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SYNTHESIS AND CHARACTERIZATION OF HYDROXYAPATITE-COLLAGEN BIOCOMPOSITE MATERIALS

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ABSTRACT

Hydroxyapatite-collagen composite is a prosperous biomaterial in reconstructive surgery for the reparation of defects within human hard tissue. Within this research, three-dimensional blocks of HAp/collagen composite biomaterial were synthesized by homogenizing the HAp/collagen mixture by cold and hot (T=60°C) pressing (P=49 MPa). The changes in the system were followed by using thermal analysis (DSC & TGA), FT-IR spectroscopy and scanning electron microscopy (SEM). The identification and kinetic analysis of appearing phase transition were done. FT-IR spectrum of the biocomposite shows that neither cold nor hot pressing had significant influence on the individual components of the composite. SEM of the fracture surfaces showed that more intimate contact between the phases happened during hot consolidation. The obtained results suggest that the native structure of collagen molecules was destroyed, but the primary structure was maintained. Such a mild modification of collagen molecules can possibly lead to elimination of antigenicity effects after implantation of this kind of biomaterial into human body.

1. INTRODUCTION

The development of hydroxyapatite(HAp)/polymer composites is considered to be one of the most interesting approaches to improved reliability and decreased stiffness of present HAp based biomaterials, as substitutes for hard bone tissue [1-12]. The properties of such composite biomaterial should also be biocompatibility, biodegradability, nontoxicity (i.e. sterility), as well as mechanical properties similar to the characteristics of natural bone. Considering that bone tissue consists mainly of HAp (78 weight %) and collagen (20 weight %), within this research, we have done the synthesis of biomaterial by using this two components. Biodegradability of such composite comes out from bioresorbility of polymer phase, i.e. characteristic of collagen molecules to degrade in body and freely involve in metabolic processes, without leaving any traces [2]. Within the process of synthesis, a special attention was paid on the influence of temperature and pressure on the possibility of producing compact HAp/collagen composites. Also, we studied degradation of collagen after the pressing of previously homogenized mixture of HAp and collagen. The relations between the characteristics of the material after cold and hot pressing, showed us the consequences of increased temperatures on thermal stability and eventual degradation of composite after synthesis. We have followed degradation or additional crosslinking of polymer phase by using FT-IR spectroscopy and thermal methods of characterization, while morphological changes of constitutive phases after hot and cold pressing of HAp and collagen was followed by using electron microscopy analysis.

2. EXPERIMENTAL PROCEDURE

HAp was produced by precipitation of $Ca(NO_3)_2$ and $(NH_3)_3PO_4$ in alkali environment, under well known procedure, described in literature, in details [11, 12]. Produced gel was dried at room temperature, and then calcinated at 1100 ^{o}C during 6 hours. Granules of calcinated, crystalline HAp with the size range from 0.2-0.5 mm were used to produce the composite material. Tendon derived collagen type II (Fluca, Germany) was used as polymer component (sample A). Composites were cold pressed at room temperatures (20°C, sample B) and hot pressed (60°C, sample C). Composites were firstly homogenized in mortar with pestle for 60 minutes, and then compacted with hot and cold pressing in cylindrical teflon dies (with diameter of 16 mm) and under pressures of 49 MPa. The samples were than pressed into pelets suitable for FT-IR spectroscopy analysis which was done on a Perkin Elmer device. The TG analysis was studied using thermal analyst Netzsch STA 409 EP. Thermal stability were studied by DSC (Du Pont thermal analyst 1090) at heating rate of 20°C/min. Microstructure of the HAp/collagen sample surface was studied by scanning electron microscopy (SEM) using a JEOL 5300.

3. RESULTS AND DISCUSSION

The spectra of a HAp/collagen biocomposite sample pressed at 20 and 60°C is shown in Fig. 1. The bands of triple structure on 585, 625 and 655 cm^{-1} which derive from the vibration of OH group of HAp, could be identified on both samples of the composite. Two absorption band peaks, one at 1060 cm^{-1}, and the other at 1100 cm^{-1} derive from PO vibrations within phosphate group of HAp.
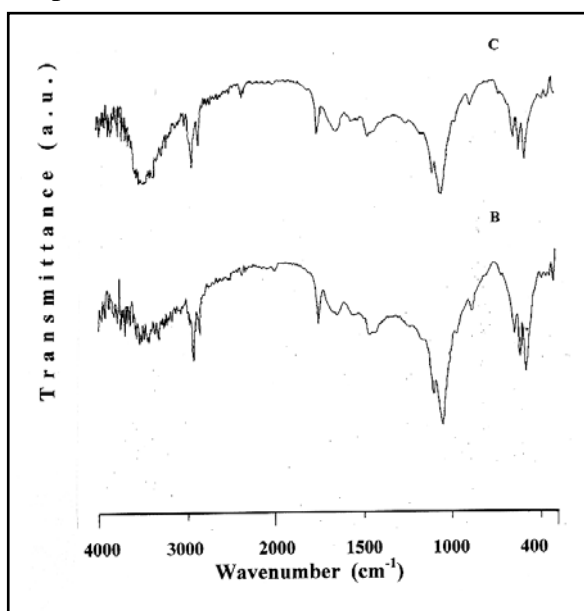

The both latter peaks cover the collagen derived peaks in the same frequency domain. The apsorption band in the range from 1400-1800 cm^{-1} are collagen derived: absorption band at 1460 cm^{-1} derives from stretching vibrations of C=O group, while the peak at 1650 cm^{-1} represents amid I bend, and apsorption band at 1760 cm^{-1} derives from the vibrations of carbonile group. It is possible to expect the appearance of symmetric and asymmetric bending vibrations of NH$_3$ group in the same domain as well as the appearance of symmetric and asymmetric stretching vibrations of C=O group. There were no intensive bands in the spectral range from 1800-2800 cm^{-1} which is a characteristic of all biological materials, and which is in accordance with previous measurements of individual components of these composites.

Figure 1. FT-IR spectrum of HAp/collagen composite biomaterials; (B) pressed at 20°C, (C) pressed at 60°C

Namely, neither HAp nor collagen showed any band in this range of IR spectrum. Two near vibrations bands at around 2900 cm^{-1} are collagen derived, and that is to say, from vibration of OH group, or which is less possible, from vibration of NH$_3$ group. Cold and hot pressed samples of the composite show almost identical IR absorption spectra, so based only upon the comparation of these absorption spectra, it is not possible to make a conclusion about possible structural and conformational changes happened under thermomechanical treatment during hot pressing. According to the obtained results, it is evident that the structural units of both components of the composite are maintained during mixing, homogenization and pressing (both hot and cold).
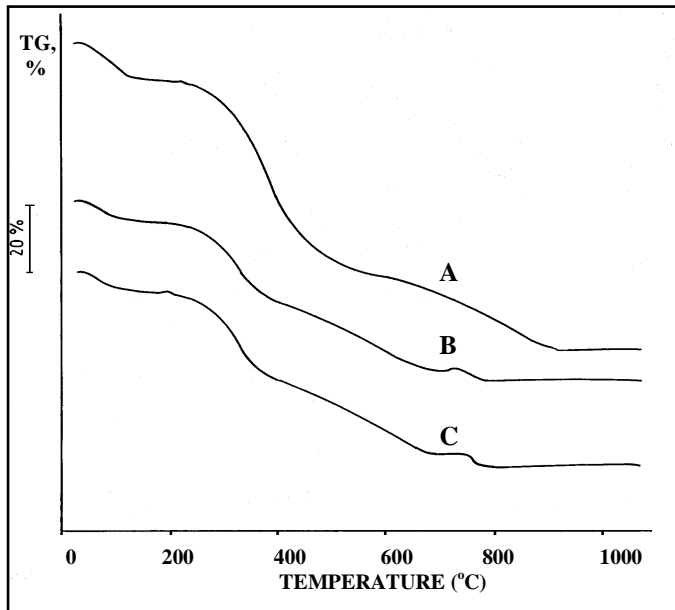
Samples A, B and C have nearly the same shape of TGA curve, which implies that complete loss of mass of the composite during its heating comes from the degradation of collagen (Fig. 2). At the same time, "pure" HAp did not show any loss of its mass during heating up to 1000°C. There did not come to more significant destabilization of collagen during the synthesis of the composite, because collagen within the composite had lost almost the same amount of quantity like alone heated collagen. This implies that there did not come to degradation of collagen during homogenization and pressing of the composite mixtures. The hot pressed samples had lost slightly more mass than cold pressed composites.

Figure 2. TGA curve of collagen (A) and HAp/collagen composite biomaterials; (B) pressed at 20°C, (C) pressed at 60°C

This small difference between the lost masses between cold and hot pressed composites does not allow us to make a precise conclusion about the influence of increased temperature in eventual degradation or additional stabilization of polymer matrix. Especially great loss of mass is observed in temperature range from 200-600°C where this effect is mostly expressed in temperature range from 200-400°C, where TGA curve shows the biggest inclination. The finish of collagen mass loosing process comes at 800°C at the composite and at 900°C at pure collagen sample. The loss of mass of pure collagen sample was not complete, because 5 % of soot remained in the container.

On the DSC diagram of collagen (Fig. 3), we have identified characteristic endothermic peaks at the temperatures of 70, 122 and 222°C as well as a complex endothermic peak with 2 separate maximums at 320 and 325°C. The appearance of 3 endothermic peaks is the characteristic of all proteins within DSC analysis. The destruction of collagen, i.e. the destruction of primary structure and consequent release of gases as products in the process of breaking covalent bonds in polypeptide phase, starts at around 290°C. This is evident on the basis of TG diagram (Fig. 2), where the loss of mass was followed, and on the DSC curve, where thermal degradation of collagen and release of gas products were observed above 290°C. Process of denaturation of collagen under influence of heating is defined with the largest and broadest endothermic peak, which appears at 122°C. A protein denaturation is a complex process, which requires breaking of Van Der Waals' and H-bonds within its native structure.
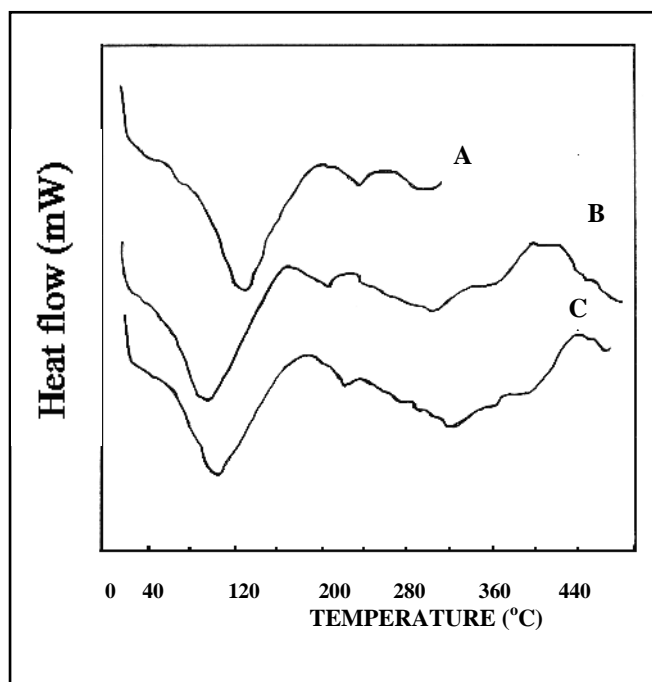

Figure 3. DSC curve of collagen (A) and HAp/collagen composite biomaterials; (B) pressed at 20°C, (C) pressed at 60°C

3

Heating of collagen leads to its dehydration. A part of the released water comes from hydroxyl groups of amino acid hydroxyprollin and as a result of thermal breakage of H-bonds which stabilize polypeptide chains.

Generally, denaturation of collagen starts with the first registration of difference in heat flux between sample and the reference sample, but according to the standard methodology, maximum of the denaturation peak is adopted as the temperature of denaturation. Collagen type II which we used in this work represents one of the most stable types of collagen, regarding its insolubility in water and increased thermal stability[13].

Effects of collagen heating can be reversible or irreversible. Mild heating results in local unfolding of polypeptide chain, which is reversible process, and so polypeptide chain returns to its initial, folded structure after restoring the normal (physiological) temperature. It is considered that these latter transformations occur due to the break of longer sequences of H-bonds, which stabilize protein helices; certain sequences along the molecule (between aminoacids residues 877 and 936) could be more sensitive to breakage of (sequential) H bonds. Heat induced breakage of crossslinking bonds between adjacent polypeptide chains can have a significant role in the process of denaturation as well [14]. During the second cycle of heating of the composite, a loss of all collagen derived denaturation peaks was observed, which means that collagen completely and permanently denaturated during the first cycle of heating up to the temperatures of 400°C and there did not come to its restructuring after returning to room temperature.

The following characteristic peaks appear on DSC diagrams of HAp/collagen composites: endothermic peak which is the result of collagen's denaturation and which appears at the temperatures between 89 and 119°C; and exothermic peak which is the result of present calcium phosphate and which temperature maximums are found between 353 and 443°C at different samples. Kinetic analysis showed us that denaturation of collagen is a reaction of first order, and its activation energy obtained by both Borhard-Daniel's and Piloyan's methods have an average value of 2,8 kJ/mol. On the other side, the phase transition between two crystalline phases of TCP is by the same method of kinetic analysis observed to be a reaction of first order with activation energy of 12,5 kJ/mol.

First of all, DSC measurements were carried out in order to indirectly follow the influence of cold and hot pressing on the structure of collagen (sample B and C) is shown in Fig. 3. Temperature maximums of collagen's denaturation peaks on the composites were shifted to lower temperature in comparison with pure collagen, which means that there did come to destabilization of collagen molecules during the pressing. In accordance with performed DSC measurements, we concluded that both cold and hot pressing have influence on the native structure of collagen, but do not have the influence on its primary structure, which was evident after FT-IR analysis, where all of the basic structure units of collagen remained conserved after thermomechanical treatment. Also, it was observed that the maximums of collagen's denaturation peaks on hot pressed composites were shifted to higher temperatures, comparing with the same temperature maximums on cold pressed composites. Thus, cold pressed composite HAp/collagen (sample B) has denaturation peak at 102°C (sample C) while hot pressed composite has denaturation peak at 109°C. Considering that cold and hot pressing partially destroy collagen's native structure, there could possibly be avoided antigenicity effect within organism in which thislike produced biomaterial would be implanted. Thus, such approach to synthesis of biocompatibile material could be proved as very prospective in the future.

The microstructure of composite biomaterials HAp/collagen pressed at 20°C and 60°C obtained by SEM is shown in Fig. 4 and 5. Fig. 4a shows a SEM image of the microstructure of HAp/collagen surface (sample B) pressed at 20°C. During watching the composite samples on SEM, it was observed that magnifies of more than 2000 times in order to make a photograph of the structure were not possible, because there did come to thermal degradation of focused collagen mnolecules by high-energy electron beams focused inside a small diameter. HAp granule coated by

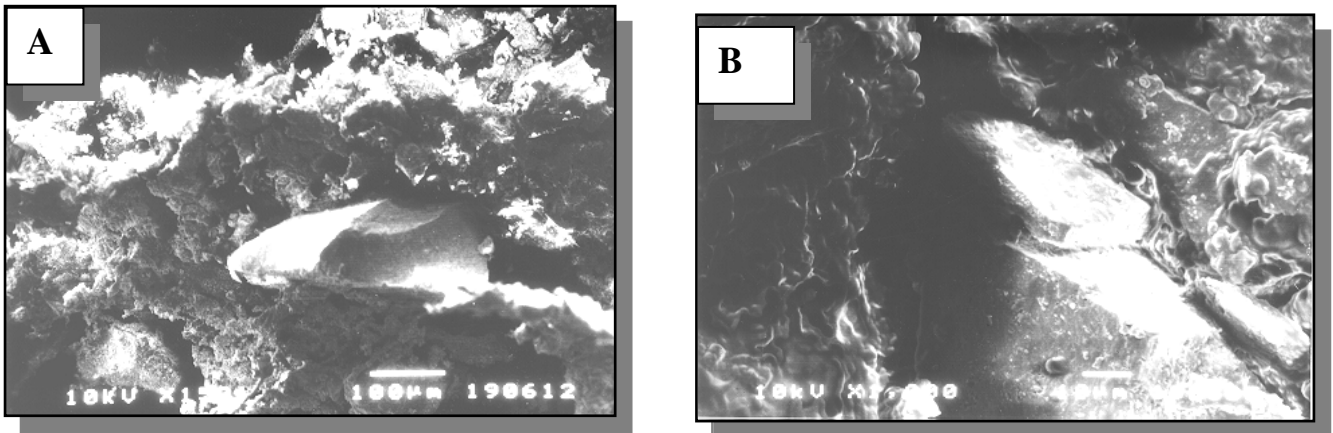collagen is show in Fig 4b. In this figure, non-intimate contact between HAp and collagen is obvious.

Figure 4. SEM image of microstructure HAp/collagen composite biomaterial pressed at 20°C: a) fracture surfaces; b) interface HAp-collagen
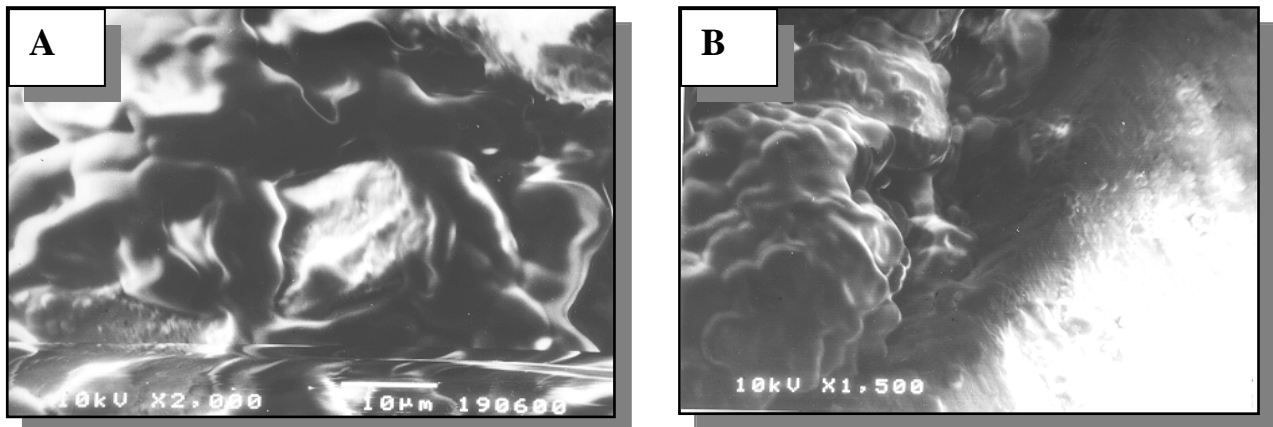

Figure 5. SEM image of microstructure HAp/collagen composite biomaterial pressed at 60°C: a) fracture surfaces; b) interface HAp-collagen

A more intimate contact on the interface between ceramic and polymer phase in hot pressed samples could be observed on enclosed SEM image in Fig. 5a. Also, higher porosity in cold pressed samples could be noted, and it is also expected from the fact that HAp did not incorporate completely inside the collagen matrix as well as they did within the hot pressed samples. Porosity is much lower within hot pressed samples because there did come to better merging of mineral phase and polymer matrix at increased temperatures as well as under higher pressures. An existence of very close interaction between HAp and collagen was observed on the interface of two phases, but higher magnifies in order to make a better and more precise interpretation of the physical nature of contact could not have been achieved, because, as was previously said, there did come to instant melting of parts of the collagen under the energy of electron beam. Contact between HAp and collagen is very close as shown in Fig. 5b.

4. CONCLUSION

HAp-collagen biocomposite material was synthesized by homogenizing the previously prepared mixture, and then by pressing the mixture at 20°C and 60°C under pressures of 49 MPa. We concluded that all of the basic structural units of collagen and HAp remained maintained after the synthesis of composite biomaterial. We came to this conclusion by observing infrared spectra of both cold and hot pressed composite, where all of the characteristic bands of individual components were identified. FT-IR results suggest that there did not come to destruction of the primary structure

of collagen. Within TG analysis, we concluded that the mass loss within the composite during its heating derives from the release of structural units of collagen in forms of evaporating gases. Within DSC measurements, the most distinctive phase transformations within composites were following peaks: endothermic peak at between 89°C and 122°C, which is the result of the denaturation of collagen; and exothermic peak, which is the result of the phase transition of TCP, which was present in small quantities inside the synthesized granules of HAp. Within the composites, collagen derived denaturation peak was shifted to lower temperatures in comparison with the pure collagen sample, which means that there did come to destabilization of native structure of collagen during the synthesis of the composite (i.e. by thermomechanical treatment). Scanning electron microscopy experiments showed that the contact between ceramic and polymer phase was much more intimate within hot pressed samples than within cold pressed samples.

REFERENCES

[1] L. L. Hench, Journal of American Ceramic Society 81 (1998), 1705 – 1728.
[2] W. Suchanek, M. Yoshimura, Journal of Materials Research 13 (1998), 94 – 117.
[3] E. Pissiotis, L. Spangberg, Journal of Endodontics, 16 (1990) 486 – 473.
[4] B. R. Constanz, S. Gunasekaran, US Patent (1995), 5455231
[5] K. Nishihara, US Patent (1999), 5 990 381.
[6] C. Gagnieu, F. Nicolas, G. Soula, US Patent (1998) 5 763 579.
[7] J. P. McIntyre, J.F. Shackelford, M. W. Chapman, R. R. Pool, Ceramic Bulletin, 70 (1991)
[8] S. H. Ree, J. D. Lee, J. Tanaka, Journal of American Ceramic Society, 83, November (2000)
[9] S. H. Rhee, J. Tanaka, Journal of Mmerican Ceramic Society, 81, (1998)
[10] C. Du, F. Z. Ciu, X. D. Zhu, K. De Groot, Journal of Biomedical Materials Research, 44 (1999) 407-415
[11] N. Ignjatovic, S. Tomic, M. Dakic, M. Miljkovic, M. Plavsic, D. Uskokovic, Biomaterials 20 (1999), 809-816
[12] N. Ignjatovic, M. Plavsic and D. Uskokovic, Advanced Engineering Materials, **2** (2000) 511-514
[13] I. A. Sicenikov, R. K. Abojanc, A. F. Dronov, L. P. Istranov, A. V. Nikolaev, A. B. Sehter, Medicina, Moskva (1978) 10-12
[14] W. Friess, G. Lee, Biomaterials 17 (1997) 2289-2294