The influence of the ceramic phase on the porosity of some biocomposites with collagen matrix used as bone substitutes

M. D. VRÂNCEANU, I. ANTONIAC*, F. MICULESCU, R. SABAN
University Politehnica of Bucharest, 313 Independentei Street, 060042, Romania

Biocomposite materials based on collagen matrix could be used for bone defects repair. Hydroxyapatite and β–tricalcium phosphate particles are generally used as filler because they are biodegradable ceramics and offer the composite materials properties similar to that of the bone. Different ratios of bioceramics were used in order to obtain collagen based sponges. A critical role in newly bone tissue formation is played by porosity and pore size of the composite biomaterials. Using different technique (Xray diffraction, FTIR and SEM) we demonstrate the influence of the bioceramic ratio on the porosity, pore size and structural properties of collagen based composite materials. Also, due to their properties, the obtained composites appear to be potential biomaterials for bone tissue engineering.

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Keywords: Composite materials, Bone substitute, Collagen type I, Hydroxyapatite, Beta-tricalcium phosphate, Porosity

1. Introduction

Bone defects repair after trauma, tumour resection and degenerative diseases [1] represent the main causes for the bone substitute's development.

Many biomaterials are used for this purpose and could be classified in different classes [2,3,4]:

-Bone substitute materials of natural origin: autogen, from same individual; allogen from same species; xenogeny from a different species; phytogen from marine origin;

-Synthetical materials (alloplastic): calcium phosphate, calcium sulphate, bioglass, degradable and nondegradable polymers;

-Composite materials, the combination of different materials (e.g. bioactive calcium phosphates and polymers);

-Bone Substitute Materials combined with growth factors: natural and recombinant growth factors used alone or in combination with other materials such as transforming growth factor-beta (TGF-beta), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and bone morphogenetic protein (BMP);

-Bone Substitute Materials with living cells, this act with cells to generate new tissue alone or are seeded onto a scaffold serving as a matrix.

These classes of biomaterials meet the requirements of an ideal bone substitute [5] such as: biocompatibility, resorbability/degrability, osteoconduction, osteoinduction, porosity, stability under stress, sterility, easy to use and cost-effectiveness.

Among these materials, HAp/Collagen has drawn a special interest because it can mimic the composition of natural bone. However, since β-TCP biodegrades more rapidly than HAp ceramics and since HAp shows minimal resorbtion, β-TCP is considered to be a more suitable candidate for obtain the products for bone tissue regeneration than HAp [6].

One important issue for the bone substitutes is porosity [7, 8] which includes increased mechanical strength with enhanced cell seeding and promoted cell interactions. Porosity and pore size of bone substitute material play a critical role in bone formation. Pore size can be divided in two different groups: microporous (<5μm pores) and macroporous (>100μm pores). Microporosity and macroporosity are important for the material bioresorbability. In addition, macroporosity plays an important role in the osteoconductivity. The minimum size pores have been quoted by many researches as 100μm [9,10,11] but subsequent studies have shown better osteogenesys for bone substitutes with pores size of 100-400μm as well as a typical porosity of 50-90% is required [12,13]. The average pore diameter is important as they will determine cells to migrate, differentiate, allowing new bone formation and mechanical properties [14].

The purpose of this paper is to develop new collagen based biocomposites with bioresorbable ceramics filler, hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP) particles, potentially used as bone substitutes and to evaluate the influence of the resorbable ceramic filler on the porosity.

2. Materials and methods

2.1. Samples preparation

The experimental biocomposite materials were obtained by adding different weight percentages (wt%) of bioceramic particles (HAp or β-TCP) into the collagen gel extracted from calf hide using freeze-drying technique

[15]. The extracted collagen is type I which is a major component of the extracellular matrix, providing natural binding sites for adhesion of osteoblast and fibroblast [16].

Bioceramic particles (purchased from Plasma Biotal Limited, UK) were added to collagen at ratios of Coll:HAp(β-TCP)=75:25, Coll:HAp(β-TCP)=50:50, Coll: HAp(β-TCP)=25:75 and then the pH was adjusted at 7.4. All gels were cross-linked with 0,25% glutaraldehyde (GA) (reported to the weight of dry collagen), then cast in polystyrene dishes and kept for 24 hours at 4°C. After the cross-linking they were freeze-dried (48 hours) in order to obtain porous materials, as follows: cooling to -40°C (4 hours), keeping up for 8 hours, then freeze-dried at -40°C and 0.1 mbar for 10 hours, then heating to +20°C for 18 hours at 0.1 mbar, then heating (6 hours) to +30°C and finally freeze-dried at -35°C at 0.01 mbar for 6 hours, using the Christ Model Delta 2–24 LSC freeze-dryer (Germany). The freezing rate and the collagen/bioceramic particles content are the most important factors in controlling pore size. Pore size is important for the biocomposite materials because will determine cell adhesion, the mechanical properties and the success of the new tissue formation [17]. A collagen matrix was used as control sample in all experiments.

2.1. Physico-chemical characterization

The bioresorbable ceramic fillers used in the experiments (hydroxyapatite and β-tricalcium phosphate) were characterized from structural and dimensional point of view using X-ray diffraction, FTIR-spectroscopy and SEM.

The X-ray diffraction analysis was performed on a XRD 6000 SHIMADZU diffractometer just to confirm the bioceramics structure. The bioceramic particles and composite materials 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.

Scanning electron microscopy determinations were made using a Philips ESEM XL 30 microscope with EDS for different purposes: the size and morphology of the bioceramics particles, integration of the bioceramic fillers in the collagen matrix, the morphological aspects and pore dimension determination for biocomposite materials. The EDS analyses revealed information on the elemental composition of samples.

The porosity was determined by the method proposed by Bundela and Bajpai [18,19]. The porosity (P) of the open pores in the investigated composites was evaluated using the following formula:

$$P=(W_1-W_0)/\rho V_0,$$

where:
W_0 and V_0 are the known weight and volume of the samples, W_1 is the weight gained by the sample after 48 hours of immersion in ethanol, ρ is the alcohol density.

3. Results and discussions

3.1. Bioceramic fillers characterization

The X-RD patterns, presented in Fig. 1 and Fig. 2, show the characteristic peaks for each sample, according to International Center for Diffraction Data database, ICDD-PDF 4+ file no. 04-007-2837 in case of HAp and ICDD-PDF 4+ file no. 01-073-4869 for β-TCP respectively. The X-RD pattern in Figure 1 indicates the presences of the majoritary phase (HAp) with a hexagonal lattice, having the main diffraction angle at 2θ = 31.804° and preferred orientation with respect to (211). The accompanying three peaks at 32.953° ((300) plane), 32.185° ((112) plane) and 25.846°((002) plane) of almost equal intensities were also detected. Other main diffraction peaks which could indicate the presences of minority phases have not been observed. In Figure 2, the X-RD pattern highlights a majority phase of β-TCP with a rhombohedra lattice and the main diffraction peaks at 2θ = 31.176°, 34.594°, 31.243° and 27.970° which are in respect to (0210), (220), (217) and (214), respectively.

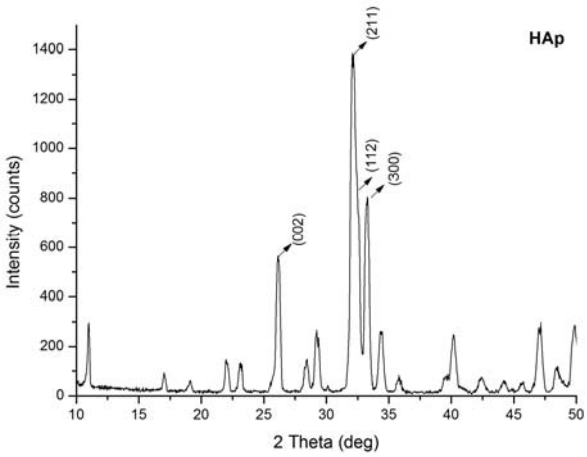


Fig. 1. XRD patterns of the investigated HAp particles

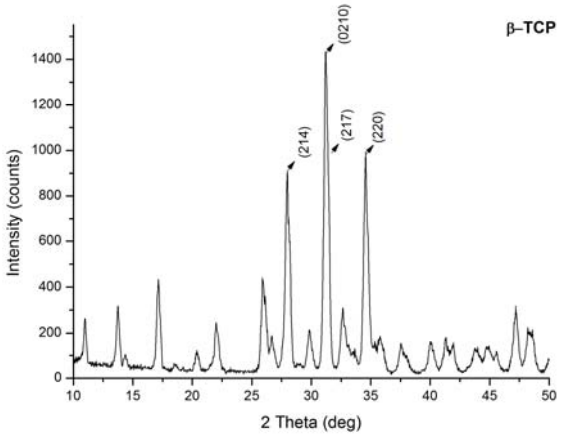


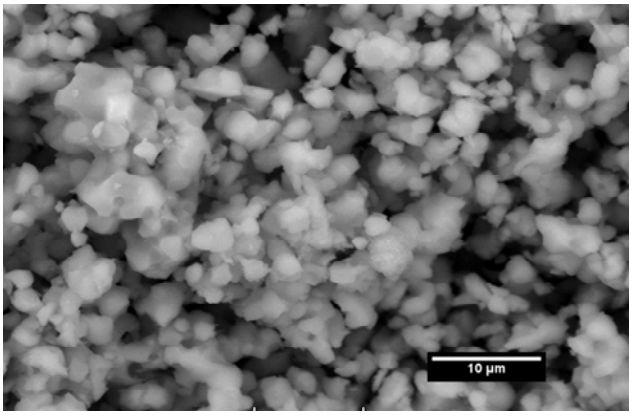
Fig. 2. XRD patterns of the investigated β-TCP particles.

The morphological aspect of the bioceramics fillers are presented in Fig. 3.

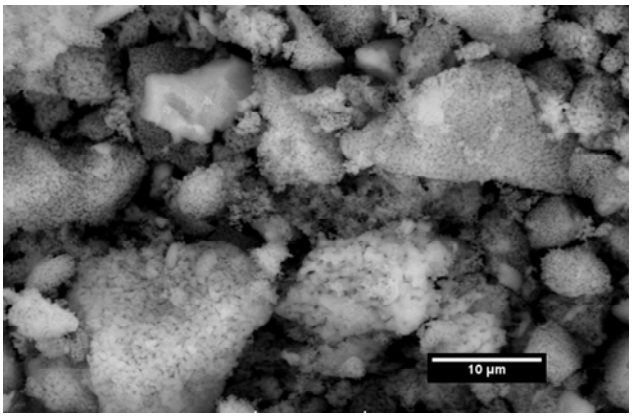
SEM analysis of the hydroxyapatite particles used to obtain composite materials collagen-hydroxyapatite are almost round and regular in shape with an average diameter of 3.9 μm.

In the case of β-TCP, as we observed in Figure 3.b, measured particle sizes was between 12.3 μm and is clearly that β-TCP particles are larger than HAp particles, with different shapes.

The EDS spectrometry has completed the study of the resorbable ceramic particles easing-up the mineral phase identification. The obtained spectrum presents a matching Ca/P ratio corresponding to the investigated powders which is 1.67 for HAp particles and 1.5 for the β-TCP particles.

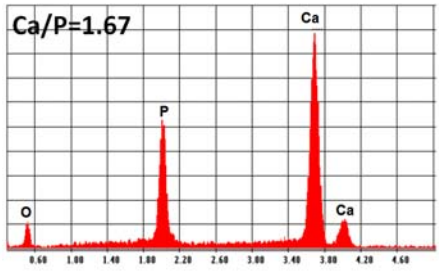


a

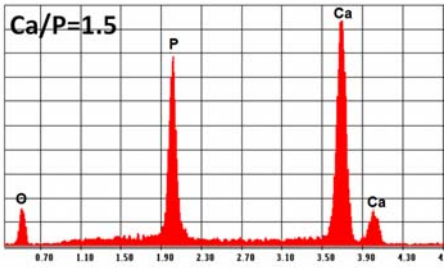


b

Fig. 3. Scanning electron microscopy investigations on the bioceramics fillers in powder state (a) Morphological aspects and particle size of the HAp particles, (b) Morphological aspects and particle size of the β-TCP particles



a



b

Fig. 4. EDS spectra of the (a) HAp powder and (b) β-TCP powder.

3.2. Biocomposite materials characterization

The representative FTIR spectra for the collagen sample, resorbable ceramic fillers and final composite materials are presented in Figs. 5 and 6.

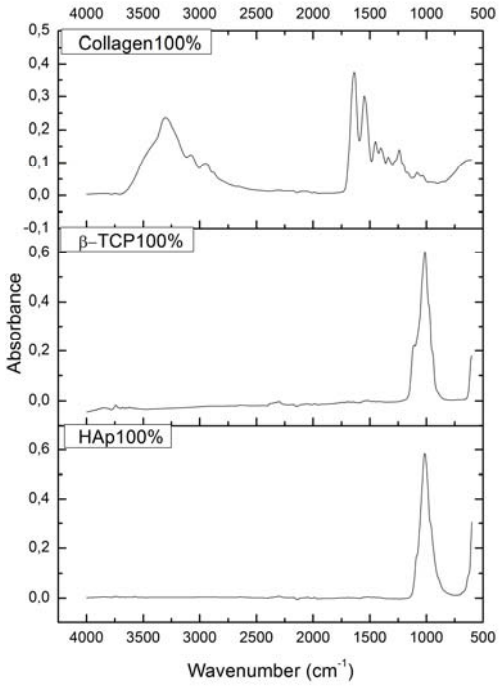


Fig. 5. FTIR spectra of the control samples (collagen, HAp and β-TCP).

For the collagen, FTIR spectrum showed the characteristic peaks as following: 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 plus the C-N stretching vibration).

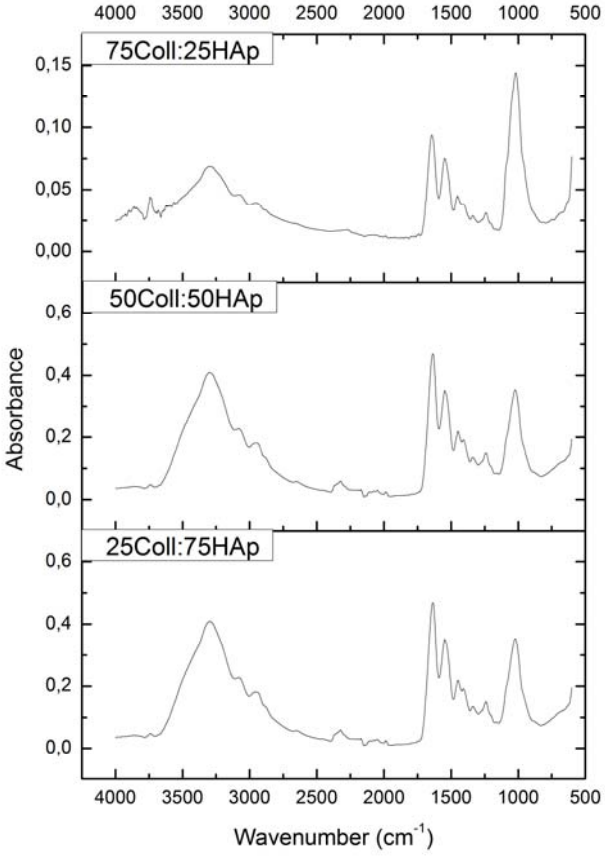


Fig. 6. FTIR spectra of the collagen/HAp composite materials

For the collagen/bioceramics composite materials, in addition to the main peaks of the collagen, the spectra also involved bands derived from HAp or β-TCP. The phosphate bands are located between 900 and 1200 cm-1. Our measurements showed peak for HAp at 1015 cm^{-1} and for β-TCP peaks at 1013, 1108 and 723 cm^{-1} associated to with the asymmetric vibration of the P-O bond from phosphoric group.

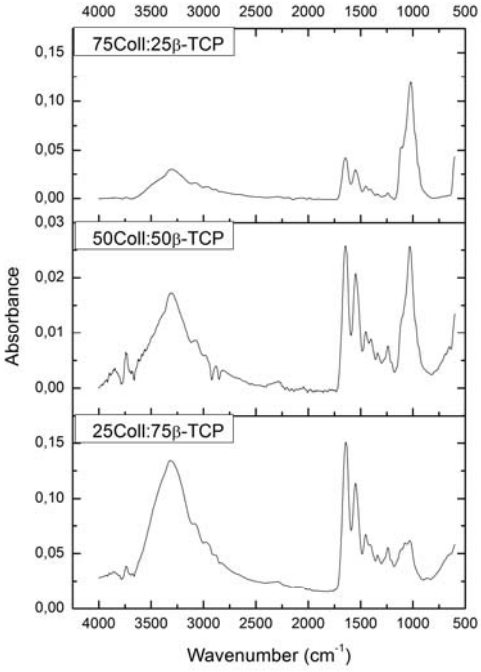


Fig. 7. FTIR spectra of the collagen/β-TCP composite materials

3.3. Biocomposite materials porosity and pore size determinations

SEM images on the composite revealed a highly porous structure, which was common in all the samples. The pure collagen (Figure 8) has a smooth surface which began to modified with the incorporation of ceramic fillers (HAp, β-TCP), gradually resulting a rough surface (Figs. 9 and 10).

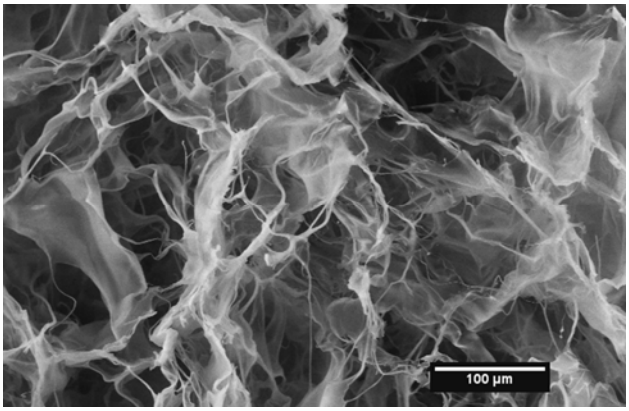
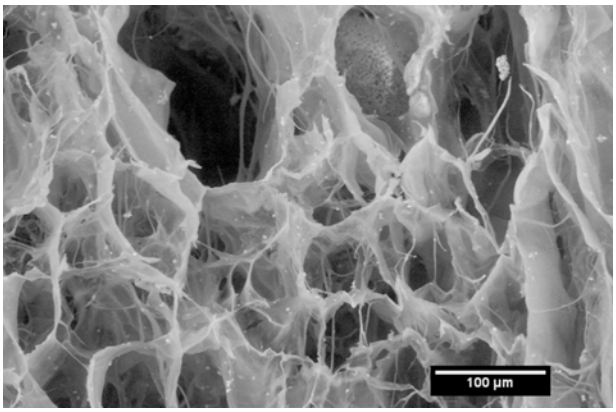


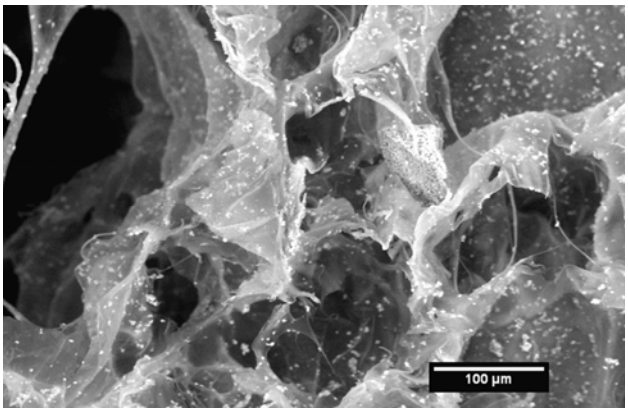
Fig. 8. SEM of porous collagen matrix.

The resorbable ceramic particles are uniformly distributed
through the collagen matrix which has interconnected pores. A small percent of the filler is well distributed on the walls of the collagen matrix. By increasing the quantity of the filler, the ceramic particles seem to agglomerate, forming clusters.

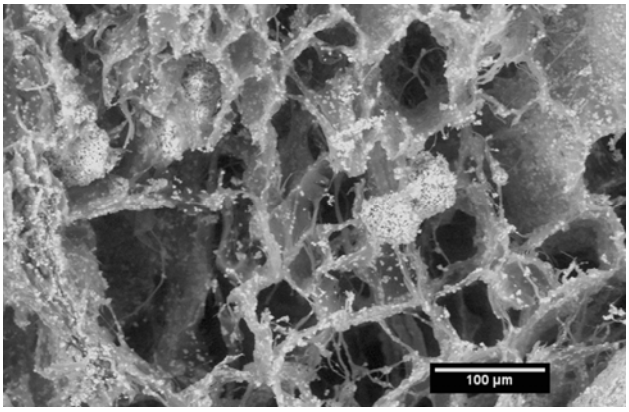
For the collagen-hydroxyapatite the pore sizes are between 85-200 μm and this did not seem to change much until the value of 75 wt% of HAp which can be seen in Fig. 9.



(a) SEM of porous composite Coll:HAp = 75:25 ratio



(b) SEM of porous composite Coll:HAp = 50:50 ratio

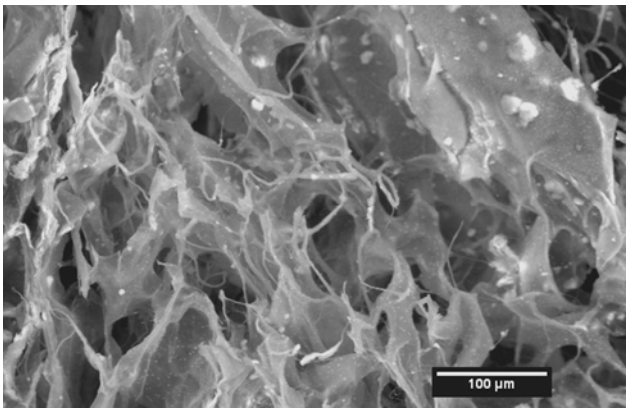


(c) SEM of porous composite Coll:HAp = 25:75 ratio

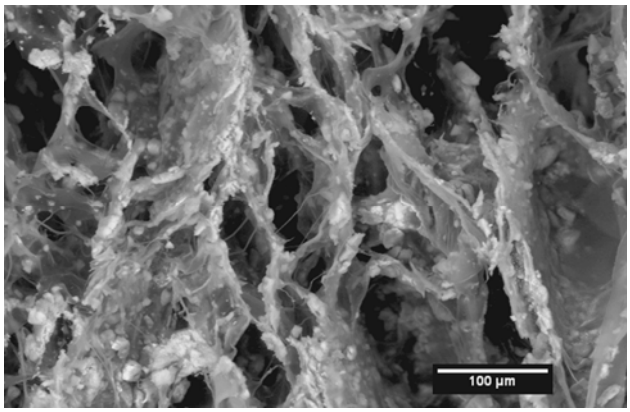
Fig. 9. SEM of porous Coll:HAp composite in different bioceramic ratio.

In the case of collagen-β-TCP composites, the pore size are in a range of 70-150 μm. Increasing the content of ceramic fillers did not disturbe the porous structure of the
composites but produces significant modification on the morphology.

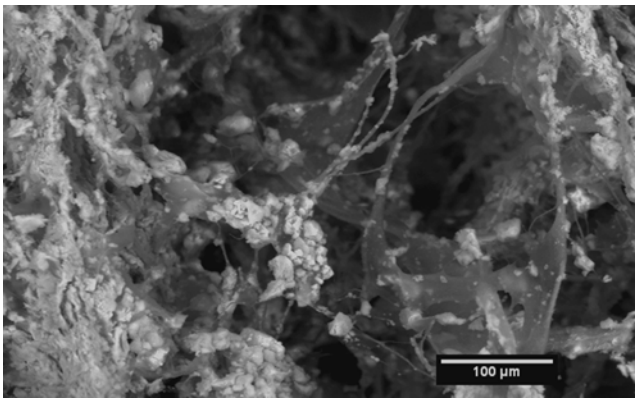
In the composites with 75% wt resorbable ceramic fillers (Fig. 10), the sizes of the pores has decreased and the walls became thicker, also the surface become rougher and the uniformity has decreased.



(a) SEM of porous composite Coll:β-TCP=75:25 ratio



(b) SEM of porous composite Coll:β-TCP=50:50 ratio



(c) SEM of porous composite Coll:β-TCP = 25:75 ratio

Fig. 10. SEM of porous composite Coll:β-TCP with different β-TCP ratio in collagen matrix (cross-section view of the walls)

The homogeneously distributed interconnected pore structure of the investigated composite materials was sustained by the high porosity values. The results are presented in Figure 11. For all samples, the total porosity was over 77%, according to similar data from literature for composite materials used as bone substitutes [20,21]. This high porosity and large pores can enhance bone ingrowths because they allow migration and proliferation of osteoblasts and mesenchymal cells, as well as vascularisation [18].

For collagen control sample were found a porosity of 98,61%. The composite with 25% and 50% HAp did not reveal a significant difference regarding porosity (98,25% respectively 97,50%), this could prove that HAp particles exist homogenously on the polymeric network and the particles bond tightly to collagen fibrils.

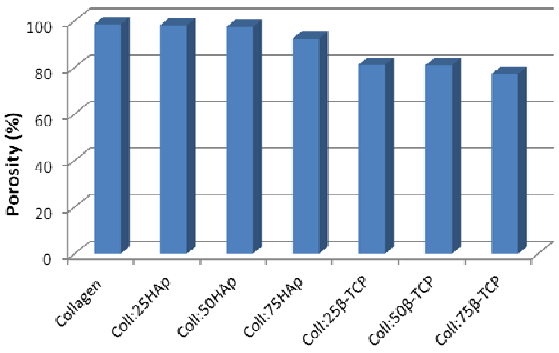


Fig. 11. The variation of the porosity with bioceramic ratio in the composite materials.

Due to the addition of HAp, a decreased porosity, was observed for the composite materials with 75%HAp (92,37%).

Even in the composite materials with β-TCP as ceramic filler can be observe the same decrease of porosity due to the addition of β-TCP. Such a decrease of porosity was expected, due to the addition of HAp, but was almost negligible until a content of HAp of 75% was met. It was also noticed that the porosity was differentiated by the type of bioceramic which had been added.

4. Conclusions

The effects of weight percents of different resorbable bioceramic fillers on the morphology, porosity and pore size of the composites type's collagen-bioceramics were clearly demonstrated. Pore size and porosity of the composite are dependent on the template which is controllable by adjusting the freezing rate.

An important aspect appear to be that in case of higher percent of resorbable bioceramic fillers (75%), because the walls of the pores could be broken and the composite material is not optimal for bone substitutes. According to our results, the ratio 50:50 between collagen and bioceramic appear to be optimal for obtaining composite biomaterials for bone substitutes. The resulted pore size distribution for each composite material was according with the needs for bone substitutes materials: between 85-200 μm for the collagen-hydroxyapatite biocomposites and 70-150 μm for the collagen-tricalcium phosphate biocomposites. The porosity was influenced not only by the quantity of the filler but also by the type of the filler (HAP or β-TCP).

The homogeneity of the structure and the interconnectivity of the pores are also some important advantages of our experimental biocomposites.

These results provide a framework for generating synthetic composites with organic/inorganic interface similar to natural bone.

Acknowledgment

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*Corresponding author: antoniac.iulian@gmail.com