materials decreased concentration of Ca^{2+} and PO_4^{3-} in SBF solution, while HAP exhibited greater uptake of these ions than FAP.

Fluoride release profile from the FAP to PBS medium with a pH value of 7.4 revealed high release rate in the first 48 h of the experiment. Maximum concentration was 0,5 ppm.

Cell culture experiment proved the significant differences between cytotoxicity of FAP and HAP granules (FIG. 2). FAP extract did not cause significant changes in cell viability after 24 and 48 hours, while HAP reduced cell viability to the level 70-80% of control.

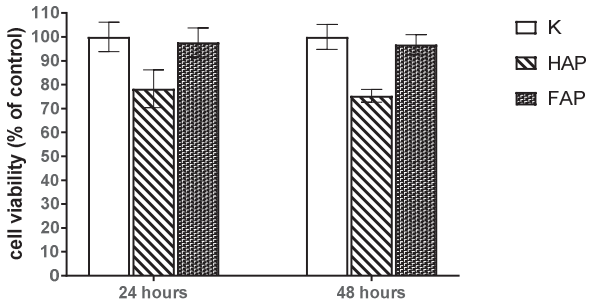

FIG. 2. Cytotoxicity evaluation of the granule extracts with the use of MTT test against human osteoblasts (hFOB 1.19).

Conclusions

In the present study, apatite modified by anionic substitution was synthesized and its properties were compared with pure hydroxyapatite. On the basis of obtained results, we suggest that fluoride-substituted apatite is a promising material for the use in bone tissue engineering.

Acknowledgments

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