The influence of the magnesium powder used as reinforcement material on the properties of some collagen based composite biomaterials

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Collagen based composite materials reinforced with different biodegradable ceramics appear to be an important category of biomaterials used for bone defects healing. In this study, we present the results regarding the obtaining and characterization of some new compositions for the collagen based composite materials reinforced with a biodegradable ceramic and magnesium powder. The experimental biocomposites were prepared by freeze-drying for 48h of collagen gels having the different weight ratio (1%, 3% and 5% wt) of magnesium powder (coded: M1, M2, M3), β-tricalcium phosphate / β-TCP (coded: T1, T2, T3) and a mixture Mg+10%β-TCP (coded: MT1, MT2, MT3) and cross-linked with glutaraldehyde. In this paper we describe the influence of the magnesium powder used as reinforcement material on the structure and properties of the collagen based composites. In order to find the appropriate properties of this composites for using in the human body we have perform different measurements: porosity, water absorption and in vitro degradation test using collagenase as medium. The water absorption and degradation characteristics have been influenced by the magnesium powder, increasing the degradation time of the experimental biocomposites.

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1. Introduction

In recent years, research and development work on new magnesium materials for bio-implant applications has increased significantly [1, 2]. Magnesium is the main component with a resorbable nature in the human environment (excess of Mg is harmless and is easily excreted with the urine), also has good biocompatibility (Mg is present in bone composition, helps the growth and strengthening of the human bone tissue) and mechanical properties [3, 4]. Staiger et al reported the advantages of magnesium as biodegradable material in orthopaedic applications and mention that the degradation behaviour and mechanical properties makes them as attractive as implants. Unfortunately magnesium and magnesium alloys corrodes too quickly in physiological environment [5].

One of the most promising biomaterials for bone substitutes appear to be composites based on collagen reinforced with calcium phosphates, because are biocompatible and structurally similar to the human bone [6]. Collagen is a natural biopolymer which is easily degraded and resorbed by the human body and allows good cell attachment [7], while the calcium phosphates have good osteonconductive and bioactive properties.

It has been noticed that by adding calcium phosphates into the collagen matrix can be provided additional advantages (improvement of the mechanical properties) [8] and that is the main reason why biocomposite materials perform better compared to the single phase materials. Also, composites type collagen-calcium phosphates have a great potential in mimicking and replacing the damaged bone tissue [9-11].

The objective of study was to show the influence of the magnesium powder used as reinforcement material on the structure and properties of collagen based composites, in order to improve some properties like degradation rate.

Another important aspect was to demonstrate that some new compositions of composites based collagen appear to have suitable properties for future use as bone substitutes.

2. Materials and methods

2.1. Samples preparation

In order to obtain the experimental composition of the biocomposite samples, we use collagen gel supplied by Collagen Department of the Romanian National Research & Development Institute for Textiles and Leather obtained using well-known method [12], magnesium powder (STREM Chemicals, Inc. U.S.A.) and β-tricalcium phosphate powder (Plasma Biotal Limited, UK). Magnesium powder and β-TCP particles were added to collagen gel at different weight ratio (presented in Table 1) and then the pH was adjusted at 7.4.

Table 1. The chemical composition of the composite materials.

Code	Composition	Ratio [g]		
		Coll.	β-TCP	Mg
T1	Collagen: β-TCP (75:52)	75	25	-
T2	Collagen: β-TCP (50:50)	50	50	-
T3	Collagen: β-TCP (75:52)	25	75	-
M1	Collagen:1%Mg	99	-	1
M2	Collagen:3%Mg	97	-	3
M3	Collagen:5%Mg	95	-	5
MT1	Collagen:1%(Mg+10%β-TCP)	99	0,1	0,9
MT2	Collagen:3%(Mg+10%β-TCP)	97	0,3	2,7
MT3	Collagen:5%(Mg+10%β-TCP)	95	0,5	4,5

The techniques that have been used in the development of the experimental biocomposites was freeze-drying (also known as lyophilization) using glutaraldehyde (Merck, Germany) as cross-linking agent, cast in polystyrene dishes and kept at 4^0C for 24 hours. After that, they were freeze-dried (48 hours) in order to obtain porous samples using the Christ Model Delta 2–24 LSC freeze-dryer (Germany), as follows: cooling to -40^0C (4 h), keeping up for 8 h, freeze-dried to -40^0C at 0.1 mbar for 10 hours, heating to $+20^0$C at 0.1 mbar for 18 h, heating for 6 h to 30^0C at 0.1 mbar and freeze-dried to $+35^0$C at 0.01 mbar for 6 hours.

2.2. Materials characterization

The experimental composites were characterized from the structural point of view by infrared spectroscopy using a JASCO 6200 type A spectrometer equipped with a ATR Golden Gate accessory. All spectra were recorded in absorption mode at 4 cm^{-1} interval and 160 scans.

The water absorption was determined by a conventional gravimetric procedure, in which weighed biocomposite samples were allowed to swell in distilled water. The experiment was performed on samples with dimensions of 10x10x10 mm^3 during 6 days. For the determination of water absorption the following equation was used:

$$\%W = \frac{W_t - W_0}{W_0} \cdot 100\ [\%]; [g/g]$$

where, W_t is the weight of the composite at t time, W_0 is the initial weight.

Enzymatic degradation of the biocomposite samples was determinate using collagenase (Sigma-Aldrich, U.S.A.) as medium, at 37°C, in phosphate buffer solution. At regular intervals the swollen biocomposites were removed from the degradation solution, dried and weighed. The percent of hydrogel degradation was determined by the following relation:

$$\%\text{weight loss} = \frac{W_i - W_t}{W_i} \cdot 100\ [\%]; [g/g]$$

where: W_i - initial weight of the sample, W_t - weight of the sample after time t.

The porosity was determined by the method proposed by Bundela and Bajpai [13, 14]. The porosity (P) of the open pores in the investigated biocomposites was evaluated using the following formula:

$$P = \frac{W_1 - W_0}{\rho V_0};$$

where:
W_0 - initial weight of the sample; V_0 – initial volume of the sample, W_1 - weight gained by the sample after 48 hours of immersion in ethanol, ρ - alcohol density.

3. Results and discutions

3.1. FTIR characterization

In order to obtain data about the structure of the experimental composites we perform FTIR analysis. The figure 1, 2 and 3 presents the FTIR spectra for all the investigated biocomposites, comparative with the collagen spectrum [15]. Characteristic peaks of the collagen was found at 3302 cm^{-1} (amide A, due to the N-H stretching vibration), 2942 cm^{-1} (assigned to C-H symmetric bond of CH_3 group from aliphatic chain of collagen), 1636 cm^{-1} (amide I, associated with the stretching vibration of carbonyl group C=O), 1547 cm^{-1} and 1239 cm^{-1} (amide II and amide III respectively, derived from the N-H in-plane bending vibration coupled to C-N stretching vibration).
The assignment of the most characteristic IR bands in the biocomposites is given in Table 2.

Table 2. Assignment of the main IR bands in the FTIR spectra of the biocomposite samples.

Wavenumber (cm^{-1})	Assignment	Group assignment
$T_{1,2,3}$: : ~3311 $M_{1,2,3}$: ~3303 $MT_{1,2,3}$: ~3305	ν (N-H)	Amide A
$T_{1,2,3}$: ~1641 $M_{1,2,3}$: ~1638 $MT_{1,2,3}$: : ~1640	ν (C=O)	Amide I
$T_{1,2,3}$: ~1549 $M_{1,2,3}$: ~1547 $MT_{1,2,3}$: ~1547	δ(N–H) ν(C–N)	Amide II
$T_{1,2,3}$: ~1239 $M_{1,2,3}$: ~1239 $MT_{1,2,3}$: ~1239	ν(C–N) δ(N–H)	Amide III
$T_{1,2,3}$: ~1030 and ~1111 $MT_{1,2,3}$: ~868 and ~1033	ν (P-O)	Phosphoric group
$T_{1,2,3}$: ~3742 $MT_{1,2,3}$: ~3745	ν (OH)	Hydroxyl group

The FTIR spectra recorded for the experimental samples containg β-TCP showed characteristic peaks of the β-TCP at ~1030 respectively ~1110 cm^{-1} associated to

with the asymmetric stretching of the P-O bond and at ~865 cm^{-1} due to the symmetric stretching of the P-O bond from phosphoric group.

Analyzing the data from Table 2 could be observed that there are not present any alterations of triple helix of collagen, because there are no changes in amide II, III and pyrrolidine ring of collagen.

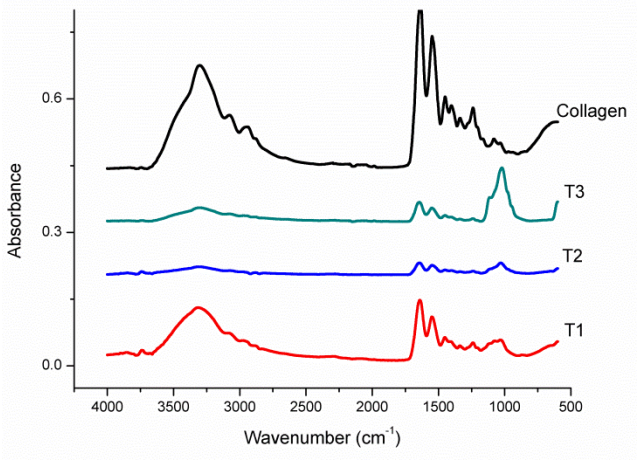

Fig. 1. FTIR spectra of the collagen:β-TCP biocomposite materials.

In case of biocomposites based on collagen and β-tricalcium phosphate the shift of amide I band is due to new chelate bonds between Ca^{2+} and C=O bond [16, 17]. The shifts from amide A towards higher wave numbers when β-TCP was added showed bonds between β-TCP and collagen.

The obtained FTIR spectra for the composites M1, M2, M3, MT1, MT2, and MT3 have revealed that the typical bands of the collagen remained practically the same; a difference was regarding the variation of the intensity.

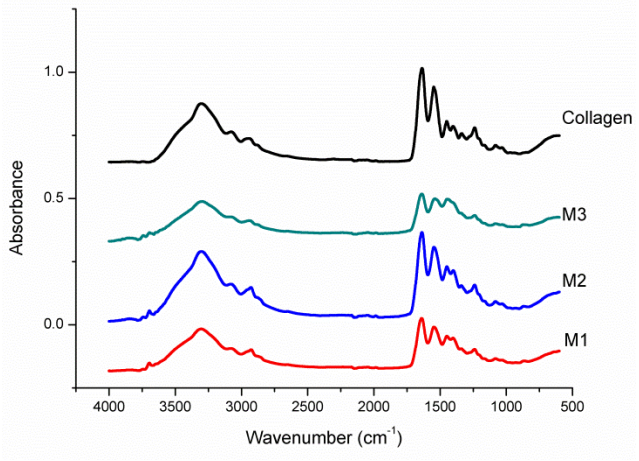

Fig. 2. FTIR spectra of the collagen:Mg composite materials.

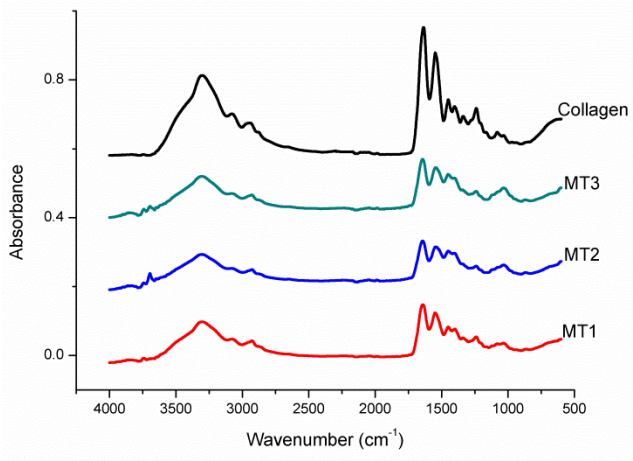

Fig. 3. FTIR spectra of the collagen:Mg +β-TCP composite materials.

3.2. Water absorption

The results related to the water-absorbing capacity of the experimental biocomposites are presented in Figs. 4, 5 and 6. Water, most likely, bonds to collagen in areas that are presents the amine and hydroxyl groups. For all investigated biocomposites we find high water absorption values in the first 2-8 hours. As it can be seen in the mentioned figures, by increasing the content of the fillers the absorbed water content is getting lower. The higher water absorption values was obtained for the collagen:β-TCP biocomposites.

The experimental data revealed that by adding magnesium to a collagen matrix, the capacity of this polymer to absorb water is reduced (Fig. 5 and 6). The deficit in water could be explained by the formation on the magnesium particles surfaces of a thin layer of $Mg(OH)_2$. A low quantity of adsorbed water could be the indirect proof for the magnesium that bonded to the carboxylic ions from the collagen structure.

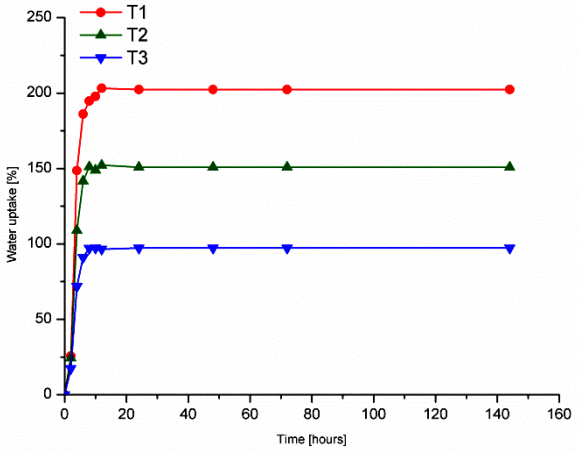

Fig. 4. Water absorption for the collagen: β-TCP composites.

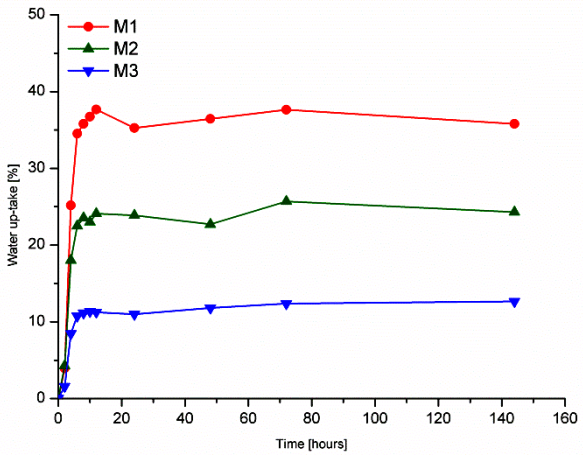

Fig. 5. Water absorption for the collagen:magnesium composites.

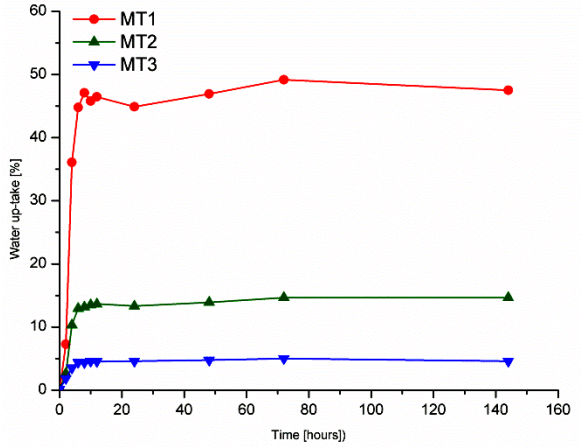

Fig. 6. Water absorption for the collagen:Mg + β-TCP composites.

3.3. Enzymatic degradation

The results obtained after the enzymatic degradation in collagenase of the experimental composites are shown in Figs. 7, 8 and 9.

An important aspect for degradation studies is to mention that during the obtaining process of the experimental composites, is very probable that a magnesium hydroxide layer appear on the surface of the magnesium particle because the magnesium powder is being added into the collagen hydrogels.

The low degradation rate can be explained by the increased quantity of the fillers in the collagen matrix, in this limiting the possibility for the collagenase to cleave the existing bonds in the bicomposites [18].

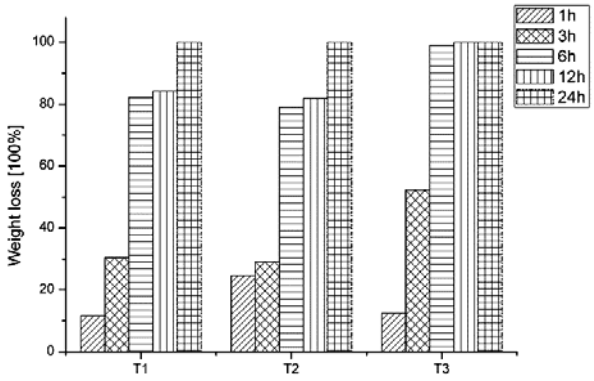

Fig. 7. Enzymatic degradation of the collagen: β-TCP composites.

The degradation rate was clearly influenced by the type and amount of the fillers. The samples T1, T2 and T3 had the highest degradation rate, and after just one day they were completed digested.

As it can be seen in Figs. 8 and 9 the composites with magnesium powder present the lowest degradation rates.

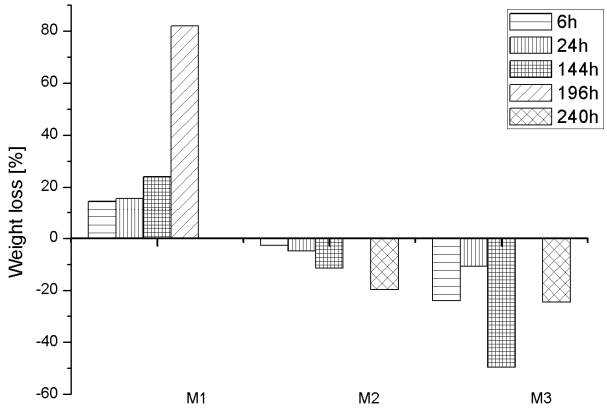

Fig. 8. Enzymatic degradation of the collagen: Mg composites.

The degradation of the collagen:Mg samples started 10 days after the immersion, time in which the samples presented absorption instead of degradation, except the samples M1 in which is a lower quantity of magnesium powder (1%). In this sense, appear to be relevant the amount of the filler used in collagen based composites.

Considering the results shown in Fig. 9, where is presented the results for experimental samples type MT, the degradation of the samples appear 3 days after the immersion. Also, the degradation rate is lower with increasing the percentage of the filler. Also, the degradation rate of the composites type MT is higher that the composites type M for the same quantity of Mg used as filler.

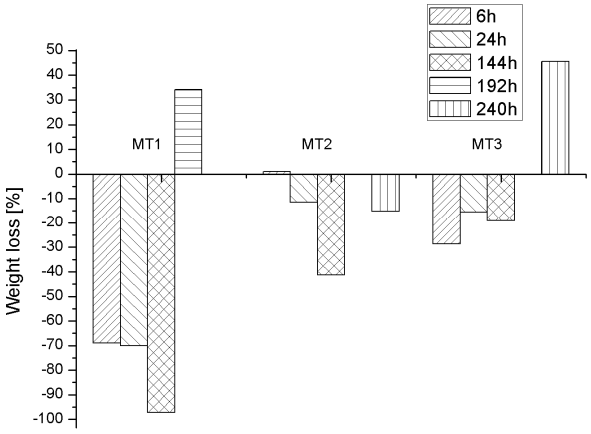

Fig. 9. Enzymatic degradation of the collagen: Mg + β-TCP composites.

According this results, is clearly that the magnesium powder influence in good sense the degradation of the collagen based composites and make this materials more suitable for create the bone substitutes from the degradation rate point of view.

After losing their structural integrity of the composites sample, in the collagenase medium were found a multitude of collagen fibrils and small particles of filler.

3.4. Porosity

In Fig. 10 are presented the results obtained after the determination of the porosity. The obtained data are influenced by the porous structure of the biocomposites which was obtained by lyophilization, which is also connected with permeability and surface morphology of the samples. According to the literature, the porosity of the trabecular bone varies from 50 to 90% [19-21] while the values for the cancellous bone is between 3 and 12 %[22].

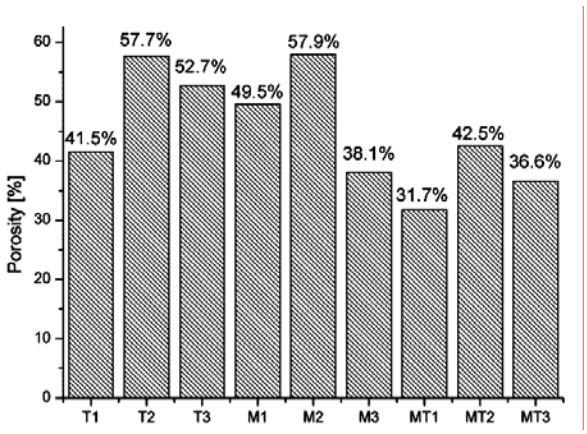

Fig. 10. The variation of the porosity with filler ratio in the experimental composite materials.

The porosity was not influenced just by the type of filler that have been used but also by the quantity.

The porosity varied from 31,7% for the MT1 sample and 57,9% for the M2 sample, the best values being obtained for the M2 composite based on collagen and magnesium filler. After our experimental results, is not possible to establish a correlation between porosity measurements and biodegradation rates.

4. Conclusions

Some new collagen based composites reinforced in different ratios with magnesium powder, β-tricalcium phosphate and a mechanically mixed combination of these powders was obtained using freeze-drying method.

The FTIR analysis revealed the chemical interaction between the collagen and the filler.

The magnesium powder used as reinforcement material influence the structure and properties of collagen based composites, and increase the degradation time of this biocomposites in collagenase. This means that the experimental composites have improved properties from the degradation rate point of view even we use a quite small amount of filler.

Based on the results obtained for all experimental measurement, we could conclude that the most promising formulation when we want to design a collagen based composite for bone substitutes using freeze-drying process appear to be the filler obtained from mechanically mixing of the magnesium powder and β-tricalcium phosphate.

According the experimental results for structural characterization, porosity, water absorption and enzymatic degradation the new composites based collagen reinforced with magnesium powder appear to have suitable properties for future use as bone substitutes. Future studies related to the mechanical and biological properties could confirm this.

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