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Bioactive vitroceramic utilized in modern reparatory medicine

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The desire of reestablishing the human organism's normal functions imposes the utilization, in modern reparatory medicine, of bioactive materials such as tricalcic phosphate, hydroxyapatite, bioglasses and biovitroceramics. These kinds of materials are well accepted by the living organism (biocompatible), they can connect to the living tissue (bioactive) but they do not posses the adequate mechanical properties. If such materials are intended to be used for making large implants that have to support high pressure, like the hip prothesis, the solution is to cover biocompatible metallic materials (titan, special steel) with biocompatible and bioactive vitroceramic coatings, the metallic structure providing the needed high mechanical properties. This work proposes to obtain such a new biovitroceramic material, within the SiO_2-CaO-MgO-Li_2O-K_2O-Na_2O-P_2O_5-TiO_2-B_2O_3 oxide system, and to test it for its physico-chemical properties that present interest (dilatation coefficient, chemical analyze, X-ray diffraction, etc). Have also been effectuated non-citotoxicity and pH dynamics tests utilized a solution that imitates the human blood plasma (SBF) and biotests of cellular proliferation using osteoblaste cells cultures.

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1. Introduction

The problem of hard tissue implant is of high interest because a large number of people need these implants. Loss of bone tissue is due to a traumatism in an accident, illnesses or age. Lost bone can be replaced either by endogenous or exogenous tissue or by an artificial implant. The first case represents the best solution but it is limited by the small quantity of such tissue and by an additional surgery act, that represents an another trauma for the patient. Exogenous tissue implant can cause a rejection phenomenon and transmission of illnesses. The only convenient solution remains using artificial implants. A hard tissue implant is ideal when it fulfills three conditions: it is biocompatible, bioactive and has adequate bone-like mechanical properties. Certain metals and their alloys (Ti, Zr, Nb), and special steels with high Cr and Ni content are the most used materials for artificial hard tissue implants. But such materials are only biocompatible and not bioactive. From the biochemical reactivity point of view, these materials are considered almost inert because they do not bind biologically or chemically to the hard tissues where they are implanted, but they are simply surrounded by the conjunctive tissue that in time provokes an incorrect distribution of the stresses, followed by the loss of the implant. Moreover, certain degradation of these materials in the living body exists. The solution of this problem is to find certain compositions of glasses and ceramics [1,2] to be both biocompatible and bioactive. The superficial reactivity of these glasses and ceramics when they come in contact with the body fluids is their most important characteristic. As a result of some complex processes like leaching, partial solving and ionic interchange between the glass surface and body fluids, a very thin layer of apatite or hydroxyapatite begins to form. This layer, having the composition of the bone, achieves a very hard connection between the implant and bone. If such materials are intended to be used for making large implants that have to support high pressure, like the hip prothesis, the solution is to cover biocompatible metallic materials (titan, special steel) with biocompatible and bioactive vitroceramic coatings, the metallic structure providing the needed high mechanical properties [3-5].

Recently, Primatarova et al. [6] have shown that ion-beam pattering on various substrates yielded the growth of a sponge-like (HA) hydroxiapatie layer on the non-ion implanted areas, and a flat dense layer on the implanted areas.

This work proposes to obtain a new biovitroceramic material, within the SiO_2-CaO-MgO-Li_2O-K_2O-Na_2O-P_2O_5-TiO_2-B_2O_3 oxide system, that can be used as coating for titan prothesis. Taking this into account, one of the important phisico-chemical properties which has been tested for the obtained material, is the thermical dilatation coefficient which has to be as close to that of the metallic support (titan), as possible to insure a good adherence between the two materials. Analysis of x-ray diffraction, chemical composition, density, etc. have also been effectuated.

Biologically, tests of non-citotoxicity and pH dynamics were effectuated, utilized a solution that imitates the human blood plasma (SBF) and biotests of cellular proliferation using osteoblaste cells cultures.

2. Materials and methods

The coating biovitroceramic synthesis was made by the classical method of melting using an electric furnace of superkanthal and raw materials of analytic purity. After

repeated melting essays in which modifications to the base composition were effectuated in the attempt to obtain a material that presents a dilation coefficient as close as possible to that of titan (the metallic support most often used in implant technology), the research focused on the 48 SiO_2; 10 CaO; 6 P_2O_5; 7 Na_2O; 7.5 K_2O; 2.9 Li_2O; 3.3 MgO; 13.2 B_2O_3; 1.3 TiO_2 (%wt.) oxide composition. To limit losses due to volatilisation, the phosphoric acid and the alkaline carbonates were excessevely used and preliminary mixes that were calcinated at 800 °C for 3 hours, were effectuated. The melting took place at 1300 ºC in alumina crucible. For the analysis were obtained prismatic samples which were annealed at 400 °C for three hours as well as glass in the form of frit.

The thermical dilatation coefficient within 20 - 300°C domain, the inferior (T_{ir}) and superior (T_{sr}) annealed temperature, the dilatometric relaxation temperature (T_D) and the vitreous transition temperature (T_g) (Table 1) were obtained utilizing a LINSEIS dilatometer with an amplifying factor of A=1000, the heating of the oven in which the sample was set (l_o = 40.12), taking place at 4 $^oC/s$.

A complete chemical analysis was realized in order to determine the real composition resulted from the melting process as well as an X-ray diffraction to identify the eventual crystallization peaks.

From the biologic point of view, using a digital PH-meter, were tested the pH modifications occurred in a solution that imitates the human blood plasma (SBF), in the presence of the synthesized vitroceramic. The SBF solution was prepared using the recipe proposed by T. Kokubo [7], which has the ionic concentration aproximatively equal to that of human plasma and the pH=7,4.

The aspect of the vitroceramic surface was visualized after the interaction with the SBF solution, by using a MOTIC® digital optical microscope. Tests of non-citotoxicity and of cellular proliferation [8,9] were effectuated by using osteoblaste cells cultures.

3. Results and discussion

The chemical analysis of the obtained material showed a composition close to that proposed and from the spectrographic analysis resulted a negligeable concentration of Al_2O_3, of 5×10^{-3} -1×10^{-2} %, due to the Al_2O_3 diffusion from the crucible used at melting. In Table 1 are presented the data obtained from the synthetised vitroceramic sample's thermogram.

Tabel 1.

Sample	T_{ir} (ºC)	T_g(°C)	T_{sr}(°C)	T_D(°C)	$\alpha_{27}^{307}\times10^{-7}$(°C^{-1})
1	430	470	480	510	95.09

A thermical dilatation coefficient ($\alpha\cdot10^{-7}$/°C) of 95.09, close to that of titan (89.49-90.16) which makes this vitroceramic suitable for usage as coating for titan prothesis. In its aspect, the obtained material is opaque and of white color.

The X-ray diffraction (Fig. 1) has showed a series of peaks attributed to some solid solutions, which were slightly moved compared to the peaks of some crystalline compounds such as : K_2TiO_3, Ca_2SiO_4, Ca_3SiO_5, $Ca_3Si_2O_7$, $Ca_7(PO_4)_2(SiO_4)_2$. This confirms the fact that the obtained material is, in fact, a vitroceramic.

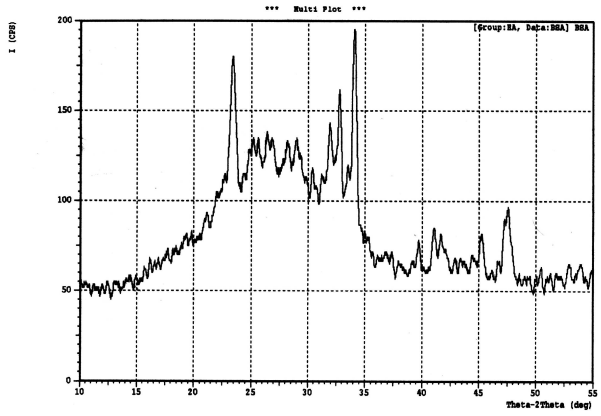

Fig. 1. The diffractogram of the obtained vitroceramic sample.

From the point of view of the biocompatibility and bioactivity tests, the vitroceramic's interaction with the SBF solution has shown the growth of hydroxyapatite crystals in carbonated form (HCA) on the surface of the samples, as it can be seen from the optical microscopy photos in Fig. 2-5. Also, in Fig. 6 is shown the variation of the pH in the SBF solution during a incubation interval of 30 days, at 37 ºC.

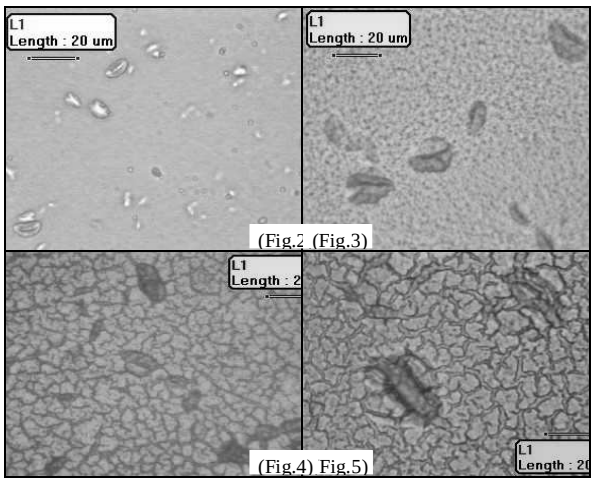

Fig. 2-5. The image of the HCA crystals on the surface of the vitroceramic samples after interacting with SBF solution, after 0 (Fig. 3), 8 (Fig. 4), 15 (Fig. 5) respectively 23 days (Fig. 6).

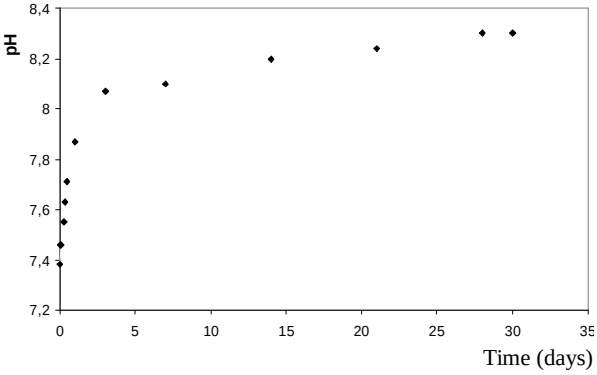

Fig. 6. Variation in the pH of the SBF solution in accordance with the incubation period when interacting with the vitroceramic sample.

Analysing the graphic from Fig. 7 it can be observed the shifting of the SBF sollution PH to more basic values with the increase of the imersion time of the sample. This phenomenon has a greater rate of evolution in beginning stages of up to 3 days of imersion, after which follows a decelleration of the process with tendencies of saturation. The variation with time of the SBF sollution's PH demonstrates that the vitroceramic material synthetised has the capacity of releasing in the implantation zone ions that accelerate the osteosynthesis process (Na^{+},Ca^{2+},P^{5+}) which gives the vitroceramic osteoinduction properties. The PH variation can also be charged to the biointeraction capability of the material with living tissue and can be a measure of the associated bioactivity degree.

The osteoblasts and the osteoclasts are specialised cells responsable for the formation and resorbtion of bones. In order to test the synthetised vitroceramic coating's biocompatibility with the bone tissue, the introduction of sterile fragments of the material was made in osteoblaste cells culture. The proliferation of human embrionary osteoblaste cells an the pieces of biomaterial was evaluated through the Digitally Optical Microscopy (DOM) analysis in phase contrast.

The proliferation of the osteoblaste cells on the surface of the samples with typical fusiform, elongated aspect (Fig. 7) resembling to the osteoblaste cells from the witness sample (Fig. 8) presented for comparison can be seen.

The samples' citotoxicity was indirectly evaluated by determination of the number of surviving cells (human embrionary osteoblasts) after the exposure to a toxic entity. The number of surviving cells was obtained by reducing the MTT-formazan colour agent and by determining the optical density at 570 nm compared to izopropanol.

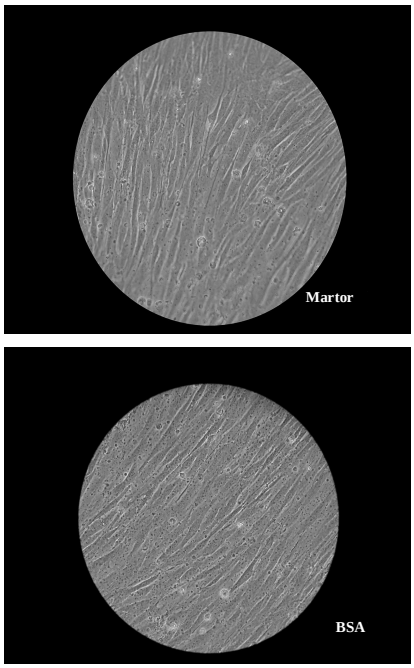

Fig. 7-8. DOM pictures, in phase contrast, of the osteoblastes in culture for the witness sample respectively for the coating vitroceramic(BSA) (×100).

The procentual viability of human embrionary osteoblasts in the presence of the vitroceramic material samples is presented in Table 2 compared to the witness sample.

Table 2.

Sample	DO_{570nm}	*% viability compared to the witness*
Witness	0.856	100
BSA	0.909	106

It can be seen that the vitroceramic material presents a very good biocompatibility with the bone tissue and even stimulates the formation of osteoblaste cells. The microscopic data for this sample (Fig. 8-9) correlate very well with those of citotoxicity (Table 2).

4. Conclusions

This work proposes to obtain a new biovitroceramic material that can be utilised in modern reparatory medicin as biocompatible and bioactive coating of metallic prothesis.

The synthetised vitroceramic is situated within the SiO_2-CaO-MgO-Li_2O-K_2O-Na_2O-P_2O_5-TiO_2-B_2O_3 oxidic system, and has been tested for the physico-chemical properties of interest (dilatation coefficient, chemical analysis, X-ray diffraction, etc) with good results.

From the point of view of the biotests the interraction manner with the SBF sollution (that imitates human blood plasma) was shown, evidenciating: growth of hidroxiapatite crystals in carbonated form on the surface of the samples and the evolution of SBF sollution's PH with time, when interracting with the vitroceramic sample.

Utilising osteoblaste cells cultures, there was also shown their proliferation on the surface of the samples and there was indirectly evaluated the citotoxicity of the vitroceramic by the determination of the number of surviving cells (human embrionary osteoblastes) after the exposure to a toxic entity.

In conclusion, the vitroceramic material obtained presents a very good biocompatibility with the bone tissue while also being bioactive.

The results of the tests for the newly obtained vitroceramic are comparable to the results presented in literature for similar vitroceramics and glasses, the utilisation of this material being, in the end, decided by the "in vitro" tests which are scheduled to be done.

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