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INTERACTION OF BIOMATERIALS CONTAINING CALCIUM HYDROXYAPATITE/POLY-L-LACTIDE WITH THE SIMULATED BODY FLUID

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The purpose of biomaterials is to replace a part or a function of the body in a safe, physiologically and economically acceptable way. The process of the reconstruction of bone defects has always been a big problem in orthopedics and maxillofacial surgery. Since hydroxyapatite (HAp) was detected as a component, the predominant constituent and the integral element of Mammalian bones, the development of the phospate ceramics as potential materials for implantation was enabled. This study investigated whether and in which way biomaterial calcium hydroxyapatite/poly-L-lactide (HAp/PLLA) interacts with the ionic composition of the human plasma. The simulated body fluid (SBF) is an artificial fluid that has the ionic composition and ionic concentration similar to the human blood plasma. HAp/PLLA was incubated for 1, 2, 3 and 5 weeks in SBF. The surfaces of both treated and untreated materials were analyzed on a scanning electron microscopy (SEM), and were also exposed to the energy dispersive X-ray spectroscopy (EDS), while SBF was submitted to the measuring of pH and electrical conductivity. However, our results indicate that the degradational changes of the material HAp/PLLA in SBF start from the surface of the treated material and that observed changes are the consequence of dissolution of its polymer component and the precipitation of the material similar to hydroxyapatite on its surface. This material shows good characteristics that place it among good candidates for the application in orthopedics and maxillofacial surgery. *Acta Medica Medianae 2011;50(4):35-39.*

Key words: *biomaterials, HAp/PLLA, SBF, SEM, EDS*

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Introduction

The purpose of biomaterials is to replace a part or a function of the body in a safe, physiologically and economically acceptable way (1). The process of the reconstruction of bone defects has always been a big problem in orthopedics and maxillofacial surgery. Because of the fact that the hydroxyapatite (HAp) was detected as a component and the predominant constituent and integral element of Mammalian bones, the development of the phospate ceramics as potential materials for implantation was enabled. The experimental and clinical research have both shown that granules and powder of HAp can be succesfully applied for the purpose of the reconstruction of bone defects (2,3). Poly-L-lactide (PLLA), a polymer obtained sinthetically, shows properties that make it interesting to be combined with HAp, and HAp can be strengthened by fibers of PLLA (4,5), because HAp itself is rigid, and the elasticity and tensile strength of natural bone are made possible by presence of the collagen fibers. By varying the synthesis parameters what is obtained is PLLAs of different molecular weight, on which are directly dependent mechanical and physicochemical characteristics of such a composite material (6-8). Estimating the biocompatibility implies the evaluation of the effects of a physiological environment on biomaterial as well as material effect on the environment.

Simulated body fluid (SBF) is an artificial fluid which has ionic composition and ionic concentration almost identical to the human blood plasma (9). Therefore, the testing of biomaterials in such enviroment, that provides the insight into the changes in materials caused by the ionic composition of blood plasma, represents simple and easy method for the testing the stability of the material in the body.

Aims

In this study, the interaction between biomaterial calcium hydroxyapatite/poly-L-lactide

(HAp/PLLA) and the simulated body fluid SBF was investigated, in order to determine whether and to what extent the ionic composition of the human plasma leads to the changes in the material mentioned above.

Materials and methods

Biomaterial: Powder of composite biomaterial HAp/PLLA was synthesized according to the procedure the detailes of which are shown in the work of Ignjatovic et al. (10). Polymeric component of material was represented by poly-L-lactide with molecular weight of 430.000 g/mol. For the purpose of this research, we used tubes of 10mm length, and 1mm width, made by mixing powder of HAp/PLLA with chloroform and passing through a biopsy needle. Just before the beginning of the experiment, the tubes were sterilised by submersing into 70% ethanol, during the period of 1 hour. Each of the tubes has been submersed in 10cm^3 of SBF, and incubated 1, 2, 3 and 5 weeks on 37 °C.

Simulated body fliud (SBF): The Simulated body fliud was prepared according to Kokubo protocol (11), by dissolving following ingredients into distilled and dionized water (500cm^3): NaCl (4.0175g), $NaHCO_3$ (0.1775g), KCl (0.1125g), K_2HPO_4 (0.1155g), $MgCl_2 \times 6H_2O$ (0.1555g), 1mol/dm HCl (19.5cm^3), $CaCl_2 \times 2H_2O$ (0.19339g), $Na_2SO_4 \times 10H_2O$ (0.0816g) and Tris (3.059g). pH of dissolution was buffered at 7.4 by adding the solution of 1 mol/dm3 HCl.

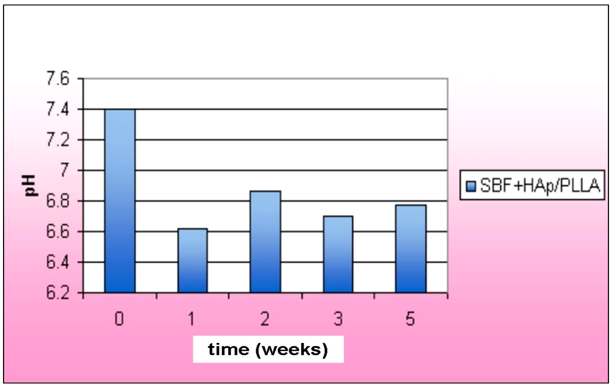
Physicochemical tests: SBF in which the samples were incubated was submitted to pH and electrical conductivity measurements. The pH value of the SBF was measured with pH meter Metrohm 827 pH lab at 37°C. Electrical conductivity of SBF was measured with Hanna EC-215 Conductivity Meter at 37°C.

SEM-EDS: The morphological changes of the material were examined by the scanning electron microscope (SEM, JEOL 5300), while the qualitative changes in the material were examined by SEM-EDS analysis.

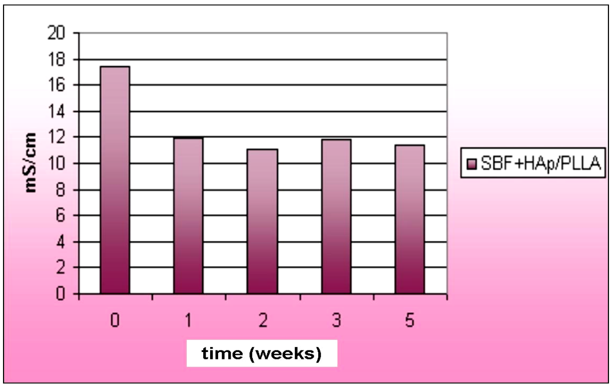
Results and discussion

Immediately after the material soaking, the initial pH value of SBF was 7.4. After one week of Hap/PLLA incubation in SBF, pH value decreases to 6.62. During the process of further incubation, the pH value of solution remains acid and oscillates around the values measured after the first week (Graph. 1).

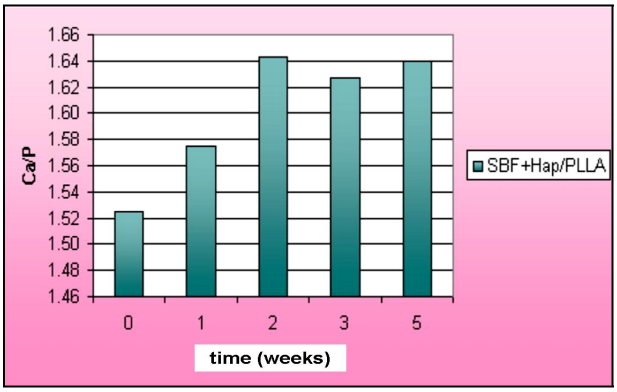
Poly-L-lactide shows spontaneous hydrolysis in aqueous solutions, in which releases lactic acid. Since SBF was proved to be the aqueous solution, during the further incubation changes in the pH values of SBF from the weak alkaline to the low-acid were expected (12).



Graph 1. Dependence of pH in function of time



Graph 2. Dependence of electrical conductivity in function of time



Graph 3. Ca/P ratio in function of time in SBF

The electrical conductivity of the solution is in a direct proportion with the ionic concentration of the present ions. The drop in the electrical conductivity of SBF from 17.4mS/cm to 11.89 mS/cm after one week of the incubation, indicates the formation of the weakly soluble compounds and their possible precipitation on the surface of the nanomaterial. Also, the obtained values recorded during the incubation of the electrical conductivity showed a similar trend as the pH-metric measurements–oscillation around the values recorded at the end of the first week (Graph 2). These results indicate that the first week of incubation is critical for the formation of weakly soluble precipitates and during the further incubation what is established is the dynamics of the ionic exchange between the material and SBF.

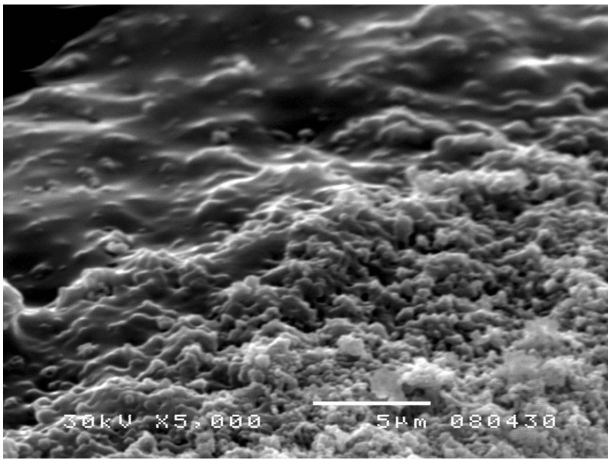


Figure 1. SEM micrography of HAp / PLLA after 1 week of incubation in SBF

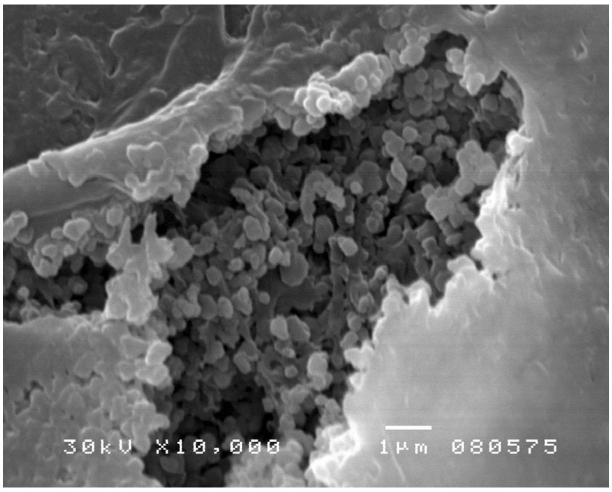


Figure 2. SEM micrography of HAp / PLLA after 2 weeks of incubation in SBF

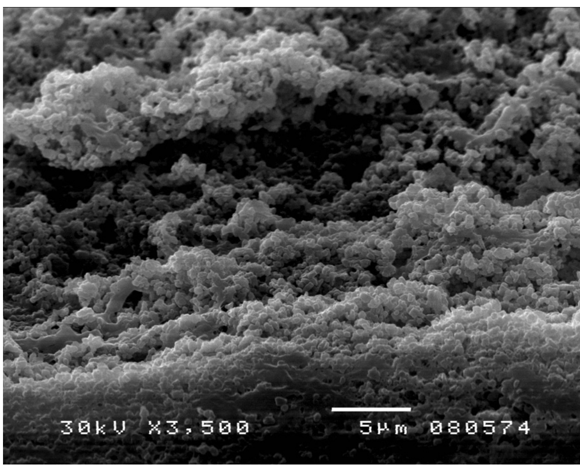


Figure 3. SEM micrography of HAp / PLLA after 3 weeks of incubation in SBF

SEM analysis of the untreated material reveals tubes of relatively smooth surfaces. No visible cracks or erosion were observed, which may indicate the compactness of material structure. On the surfaces of the treated materials, we observed fleet, wide, irregular-shaped fields of the erosion, which bottom was covered with clearly visible granules of the HAp. We also noticed narrow cracks that penetrate deep into the materials. The number, size and depth of erosion and cracks increases with the duration of incubation in the SBF, which clearly indicates that degradation in SBF begins from the surface of the material and that the degradation is the consequence of chemical interactions between the components of SBF and the material components. (Figure 1, 2 and 3)

The Ca/P ratio of untreated HAp/PLLA obtained by SEM-EDS analysis was 1.53. By calculating the mean ratio of Ca/P for each sample, there is a slight upward trend in the Ca/P ratio during the incubation in the SBF (Graph 3.). Considering that the values of the individual measurements, and that the mean ratio of Ca/P for each sample were close to those recorded in different apatites (1.5 -1.75) (13), we are able to discuss about precipitation of apatite-like material on the surface of the treated tubes.

The cleveage of ester bonds in poly-L-lactide give rise to carboxylic groups, which can be transfered to carboxylate anions at the buffered solution at a pH value around 7.4. They provide a negatively charged surface which adsorb positive calcium ions in the surroundings. The local excess Ca^{2+} then induces apatite nucleation by combining phosphate ions in the vicinity. The apatite grew by consuming the Ca^{2+} and HPO_4^{2-} in the supersaturated solution, which could explain the results of our investigation. (14,15).

In order for an artificial material to be attached to the living bone, the layer similar to apatite must be formed first on its surface (16). As we demonstrated in our study, on the surface of this material in the environment that is similar to the human body, a layer similar to the apatite is formed. The results indicate that HAp/PLLA alone, or in the combination with the blood or bone marrow, may be an adequate candidate for the potential application in the orthopedics and maxillofacial surgery (17).

Conclusion

On the surfaces of the treated materials, what was observed were actually fleet, wide, irregular-shaped fields of the erosion, whose bottom was covered with clearly visible Hap grains. There were also narrow cracks that penetrated deep into the material. The number, size and depth of erosion and cracks increases with the duration of incubation in the SBF, which clearly indicates that degradation in SBF begins from the surface of the material and that the degradation is the consequence of chemical interactions between the components of SBF and the material components.

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INTERAKCIJA BIOMATERIJALA NA BAZI KALCIJUM HIDROKSIAPATITA /POLI-L-LAKTIDA I SIMULIRANE TELESNE TEČNOSTI

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Svrha biomaterijala je da zamene deo ili funkciju dela tela na siguran, fiziološki i ekonomski prihvatljiv način. Rekonstrukcija koštanih defekata oduvek je predstavljala veliki problem ortopedije i maksilofacijalne hirurgije. Otkrićem hidroksiapatita (Hap) kao sastavnog i najzastupljenijeg dela kostiju sisara omogućen je razvoj fosfatnih keramika kao potencijalnog materijala za implantaciju. U ovom radu ispitivano je da li i na koji način biomaterijal kalcijum hidroksiapatit/poli-L-laktid (HAp/PLLA) interaguje sa jonskim sastavom ljudske plazme. Simulirana telesna tečnost (SBF) je veštački napravljena tečnost jonskog sastava i jonske koncentracije bliske ljudskoj plazmi. HAp/PLLA je inkubiran 1, 2, 3 i 5 nedelja u SBF-u. Površina tretiranog i netretiranog materijala ispitivana je skening elektronskom mikroskopijom (SEM) i EDS analizom (Energy dispersive X-ray spectroscopy), dok je SBF u kome je materijal inkubiran podvrgnut merenju električne provodljivosti i pH vrednosti.

Dobijeni rezultati pokazuju da u SBF-u degradacija materijala HAp/PLLA kreće od površine materijala i da je uzrokovana rastvaranjem njegove polimerne komponente i precipitacije materijala sličnog hidroksiapatitu na njegovoj površini. Ovaj materijal pokazuje karakteristike koje ga svrstavaju u kandidate za primenu u ortopediji i maksilofacijalnoj hirurgiji. *Acta Medica Medianae 2011;50(4):35-39.*

Ključne reči: *biomaterijali, HAp/PLLA, SBF, SEM, EDS*