New Biocomposite Matrices Structures Based on Collagen and Synthetic Polymers Designed for Medical Applications

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Among the composition and morphological structure field of biomaterials, the biodegradable biocomposites based on, synthetic and natural polymers as biocompatible spongious matrices has been studied. The biodegradable synthetic polymers have good mechanical properties, low toxicity and a controlled degradation kinetic. However, these polymers are hydrophobic and do not exhibit adequate surface for cells attachment and proliferation because of receptors absence for cells recognition. The natural polymers show good cellular interaction and high hydrophilicity. The advantages of biocomposites made of both synthetic and natural polymers are holding both physico-chemical properties of synthetic polymers and biocompatibility, cellular adhesion properties and variety of biological interactions induced by the contact between cells and natural polymeric support. The aim of this study was the synthesis of biocompatible porous structures by compounding synthetic polymers - (poly(L-lactic acid) (PLLA), poly(D,L-lactic-co-glycolic acid) (PLGA) which are biocompatible, biodegradable but hydrophobic, with natural polymer – collagen, the main protein of extracelullar matrix. The biocomposites were prepared in a polymer: collagen ratio of 2:1 by adding synthetic polymer solution, solved in chloroform, to aqueous collagen gel. The synthesis of biocomposite matrices was achieved by lyophilizing of synthetic polymer/collagen blends after dialyse against 1M KCl. Both uncrosslinked and crosslinked samples were prepared. As standard sample the uncrosslinked and crosslinked collagen matrices were chosen. The morphology of biocomposite matrices PLLA/collagen, PLGA/collagen was characterized by SEM in order to investigate the macro-, micro and nanoporosity. Physico-chemical characterization was focused on thermal stability (DSC and TGA) and surface hydrophilic character by water vapours absorption, water vapours permeability and water absorption.

Keywords: biocomposites, collagen, biodegradable synthetic polymers

The biomaterial composites field is large and developed due to both variety of combinations between synthetic and natural polymers and increasing request for implants, prostheses and artificial organs achieved by tissue engineering with specific biofunctionality for substituted tissue [1-3]. The most used polymers are biodegradable synthetic polymers - (poly(L-lactic acid) (PLLA), poly(D,L-lactic-co-glycolic acid) (PLGA) or natural polymers – collagen, hyaluronic acid. Both type of materials exhibits advantages and disadvantages [4, 5].

The biodegradable synthetic polymers exhibit good mechanical properties, low toxicity and a controlled degradation kinetic. However, these polymers are hydrophobic and haven't adequate surface for cells attachment and proliferation because of receptors absence for cells recognition [6]. The natural polymers show good cellular interaction and high hydrophilicity but they haven't adequate mechanical strength for maintaining form of implant until mature tissue formation [7, 8]. The advantages of biocomposites composed of synthetic and natural polymers are holding both physico-chemical properties of synthetic polymers and biocompatibility, cellular adhesion properties and variety of biological interactions induced by the contact between cells and natural polymeric support [9, 10].

In this paper, the biodegradable biocomposites based on synthetic and natural polymers made as biocompatible spongious matrices were studied. This scaffold combines the biocompatibility collagen properties with high mechanical strength of synthetic polymers thus the biocomposite being used for repair of conjunctive tissue small defect. Three-dimensional porous matrix plays an important role in tissue engineering and is used as an adequate support for attachment, proliferation and differentiation of cultured cells. After a certain cellular density on surface and *in situ* is reached, the scaffold become a bioactive one and it is implanted into body, where it induces growing of host tissue due to release of chemotactic agents which attract own cells in porous network. Attached cells proliferate and secrete extracellular matrix and growth factors in polymeric network which ensures structural and mechanical stability for a long time. New formed tissue penetrates matrix pores and the cells reorganize as own tissue during the polymeric matrix biodegradation.

Materials and methods

Type I collagen gel was extracted from derma of bovine hides by a technological process which allows collagen molecules extraction with triple helix native conformation. Biodegradable synthetic polymers used were (poly(L-lactic acid) (PLLA M_w 124000, Fluka), poly(D,L-lactic-co-glycolic acid) (PLGA, 50:50, M_w 15000, Aldrich). Polymers were solubilized in chloroform by continue stirring for two hours. Aqueous collagen gel was used for synthesis of biocomposites.

Synthetic polymer and collagen blends were obtained in 2:1 ratio by 4% polymer solution dropping into 1.1% aqueous collagen gel by continue stirring at 20^0C for 5-8 min. The chloroform was removed from the blend through dialyze using 1M KCl solution and then being neutralized at 7.2 pH. Both uncrosslinked samples and crosslinked ones with 0.2% formaldehyde to collagen dry substance were prepared. Spongious biocomposite matrix was

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obtained by lyophilizing of blends. The lyophilizing process consists in freezing the samples from -20°C down to -70°C for 12 h and ice sublimation in advanced vacuum directly in vapor phase for 36 h. As references the uncrosslinked and crosslinked collagen matrices were chosen (fig. 1).

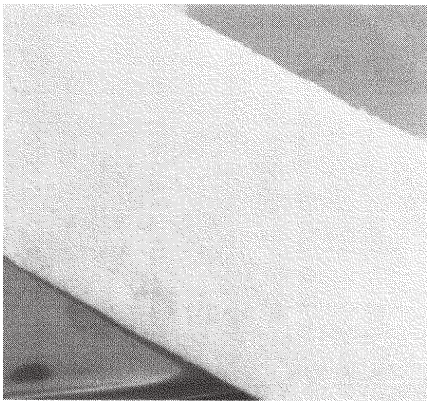


Fig. 1. Collagen matrix

The morphology of biocomposite matrices PLLA/ collagen, PLGA/collagen was characterized by SEM in order to investigate the macro-, micro and nanoporosity.

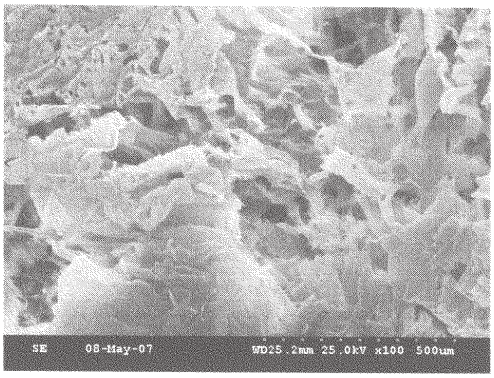
Physico-chemical characterization was focused on thermal stability (DSC and TGA) and hydrophilic characteristics of surfaces by water vapours absorbtion, water vapours permeability (according to SR-5048/2:1999) and water absorbtion. The DSC curves were registered on an LINSEIS DSC PT10 instrument by samples heating up to 400°C with 10°C/min**.** The TGA graphs were recorded on a TA Q500 instrument by sample heating (10 mg) in N_2 atmosphere up to 400°C, using a heating rate of 10°C/min.

Results and discussion

The most important features of a scaffold with applications in bone tissue engineering are given by the appropriate surface for cells attachment, proliferation and differentiation and high porous three dimensional architecture with interconnected pores which allows the cells migration and the transport of nutrients and metabolic products.

The obtained matrices are 3D porous structures with the pores size ranges between macropores ($\phi > 50\mu m$) and nanopores ($\phi < 10\ \mu m$) (fig. 2).

Microscopy images show that the biocomposites have a similar morphology with the collagen matrices even using a high synthetic polymer ratio (60%). Concerning the homogeneity, one may observe agglomeration of synthetic polymer on the collagen fibrils in biocomposites, which leads to a less compact structure and a specific fragility of the final product.



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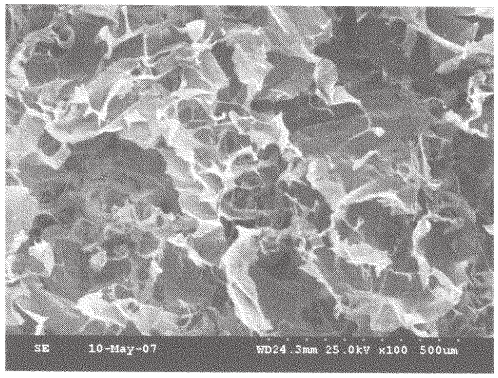

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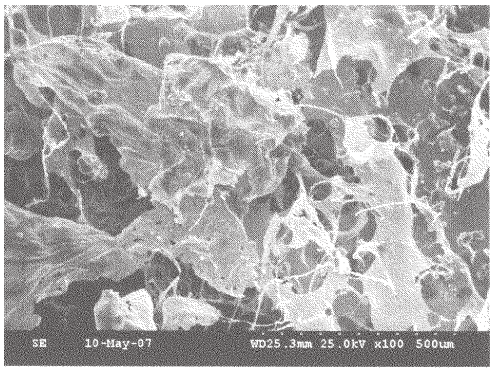

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Fig. 2. SEM images of surface: a) collagen crosslinked matrix reference; b) PLLA/ crosslinked collagen matrix; c) PLGA/ crosslinked collagen matrix

The biocomposites matrices exhibit an aerogel structure, their specific weight range is between 0,031-0,041 g/cm^3 and the specific weight for collagen matrix is 0,021 g/cm^3.

The physico-chemical characterization of the biocomposites matrices focused on the surface hidrophilicity, one of the most important parameters for biocompatibility. It was determined the water vapours absorption in 48 h (fig. 3) as well as water vapours permeability in 24 h according to SR 5048/2:1999 methods. Also it was revealed the water absorption through immersion of the sample in the water for 2 h.

The microporous structures of the biocomposites ensure themselves a specific hydrophilicity either water vapours permeability or water absorption (swelling) and, to a certain extent, water vapours absorption which is

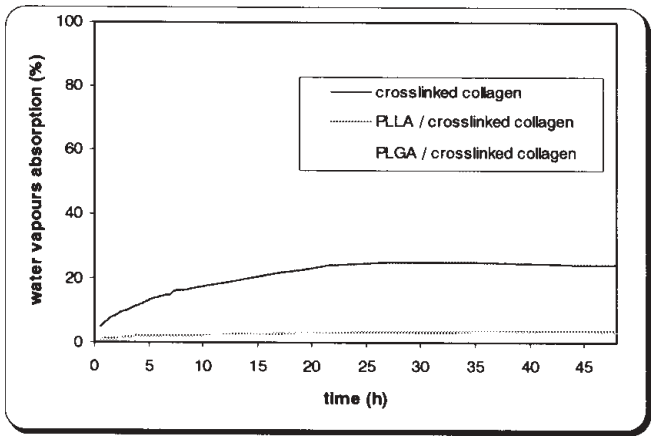


Fig. 3. Water vapours absorption kinetics in 48h

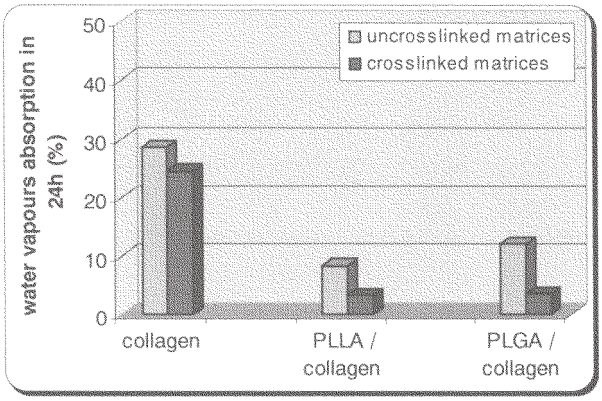


Fig. 4. Water vapours absorption of the biocomposites

influenced by the reactive groups on the surface capable to link water.

A high ratio of synthetic polymer in the biocomposites matrices decreases to half the water vapours absorption comparing to the collagen matrix, this effect being enhanced if the samples are crosslinked (fig. 4). This may be explained by the hydrophobic nature of the synthetic polymer and by the decrease of the polar groups from the crosslinked collagen, which are capable to link water.

The obtained results in water vapours permeability analyses are more significant for the microporous structure than the surface hidrophilicity. Therefore, the uncrosslinked biocomposites samples have similar features with the collagen matrix (fig. 5). The water vapours permeability of the crosslinked biocomposites decrease more, especially, for the PLGA/collagen samples. This fact is sustained by the SEM images, which show a very dense structure for PLGA/collagen biocomposite.

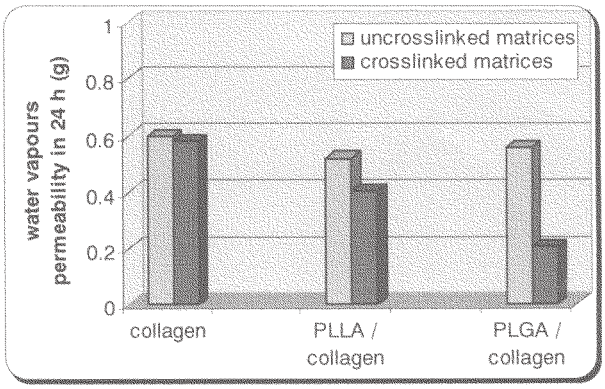


Fig. 5. Water vapours permeability of the biocomposites

The PLLA/ collagen uncrosslinked composite is less hydrophilic than the PLGA/ collagen uncrosslinked composite. After the crosslinking process the biocomposite samples reach almost the same value of the water vapours absorption (~3%). However, they exhibit different water vapours permeability 0,4g respectively 0,2g/24h.

The water absorption of biocomposites (fig. 6) reveals similar hydrophilicity features determined from the other methods. The uncrosslinked composites exhibit water absorption of 3-4 times lower than the uncrosslinked collagen matrices, and the crosslinked composites exhibit water absorption of 2-3 times lower than the crosslinked collagen matrices. The higher values of the water absorption noticed at the crosslinked biocomposites comparing to the uncrosslinked ones may be a result of the specific microporous structure.

The thermal stability of biocomposites is an important feature for their application in tissue engineering. The thermal stability was determined by Differential Scanning

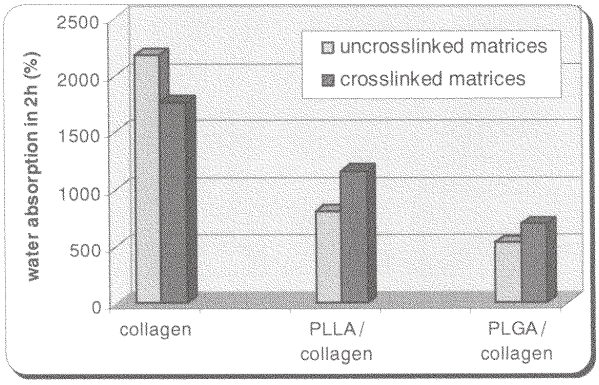
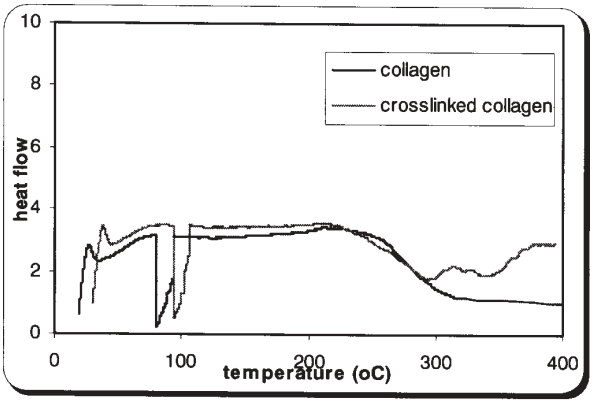


Fig. 6. Water absorption of biocomposites comparing to reference collagen matrix



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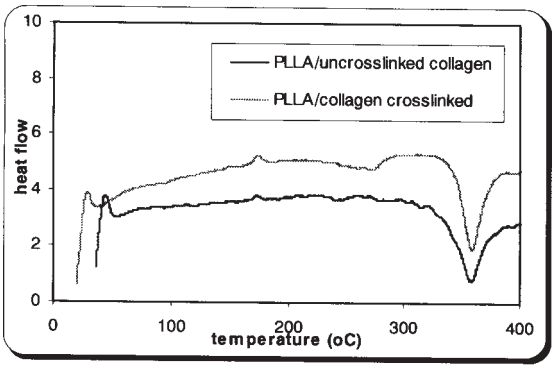

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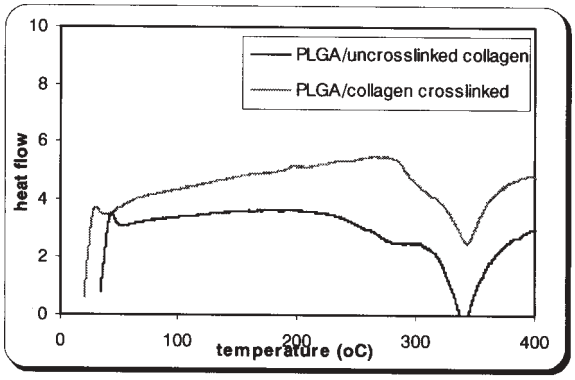

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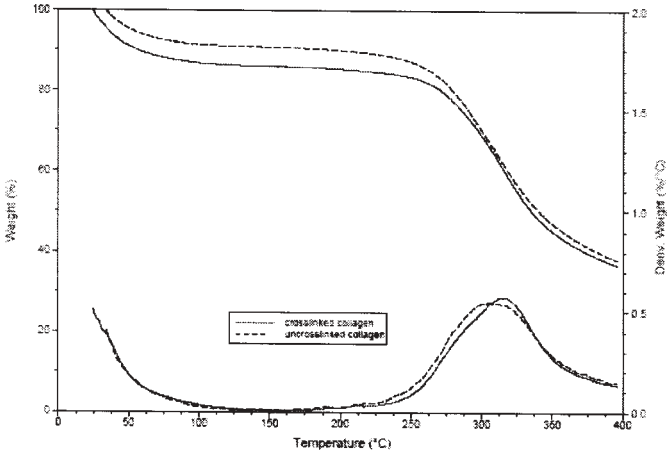
Fig. 7. The DSC curves of synthetic polymer / collagen (2:1 ratio) biocomposites: a)collagen / crosslinked collagen; b) PLLA/collagen; c) PLGA collagen

Table 1
THE ENTHALPY AND THE MAXIMUM OF DENATURATION AND DEGRADATION TEMPERATURE OF BIOCOMPOSITES

No.	Biocomposite matrix	Synthetic polymer/ collagen ratio	T_{max} (^{0}C)	Enthalpy (J/g)
1	Collagen	1:0	93,3 (denaturation)/ 294 (degradation)	[22] / [156]
2	Crosslinked collagen	1:0	94 (denaturation)/ 296 (degradation)	[23] / [175]
3	PLLA/collagen	2:1	358	[36]
4	PLLA/ collagen crosslinked	2:1	359	[37]
5	PLGA/ collagen	2:1	340	[58]
6	PLGA/collagen crosslinked	2:1	343	[81]



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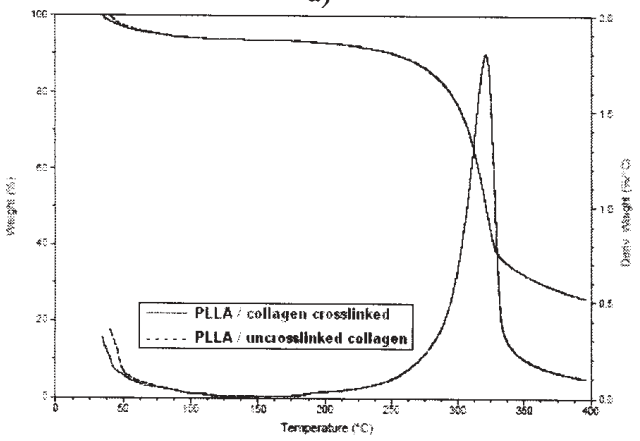

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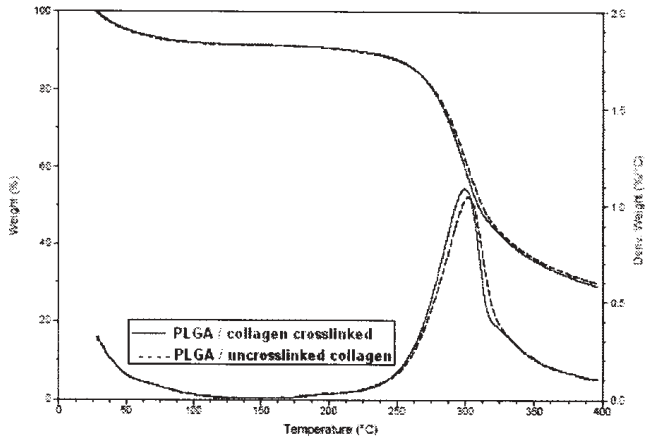

c)

Fig. 8: DTA/TGA curves of the synthetic polymer / collagen (2:1 ratio) biocomposites: a) collagen / collagen crosslinked ; b) PLLA/collagen; c) PLGA/collagen

Table 2
THE WEIGHT LOSS (%) OF BIOCOMPOSITES

Nr. Crt.	Biocomposite matrix	% weight loss
1	Collagen	61.48
2	Crosslinked colagen	62.58
3	PLLA/collagen	74.05
4	PLLA/ collagen crosslinked	74.02
5	PLGA/ collagen	69.97
6	PLGA/ collagen crosslinked	70.80

Calorimetry (DSC) (fig. 7, table 1) and by Termogravimetric Analysis (TGA) (fig. 8, table 2).

The obtained biocomposites exhibit a higher thermal stability than collagen matrix reference due to the high synthetic polymer content. Biopolymeric matrices are stable in a large range of temperatures, between 30°C - 300°C. This fact opens new perspectives in medical field. The degradation process for biocomposites begins at 300-350°C temperature. The collagen crosslinking process doesn't influence significantly the thermal stability of biocomposites. The weight loss noticed at 100-120°C corresponds to loss of water linked to collagen.

From the data shown in figure 8 and table 2 it can be observed that the nature of the synthetic polymer within the biocomposites slightly influences its thermal stability. Thus the biocomposites based on PLLA exhibit a less stability than those based on PLGA.

Conclusions

The spongious structure of biocomposite matrices obtained from synthetic polymer and collagen ensures itself a specific hidrophilicity either water vapours permeability or water absorption (swelling) and, in a certain extent, by water vapours absorption which is more influenced by the reactive groups of collagen capable to link water.

The matrices hydrophilicity influences both the surface reactivity to water and micro and nanoporous structure, noticed from SEM analysis. This structure allows water penetration and facilitates physiological fluid transport and cells development when the matrices are used as tissue substitutes. In addition, the thermal stability of the biocomposites matrices is very large, between 30°C and 300°C.

Biocomposite matrices will undergo *in vitro* tests by growth fibroblasts and osteoblasts cell culture in order to establish the biocompatibility. More synthetic polymer / collagen ratio will be studied for comparing the influence of synthetic polymer hydrophobic character against biocompatibility because the opened pore structure facilitates integration with host tissue.

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Manuscript received: 28.08.2007