

UDK 666.3-184: 532.74

Structure and Characteristics of the Hot Pressed Hydroxyapatite/poly-L-lactide Composite

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(Dedicated to Professor Dr. Drago Kolar in memory of a great scientist and a great man)

Abstract: Hydroxyapatite/poly-L-lactide (HAp/PLL) composite biomaterial can be obtained by different processing methods. Three-dimensional blocks of HAp/PLLA composite biomaterial with mechanical characteristics close to the natural bone tissue can be obtained by hot pressing procedure. Effects of synthesis and compacting on the structure and characteristics of the HAp/PLLA composite biomaterial were studied in this work. Using wide angle X-ray structural analyses (WAXS), differentially scanning calorimetry (DSC), thermogravimetric analysis (TGA) and infrared (IR) spectroscopy, the changes occurring in the material during synthesis and hot pressing were monitored. Surface microstructure was analyzed by scanning electronic microscopy (SEM) coupled with electron-dispersion analysis (EDX). The results obtained indicate a possible decrease in the degree of crystallinity with hot pressing time increase. A block of HAp/PLLA composite biomaterial with 1.6 times lower crystallinity of the polymer phase was obtained by hot pressing in a given time interval with a maximum of 60 minutes. Results of TG analysis show that PLLA stability decreases with increasing hot pressing time, and vice versa. IR study proved that neither destructive changes in constituents nor formation of new phases occurred during hot pressing.

Keywords: Composite Biomaterial; Hydroxyapatite/poly-L-lactide; Structure; Degree of Crystallinity.

Резюме: Композиционный биоматериал гидроксиапатит/поли-L-лактид (HAp/PLLA) можно получить с применением различных методов обработки. Методом горячего прессования получают трехмерные блоки композиционного биоматериала HAp/PLLA, механические свойства которых близки к человеческой костной ткани. В данной работе исследованы влияния синтеза и прессования на структуру и свойства композиционного биоматериала HAp/PLLA. С применением метода рентгеноструктурного анализа, дифференциальной сканирующей калориметрии, термогравиметрии и инфракрасной спектроскопии наблюдали основные изменения, проходящие в материале в ходе процессов синтеза и горячего прессования. Микроструктуру поверхности исследовали при помощи сканирующей электронной микроскопии с применением электрон-

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дисперсионного анализа. Полученные результаты указывают на возможность уменьшения степени кристалличности с продолжением времени горячего прессования. В интервале времени 60 минут нами получен блок композиционного биоматериала HAр/PLLA, в полимерной фазе которого кристалличность уменьшена на 1,6 раз. На основании термогравиметрического анализа установлено, что с продолжением времени горячего прессования устойчивость PLLA уменьшается и наоборот. Исследования методом инфракрасной спектроскопии показали, что в процессе горячего прессования не происходят разрушение конституэнтов, а также появление новых фазы не появляются.

Ключевые слова: Композиционный материал; гидроксипатит/поли-L-лактид; структура; степень кристалличности.

Садржај: Композитни биоматеријал хидроксипатит/поли-L-лактид (HAр/PLLA) може се добити применом различитих поступака процесирања. Поступком топлог пресовања могу се добити тродимензионални блокови композитног биоматеријала HAр/PLLA са механичким својствима блиским природном коштаном ткиву. У овом раду испитан је утицај синтезе и компактирања на структуру и својства композитног биоматеријала HAр/PLLA. Применом рендгеноструктурне анализе (WAXS), диференцијално сканирајуће калориметрије (DSC), термогравиметрије (TGA) и инфрацрвене (IR) спектроскопије праћене су основне промене које се дешавају у материјалу током синтезе и топлог пресовања. Микроструктура површине анализирана је скенирајућом електронском микроскопијом (SEM) уз примену електрондисперзионе анализе (EDX). Добијени резултати указују на могућност смањења степена кристалиничности са повећањем времена топлог пресовања. У анализираном временском интервалу топлог пресовања, са максималним временом од 60 минута, добијен је блок композитног биоматеријала HAр/PLLA у чијој се полимерној фази кристалиничност смањила за 1,6 пута. На основи TG анализе утврђено је да се са повећањем времена топлог пресовања стабилност PLLA смањује и обрнуто. IR истраживања утврдила су да током топлог пресовања не долази до деструктивних промена конститутивних компонената и појаве нових фаза.

Кључне речи: Композитни материјал; хидроксипатит/поли-L-лактид; структура; кристалиничност.

1. Introduction

Substitution and repair of hard bone tissue can be made by biomaterials which properties such as biocompatibility, non-toxicity, and numerous biomechanical ones have to satisfy the requirements of a defect to be repaired. Good adhesion of the surrounding cells with the biomaterial surface to establish a hard connection in the implant-tissue interlayer is a prerequisite for the successful repair.

Bone tissue repairs have been performed by now using a number of different biomaterials [1]. Ceramic/polymer composite biomaterials have mechanical characteristics very close to those of the natural bone tissue [2]. Reinforcement of the ceramic phase by bioresorptive polymers results in formation of bioresorptive composites, whose ceramic phase after application remain unchanged while the polymer resorbs with time leaving space to new body tissue formed [3].

Chemically synthesized calcium hydroxyapatite (HAp) is similar to the ceramic component of human bone tissue [4]. Bioresorptive polylactides (PLA) and their co-polymers belong to the group of nontoxic polymers, because the final products of their degradation (CO_2 and H_2O) enter without difficulty three-carboxylic acids cycle, not disturbing the metabolism of the surrounding tissue in the process [5]. Compared with pure hydroxyapatite, composite biomaterials of HAp/PLA type (or its co-polymers) induce, on their surface, formation of a larger number of osteoblasts necessary for repair of bone defects. Concentration of alkaline phosphatase, needed for osteoblast differentiation, is higher near the HAp/PLA composite biomaterial (or its co-polymers) than near pure PLLA [6]. Chemically synthesized HAp and poly-L-lactide (PLLA) enable on their surfaces good adhesion, differentiation and gene expression of osteoblasts, as well as absorption of proteins responsible for the adhesion [7].

Response of the organism to the composite biomaterial implanted depends on numerous factors; the most important among them are biocompatibility, porosity (size and distribution of pores), surface microstructure, etc. In addition, the behavior of HAp/PLLA composite biomaterial depends on the characteristics of HAp and PLLA, too. Bioresorption time of PLLA depends on the ratio and distribution of amorphous/crystalline phase in the polymer, their molecular weight, polymer porosity, etc. Generally, decrease in polymer crystallinity decreases the bioresorption time [8]. HAp/PLLA composite biomaterial was obtained by synthesis and subsequent polymerization or forging of a HAp and PLLA mixture [3, 9]. By hot pressing of HAp/PLLA at the PLLA melting temperature, we obtained blocks of required densities and mechanical characteristics [10-12]. The obtained blocks exhibit extremely high mechanical characteristics (compressive strength of 140 MPa, elasticity modulus of 10 GPa) [13]. During hot pressing of highly porous HAp/PLLA composite biomaterial and its transformation into nonporous material, physicochemical changes occur in the PLLA phase, while the HAp phase remains stable. PLLA molecular weight decreases during heat and mechanical treatment [14].

In this work, blocks of HAp/PLLA composite biomaterial were synthesized by hot pressing. The influence of hot pressing on the structure and characteristics of HAp/PLLA composite biomaterial was studied by several methods: X-ray structural analyses (WAXS), differentially scanning calorimetry (DSC), thermogravimetric analysis (TGA), infrared spectroscopy (IR) and scanning electronic microscopy (SEM) with electron-dispersion analysis (EDX). With the applied methods we examined the influence of hot pressing on the designing of degree of crystallinity and stability of the material, very important properties for *in vitro* and *in vivo* as well as for clinical use of this biomaterial.

2. Materials and methods

Calcium-hydroxyapatite was obtained by precipitation of calcium nitrate and ammonium phosphate in an alkaline medium according to the reaction:



The gel was dried at room temperature and calcined at 1100°C for 6 hours. HAp powder of a 100 nm average particle size obtained by grinding the HAp granules, was used as a constituent in processing of the composite biomaterial. Commercial poly-L-lactide (Fluka, Germany) with a molecular weight of 100,000 g/mol was used as a polymer component.

After complete dissolution of PLLA in chloroform, HAp powder was added and thus obtained mixture was vacuum evaporated at 10 MPa pressure, according to the previously described procedure [10].

The HAp/PLLA mass ratio in all samples was 80/20. Highly porous HAp/PLLA composite was then compacted by hot pressing using cylindrical dies 10 mm in diameter on a CARVER, Inc. Auto "C" Model 3889 press, at $176 \pm 1^\circ\text{C}$ temperature and 98.1 MPa pressure, for 30 and 60 minutes. Pressing was performed at the PLLA melting temperature. The amount of 2 g of HAp/PLLA composite biomaterial was poured into the die heated to a given temperature. Then, the given pressure was reached in 30 seconds; and the pressing performed for 30 and 60 minutes. After pressing, the pressure was lowered to the atmospheric for 20 seconds and the samples were extracted out of the dies during 1 minute.

Porosity, i.e., relative density, of the HAp/PLLA composite biomaterial blocks was determined geometrically from the mass to volume sample ratio, while the mechanical characteristics were tested using an INSTRON M 1185 instrument. Microstructure characterization was made by a JSM 5300 scanning electronic microscope coupled with a QX2000 Analyzer System (Oxford Instruments, UK) for the electron-dispersion analysis (EDX). For SEM analyses, the fracture surfaces of samples were covered by a thin layer of gold, under vacuum evaporation. Wide-angle X-ray structural (WAXS) analyses of HAp, PLLA and HAp/PLLA composite biomaterial blocks were made by a Philips PW 1710 diffractometer. The beam of x-rays was filtered with $\text{CuK}_\alpha\text{-Ni}$ at 0.1542 nm wavelength, the recording speed was $0.2^\circ/\text{min}$, in the range of angles of $2\Theta = (4.00-90.00)^\circ$. Calorimetric (DSC) tests were done by a Perkin Elmer DSC-2 differential scanning calorimeter in the 47-247°C temperature interval, with a heating rate of $20^\circ\text{C}/\text{min}$, in nitrogen atmosphere.

Enthalpy of melting obtained by DSC was proportional to the area below the peak that defines the melting of the PLLA polymer:

$$\Delta H = \int \frac{H}{t} dt = 1/v_g \int_{71}^{72} \frac{H}{t} dt,$$

where v_g is the heating rate.

Degree of crystallinity of the PLLA polymer was determined by the ratio of tested PLLA melting enthalpy to 100 % crystalline PLLA melting enthalpy:

$$\chi = \frac{\Delta H}{\Delta H_f},$$

where χ is the degree of crystallinity of PLLA, ΔH is the tested PLLA melting enthalpy, ΔH_f is the 100 % crystalline PLLA melting enthalpy (93.7 J/g) [9].

Thermogravimetric (TGA) analyses were made in a nitrogen atmosphere by a Perkin Elmer TGA-2 device, in the 20-500°C temperature range, with a heating rate of $20^\circ\text{C}/\text{min}$, in nitrogen atmosphere. Infrared (IR) spectra were obtained by the KBr pastille method in the 4000-400 cm^{-1} wave number range using a Specord 75 IR device.

3. Results and discussion

3.1. WAXS analysis of the hot pressing effects on the HAp/PLLA composite biomaterial structure

Effects of hot pressing on the structural changes occurring in the material, mostly changes in crystallinity of PLLA, were studied by WAXS.

HAp powder, whose roentgenograms and microstructure were examined in our previous papers [10-11], was synthesized by precipitation. Diffraction spectra of PLLA (Fig. 1a) have characteristic shape where, besides a wide peak corresponding to the amorphous phase, diffraction line at 16.89° dominates as well as three less intense lines at 15.41° , 19.11° and 22.43° . The results are in agreement with those reported by van der Witte [15]. WAXS confirmed the existence of a pure HAp phase (Fig. 1b) [14].

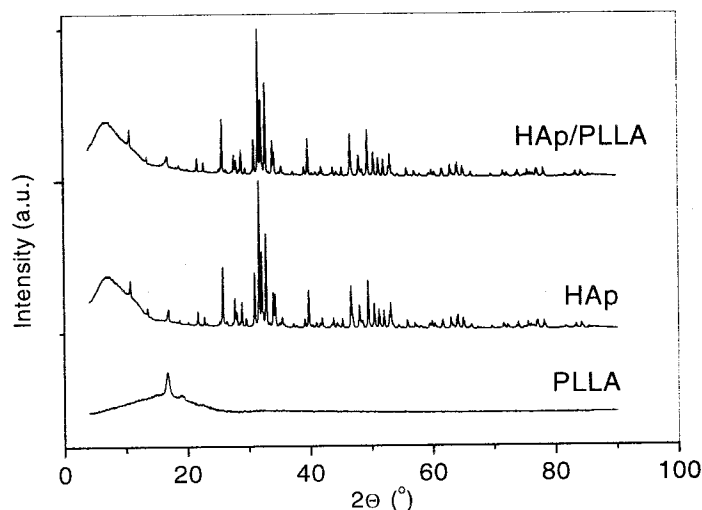


Fig. 1 Diffractograms of a) PLLA, b) HAp and c) HAp/PLLA composite biomaterial.

After complete dissolution of PLLA in chloroform and addition of HAp particles, a suspension was obtained subsequently vacuum evaporated and cold pressed. Diffraction spectra of as-obtained HAp/PLLA are shown in Fig. 1c. It is obvious that three very weak peaks arising from PLLA and its amorphous phase cannot be distinguished from the basic line, while the most intense peak lies in the very narrow angle range together with a doublet of diffraction lines of HAp. Therefore, changes in this intense PLLA peak as well as in two HAp peaks were analyzed and compared with the composite biomaterial spectrum. No changes in angles, intensities and profiles of the diffraction peaks were observed by comparing HAp diffraction spectra recorded before and after hot pressing. It shows that applied pressure and the presence of PLLA do not affect the HAp crystal structure (single cell symmetry and crystallite size). Therefore, only changes in PLLA peak, which is now easy to follow, indicating changes in PLLA during composite biomaterial compacting were analyzed.

Hot pressing of highly porous HAp/PLLA composite biomaterial at the PLLA melting temperature for 60 minutes, yielded a composite biomaterial of very high compressive strength of 140 MPa and elasticity modulus around 10 GPa [13]. No changes in HAp peaks were observed by comparing HAp/PLLA composite biomaterial diffraction spectra before and after 60 minutes of hot pressing (Figs. 1-2). Namely, all diffraction peaks of HAp in the composite biomaterial appear at the same angles and intensity to profile ratio values before and after 60 minutes of hot pressing. Differences observed in these spectra originate exclusively from the peaks characteristic of PLLA. This is in accordance with our previous findings when differential thermal analyses (DTA) were used to study the changes in PLLA phase and stability of HAp during hot pressing [14]. Therefore, it is enough to observe the changes in triplets of diffraction lines characteristic of composite biomaterial (Fig. 2) to determine the changes occurring in PLLA during the melting and pressing processes.

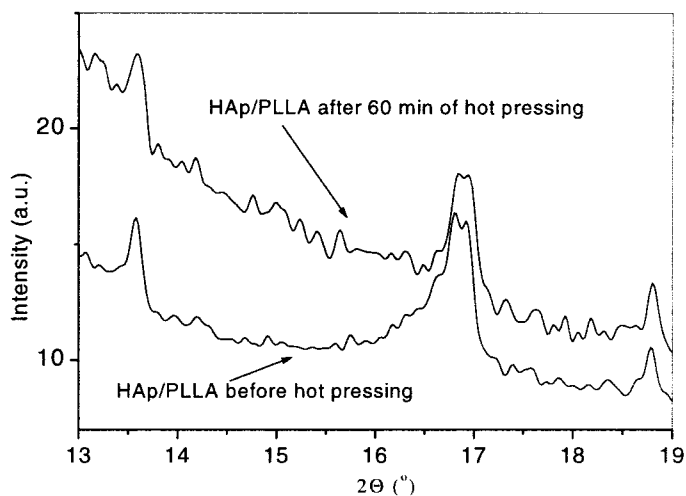


Fig. 2 Characteristic triplets in HAp/PLLA composite biomaterial diffraction spectra before and after hot pressing.

For the purpose, a software was developed to fit experimentally obtained X-ray spectra with the sum of three asymmetric pseudo-Voight profiles accompanying each diffraction peak of the triplet observed, in the given angle range (2Θ). Namely, experimentally obtained diffraction spectrum was simulated by the sum of three functions as follows:

$$I = \sum_{N=1}^3 I_N \left[U_N \exp(-\ln 2 F_{N,I}^2) + \frac{(1-U_N)}{(1+F_{N,I}^2)} \right]$$

$$F_{N,M} = \sum_M \left[\frac{2(\theta_M - \Theta_N)}{W_N} + A_N (\theta_M - \Theta_N) / \Theta_N \right],$$

where I is the total intensity of three peaks (denoted by N index) in the whole angle range of diffraction spectrum (denoted by M index). Each of these three peaks is presented by the sum of asymmetric pseudo-Voight functions, which include percentages of asymmetric Gauss' to Cachy's profile ratio.

The fitting procedure started with subtraction of the diffraction spectrum of the reference sample (without carrier) from the diffraction spectrum of the material itself, then the Fast Furrie transform method was applied, followed by normalization to the 100 % value of the most intense peak. After that, fitting of the sum of three pseudo-Voight asymmetric functions to experimentally recorded spectrum was made. Where, intensity (I_N), angle (Θ_N) and width on half-height (W_N), as well as asymmetry coefficient (A_N) and percentage of asymmetric Gauss' and Cachy's function in the overall profile (U_N) of each peak were used as variables. Note that during the fitting, changes in A_N and U_N for both HAp peaks were the same and that the quotient of their intensities (I_2/I_3) was kept constant. Other parameters changed freely within realistically specified intervals. The quality of fitting was checked by remainder function defined by the relation:

$$R = \sqrt{\sum_I^{2\Theta} (\Delta I_I)^2}; \quad \Delta I_I(\theta_I) = I_I^{exp}(\theta_I) - I_I^{fit}(\theta_I).$$

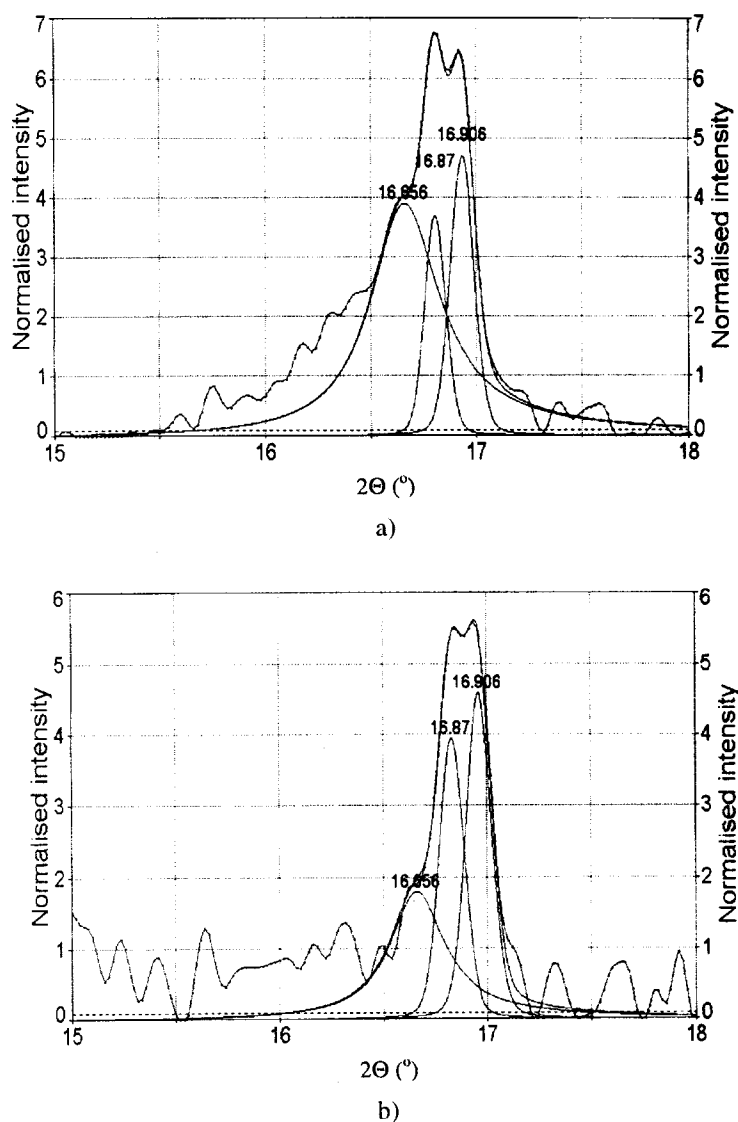


Fig. 3 Fitted X-ray spectrum of the HAp/PLLA composite biomaterial: a) before hot pressing, b) after 60 minutes of hot pressing.

In accordance with our previous PLLA X-ray study, the peak 1 at an angle of 16.7° corresponds to PLLA, while the peaks 2 and 3 originate from HAp. Tab. I presents the parameters of peaks 1, 2 and 3 originating from the HAp/PLLA composite biomaterial before and after 60 minutes of hot pressing.

Tab. I Parameters of the peaks for the HAp/PLLA composite biomaterial before and after 60 minutes of hot pressing.

Samples	Number of peak	Center	Area (%)
HAp/PLLA before hot pressing	1	16.66	64.80
	2	16.87	14.33
	3	16.91	20.85
HAp/PLLA after 60 min of hot pressing	1	16.66	37.73
	2	16.87	28.11
	3	16.91	34.15

Decreased intensity and changed shape of the peak 1, arising from PLLA, are evident by comparing Figs. 3a and b. According to the data given in Tab. I, the peak 1 occupy 64.8 % of the total triplet area. After 60 minutes of hot pressing, its area decreases 1.7 times, which amounts to 37.7 %. Also, its shape is changed so that its width at the half height before the hot pressing is 0.400 nm and after 60 minutes of hot pressing it is 0.325 nm. The results given indicate decrease in PLLA crystallinity during the hot pressing.

3.2. DSC analysis of the hot pressing effects on the HAp/PLLA composite biomaterial structure

To get a more complete picture of the quantitative changes registered by WAXS, DSC measurements were performed with pure PLLA as well as with HAp/PLLA biocomposite before and after 30 and 60 minutes of hot pressing. The results are illustrated in Fig. 4.

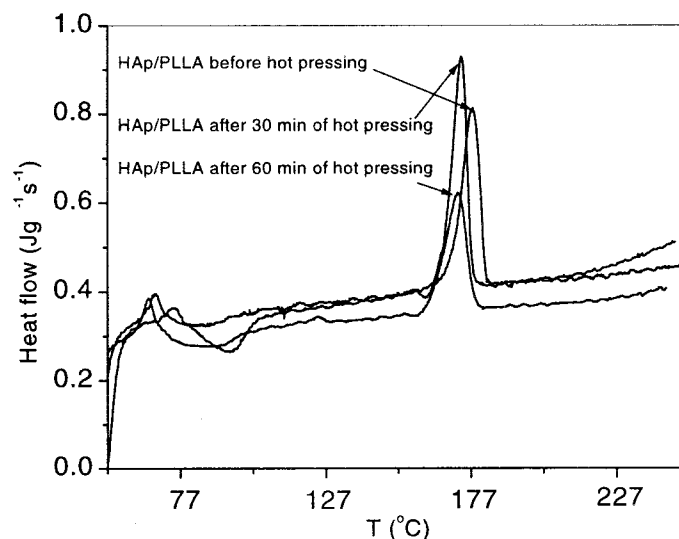


Fig. 4 DSC curves of the HAp/PLLA composite biomaterial before and after 30 and 60 minutes of hot pressing.

Fig. 4 shows characteristic peaks arising from the vitrified and molten PLLA within the composite. All the changes recorded in the biocomposite originate from PLLA, because the HAp phase in the temperature range studied is stable [14]. DSC curves obtained are similar to the ones obtained for pure PLLA by other authors [16-17]. Generally, DSC curves (Fig. 4) beside certain similarities, have significantly different shapes, positions and peak areas of molten HAp/PLLA composite biomaterial.

Tab. II summarizes melting temperatures T_m , melting enthalpies ΔH_{TM} and crystallinity degrees χ of pure PLLA, as well as of PLLA in the composite biomaterial before and after 30 and 60 minutes of hot pressing. Crystallinity degree (χ) of the polymer was calculated as a quotient of the enthalpy of PLLA melting and the enthalpy of 100 % crystalline PLLA melting, which amounts to 93.7 J/g [9]. The enthalpy of melting ΔH_{TM} presented as the area below the peak, was calculated by integrating this area.

Tab. II T_m , ΔH_{TM} and χ of pure PLLA and PLLA in HAp/PLLA composite biomaterial before and after 30 and 60 minutes of hot pressing.

Samples	T_m (°C)	ΔH (J/g)	χ (%)
PLLA	178.7	50.2	53.5
HAp/PLLA before hot pressing	177.9	44.1	46.9
HAp/PLLA after 30 min of hot pressing	174.8	45.3	48.3
HAp/PLLA after 60 min of hot pressing	172.2	32.4	34.5

In the case of pure PLLA, at a given heating rate of 20 K/min, the values obtained for the melting temperature ($T_m = 178.7^\circ\text{C}$), melting enthalpy ($\Delta H_{TM} = 50.2$ J/g) and crystallinity degree ($\chi = 53.5$ %) are in agreement with the literature data [8, 15].

Highly porous HAp/PLLA composite biomaterial, further on compacted by hot pressing, was obtained by mixing completely dissolved PLLA with HAp particles. Precipitation and crystallization of PLLA on HAp particles occurred during vacuum evaporation of the as-obtained mixture. Readsorption and crystallization of PLLA on the HAp particle surfaces changes PLLA melting temperature, its enthalpy and crystallinity. PLLA in the composite is around HAp particles and, compared with pure PLLA, its melting point lowers (from 178.7 to 177.9°C), melting enthalpy (from 50.2 to 44.1 J/g) and crystallinity degree (from 53.5 to 46.9 %), as well. HAp particles play a significant role in these changes. Precipitation and crystallization of PLLA occurring during vacuum evaporation are limited and last relatively short to provide higher crystallinity degree.

Hot pressing effects on the properties of PLLA in HAp/PLLA composite biomaterial are also evident in Fig. 4 and Tab. II. After 30 minutes of hot pressing, more compact HAp/PLLA composite biomaterial (with PLLA whose $T_m = 174.8^\circ\text{C}$, $\Delta H_{TM} = 45.3$ J/g and $\chi = 48.3$ %) was obtained. Decrease in porosity and improvement of mechanical properties of the biomaterial are the result of hot pressing due to decreased density and easier flow of PLLA around HAp particles and high pressure applied [11-12]. Due to thermo-mechanical factors, breaking of chains and, therefore, decrease in PLLA molecular weight occurred during hot pressing [14]. After 30 minutes of hot pressing, PLLA of lower molecular weight was obtained whose crystallization proceeds according to mechanism different from that for untreated PLLA. Different mechanisms of crystallization of PLLA in HAp/PLLA (from the melt and solution) will lead to different appearance, patterns and size of crystals. This will reflect on the crystallinity degree (which is somewhat higher in the case of crystallization of HAp/PLLA obtained by 30 minutes of hot pressing) and given composite melting temperature.

The most significant effects caused by 60 minutes of hot pressing are the changes in the properties of PLLA in the HAp/PLLA composite biomaterial. After 60 minutes of hot pressing, HAp/PLLA biocomposite was obtained with PLLA having $T_m = 172.2^\circ\text{C}$, $\Delta H_{TM} = 32.4$ J/g and $\chi = 34.5\%$. The obtained melting enthalpy and crystallinity degree is considerably lower than that of untreated and 30 minutes hot pressed HAp/PLLA composite biomaterial. These results are in accordance with the given WAXS results. After 30 minutes of hot pressing, blocks with porosity of 1.4 ± 0.2 % were obtained. After 60 minutes of hot pressing, the blocks produced have a density close to the theoretical density and porosity of 0.4 ± 0.2 %. Besides previously specified factors, the porosity as supposed affects the crystallization mechanism, melting enthalpy and crystallinity degree as well. Under the

pressure applied, it comes to the strain in PLLA globular molecules, their unfolding and the generation of linear forms [18].

A decrease in crystallinity of the PLLA phase surrounding HAp particles under high pressure was reported earlier by Shikinami and Okuno [9]. Lower crystallinity of PLLA in the HAp/PLLA composite compared with pure PLLA and formation of larger amorphous regions were found to occur due to separation and decrease in PLLA crystal regions caused by HAp particles under high pressure.

3.3. SEM analysis of HAp/PLLA composite biomaterial fracture surfaces before and after hot pressing

HAp/PLLA composite biomaterial before and after hot pressing was studied by WAXS, DSC and SEM, too. In order to test the influence of hot pressing time on the microstructure and distribution of HAp and PLLA phases, the fracture surfaces of HAp/PLLA composite biomaterial blocks were analyzed by SEM.

Microstructure of highly porous HAp/PLLA composite biomaterial surface (before hot pressing) is shown in Fig. 5a. HAp/PLLA composite biomaterial surface has spherical and ellipsoidal-cylindrical pores, approximately the same in diameter (2 to 4 μm), formed by removal of the solvent during vacuum evaporation. Microstructure of the fracture surface shown in Fig. 5b was obtained after 60 minutes of hot pressing of highly porous HAp/PLLA composite biomaterial. After 60 minutes of hot pressing, nonporous HAp/PLLA composite biomaterial blocks with HAp and PLLA in very close contact were obtained. Very fine distribution of HAp particles in the 3D PLLA base can be seen in Fig. 5b. After hot pressing, PLLA is in intimate contact with HAp. SEM images proves penetration of PLLA into the porous surface of HAp granules [11].

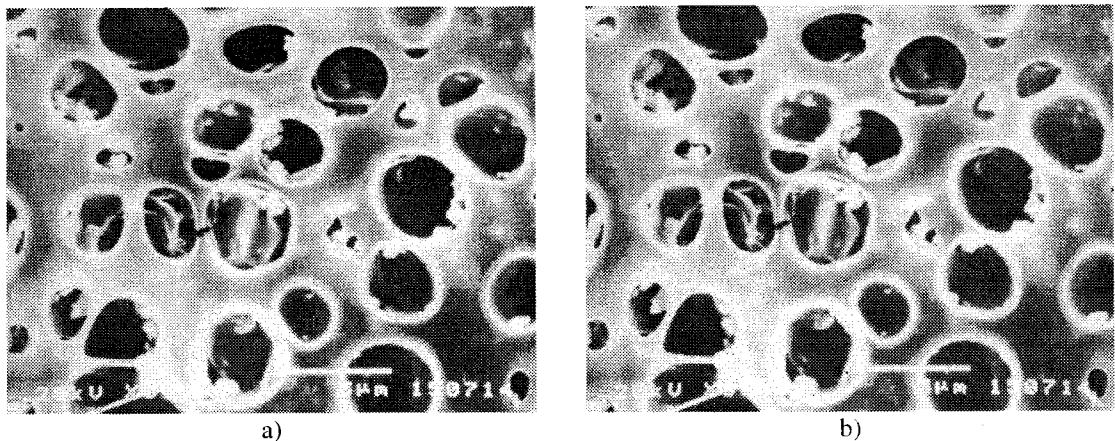


Fig. 5 SEM images of HAp/PLLA composite biomaterial: a) before hot pressing; b) after 60 minutes of hot pressing.

EDX spectra of HAp and PLLA are presented in Fig. 6. A spectrum shown in Fig. 6a has peaks originating from HAp. PLLA phase is defined by a spectrum with corresponding peaks shown in Fig. 6b. The spectra presented in Fig. 6 define HAp and PLLA distribution in the fracture surfaces illustrated by SEM images in Fig. 5.

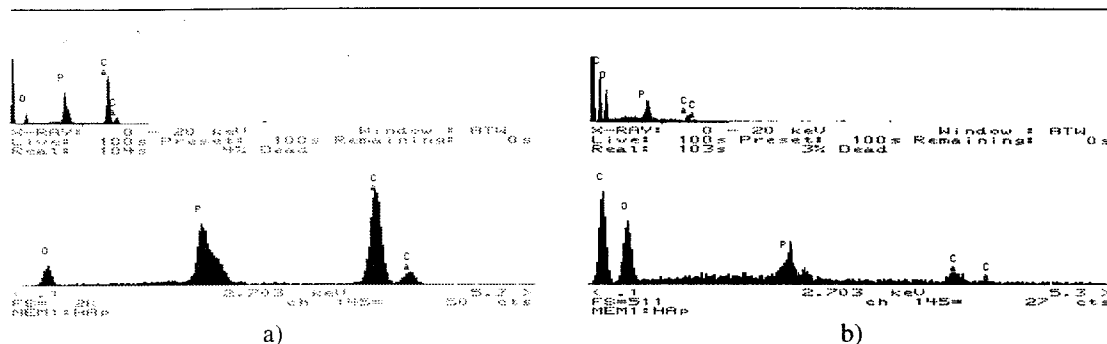


Fig. 6 EDX spectra: a) HAp; b) PLLA.

3.4. Thermogravimetric (TG) analysis of the hot pressing effects on the thermal stability

Effects of hot pressing time on the HAp/PLLA composite biomaterial thermal stability were determined by thermogravimetric (TG) analyses. Compacting of the HAp/PLLA composite biomaterial at the PLLA melting temperature affects the PLLA thermal stability only, because the HAp phase remains stable [14].

Fig. 7 shows TGA curves of PLLA from HAp/PLLA composite biomaterial before and after 30 and 60 minutes of hot pressing. TGA curves for HAp/PLLA composite biomaterial were compared with TGA curves for pure PLLA because, in the given temperature interval, the HAp phase is constant and only mass loss of PLLA was registered [14]. PLLA from the HAp/PLLA composite biomaterial, compared with pure PLLA, shows greater stability at temperatures up to 200°C. The reason for such a behavior are most probably HAp particles, which in the composite act as barriers preventing heat transfer, as well as the cavities which determine the system porosity, due to differences in heat transfer coefficients [19]. Because of non-stationary heat conductivity in the observed temperature range, HAp particles and porosity cause seemingly higher thermal stability of the PLLA. Above 200°C and at the heating rate of 20°C/min, stationary heat transfer through the sample and thermal degradation to complete mass loss of PLLA occur. Thermal stability of the PLLA from the composite is different and it decreases with increasing hot pressing time. PLLA in the composite biomaterial obtained after 60 minutes of hot pressing shows the lowest stability. Mass loss of pure PLLA was registered at the temperature of 360°C. The highest stability exhibits pure PLLA whose 100 % mass loss was registered at 435°C, as can be seen in Fig. 7.

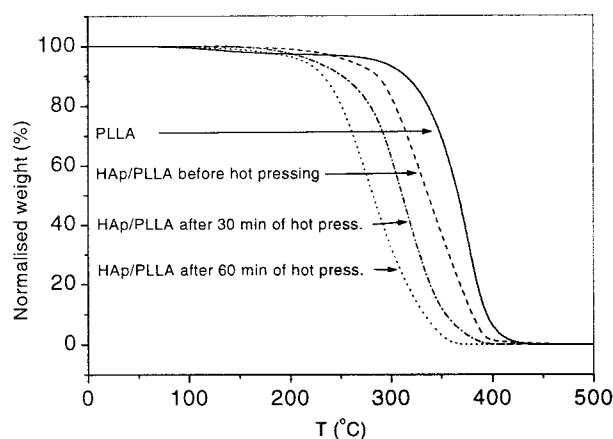


Fig. 7 TGA curves of PLLA and HAp/PLLA composite biomaterial before and after 30 and 60 minutes of hot pressing.

PLLA is a thermally very sensitive polymer. During thermal degradation of polymers, it comes to the breaking of bonds in the basic chain, which results in formation of oligomeric fragments that further on degrade [20]. As a consequence, molecular weights of polymers decrease while in the subsequent degradation stages, monomers and gas-products are formed. Our previous study confirmed decrease in PLLA molecular weight after 60 minutes of hot pressing, while total degradation was not registered [14]. Owing to the planar conformation of PLLA and its basic structure defined as $(-O-CH(CH_3)-CO-)_n$ one can predict where the breaking of bonds in the basic chain will occur [21]. Thermal degradation of PLLA is a one-step process with the first order reaction kinetics [22]. According to the model given by McNeil and Lepier [23], cyclic oligomers, lactide, acetaldehyde, and carbon monoxide are final products of PLLA thermal degradation. Thermal degradation is dictated by the final OH groups that “attack” the basic chain, which bends under the influence of thermal energy. Such a reaction can start at temperatures higher than 230°C [24]. In each of these reactions, the OH group regenerates and the process continues. Depending on the basic chain spot “attacked” by the OH group, different products are obtained. Consolidation by hot pressing is characterized not only by high temperature but high system pressure as a function of time, as well [11]. Due to mentioned reasons, the time of hot pressing has important influence on the PLLA molecular weight and, therefore, stability of the blocks obtained. Decrease in PLLA molecular weight is induced by hot pressing [14]. With molecular weight decrease, the PLLA chains become more flexible and movable and, therefore, thermally less stable. Longer chains are less mobile but more thermally stable [25]. According to TGA curves given in Fig. 7, HAp/PLLA composite biomaterial blocks get energy during hot pressing from outside (pressure, heat) which causes decrease in size of PLLA chains and, therefore, their stability. The obtained blocks become less stable if the time of energy induction is longer (longer hot pressing time). PLLA mass loss of 10 % was registered at 233°C in the case of the composite block obtained after 60 minutes of hot pressing. While the pure PLLA mass loss of 10 % was registered at higher temperature of 314°C. PLLA from the composite biomaterial obtained after 30 minutes of hot pressing, loses 10 % of its mass at 254°C, at temperature in between the two earlier mentioned.

3.5 IR spectroscopic analysis of the stability during hot pressing

Decrease in PLLA molecular weight occurs during hot pressing up to 60 minutes [14]. Dependence of the stability of HAp/PLLA composite biomaterial blocks on the hot pressing time (30 and 60 minutes) was studied by IR spectroscopy. IR spectra of HAp/PLLA composite biomaterial before and after 30 and 60 minutes of hot pressing are presented in Fig. 8.

A spectrum of HAp/PLLA composite biomaterial before hot pressing consists of spectral lines originating from its constituents HAp and PLLA as explained earlier [10, 26-27]. PLLA is characterized by an absorption band at 1760 cm^{-1} arising from the stretching vibrations of (C=O) group, bending vibrations of (CH₃) group at 1386 and 1453 cm^{-1} and stretching vibrations of (C-H) group at 2986 and 2933 cm^{-1} . HAp is characterized by a triplet of absorption bands at 626, 600 and 566 cm^{-1} arising from (OH) groups, a doublet at 1093 and 1040 cm^{-1} from (PO₄) groups and an absorption band at 3573 cm^{-1} arising from (OH) group. The spectra of HAp/PLLA composite biomaterial obtained after 30 and 60 minutes of hot pressing are slightly different from those of the same biomaterial but before hot pressing.

Positions and intensities of absorption bands are also slightly different for all three composite biomaterials. According to IR spectra, after 60 minutes of hot pressing, formation of new phases and disappearance of PLLA were not recorded. The changes occurring in PLLA during 30 and 60 minutes are those already noted, i.e., decrease in molecular weight [14] and changes in temperature, melting enthalpy and crystallinity degree. Non-appearance of new absorption bands in the range of wave numbers from 400 to 4000 cm^{-1} in the spectra of HAp/PLLA composite biomaterial samples after 30 and 60 minutes of hot pressing, confirmed insignificant influence of hot pressing on the qualitative stability of the material.

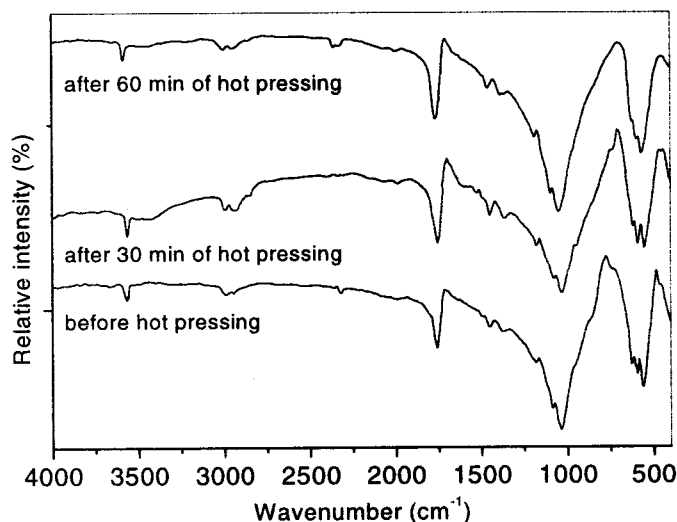


Fig. 8 IR spectra of HAp/PLLA composite biomaterial before and after 30 and 60 minutes of hot pressing.

HAp/PLLA composite biomaterial blocks with a compressive strength of about 140 MPa, the value close to that for natural bone were obtained by 60 minutes of hot pressing [13]. Prior *in vivo* studies [26-27] were made with the blocks here denoted as untreated HAp/PLLA. This composite has higher degree of crystallinity than hot pressed blocks, and therefore their bioresorption is potentially longer. Because of that, it would be necessary to examine, in *in vivo* conditions, the properties the HAp/PLLA composite biomaterial blocks obtained after 30 and 60 minutes of hot pressing, which will be the subject of our further work.

4. Conclusion

By hot pressing of highly porous HAp/PLLA composite biomaterial at the PLLA melting temperature, blocks were obtained with relatively uniform distribution of phases. Designing of HAp/PLLA composite biomaterial block properties was provided by combining high pressure, temperature and time.

Prolonged hot pressing time causes decrease in crystallinity of PLLA in the HAp/PLLA composite biomaterial as proved by WAXS analysis. DSC analyses confirmed not only changes in crystallinity degree but also in the melting temperature and melting enthalpy of the PLLA polymer. Varying the hot pressing time the intensity of these changes can be controlled. For the given hot pressing time, with the maximum of 60 minutes,

HAp/PLLA composite biomaterial block was obtained with 1.6 times lower crystallinity of its polymer phase. TG analyses identified the influence of hot pressing time on the thermal stability of the material. Longer hot pressing time causes lower PLLA stability and vice versa. In the given time interval of hot pressing from 0 to 60 minutes, insignificant qualitative changes in the HAp and PLLA phases were recorded by IR spectroscopy. During hot pressing of 60 minutes, complete degradation of PLLA that would have been followed by the appearance of new phases did not take place.

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