

Biomimetically Synthesis of Collagen / Hydroxyapatite Composite Materials

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The aim of this work is to obtain and characterize some collagen/hydroxyapatite composite materials obtained by collagen matrix immersion in SBF, for different immersion times. The obtained composite materials were characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) coupled with energy dispersive spectroscopy detector (EDS) and complex thermal analysis. The main differences between the composites obtained by different immersion times can be visualized by SEM images. The increasing immersion time lead to a more compact composite material, with lower porosity as exhibited in the recorded SEM images. On the other hand, not only the morphology is changed but also the composition of the composites. At high immersion time, onto the composite materials, sodium chloride (NaCl) crystals can be observed. The presence of NaCl can be easily identified by XRD and, based on the relative characteristic peaks intensity can be quantified.

Keywords: SBF immersion, collagen matrix mineralization, morphological changes versus immersion time

Bone graft materials represent a high interest for the researchers because of the increasing demand for these materials [1]. Bone is a remarkable biocomposite containing collagen-COLL and apatite (mainly hydroxyapatite-HA) as major components [2-4]. Many synthetic methods were developed and some of them were improved in different ways [5-13].

In present, research activities are concerning on biomimetic synthesis of these composite materials [14-16]. The main advantage of these “biomimetically” obtained materials is the better biocompatibility, mainly due to the *in vivo* simulated conditions [17]. Due to the advantages of these materials, the classical obtained materials are often “biomimetically treated” in order to improve their properties. For instance, titanium [18], alumina [19], Ti-6Al-7Nb alloy [20], CoCrMo alloy [21] can be coated with a thin apatite layer.

Biomimetic conditions are achieved by SBF immersion at 37°C. SBF is obtained by dissolution of various amounts of inorganic salts in distilled (better demineralized) water and usually, adjusting the pH at 7.2 – 7.4 by tris(hydroxymethyl)aminomethane buffer (TRIS). In terms of the desired composition of the targeted biomaterial the composition of the SBF can vary in relative large range; for instance, the apatite formation is favored by a phosphate enriched SBF. The control of the amount of deposited mineral phase can be made by many factors, such as: immersion time, temperature, pH and so on.

The aim of this work was to obtain COLL/HA composite materials by SBF immersion of collagen matrices and to characterize these materials especially, from the point of view of morphological modification versus immersion time.

Experimental part

Type I fibrillar collagen was obtained from calf pelt by a chemical treatment as gel form. The obtained gel, with molecular weight average of 350000 Da and concentration of 0.8% collagen reported to dry substance was adjusted

at pH 6.8 with 25% ammonia solution and then freeze-dried using the Christ Model Delta 2-24 KD lyophilizer.

The SBF was obtained by dissolving the corresponding amount of NaCl, NaHCO₃, KCl, Na₂HPO₄·2H₂O, MgCl₂·6H₂O, HCl, CaCl₂, Na₂SO₄ and TRIS (NH₂C(CH₂OH)₃) in distilled water and adjusting the pH to 7.4. The final concentration of each species is given in table 1.

Some COLL/HA composite materials were obtained started from collagen matrices by immersion in SBF. The immersion time varied from 1h and up to more than 300h.

Table 1
COMPOSITION OF SBF AND HUMAN PLASMA

Species	Concentration; mM	
	SBF	Human plasma
Na ⁺	142	142
K ⁺	5	5
Mg ²⁺	1.5	1.5
Ca ²⁺	2.5	2.5
Cl ⁻	120	103
HCO ₃ ⁻	27	27
HPO ₄ ²⁻	2.27	1.0
SO ₄ ²⁻	0.5	0.5
TRIS	50	-

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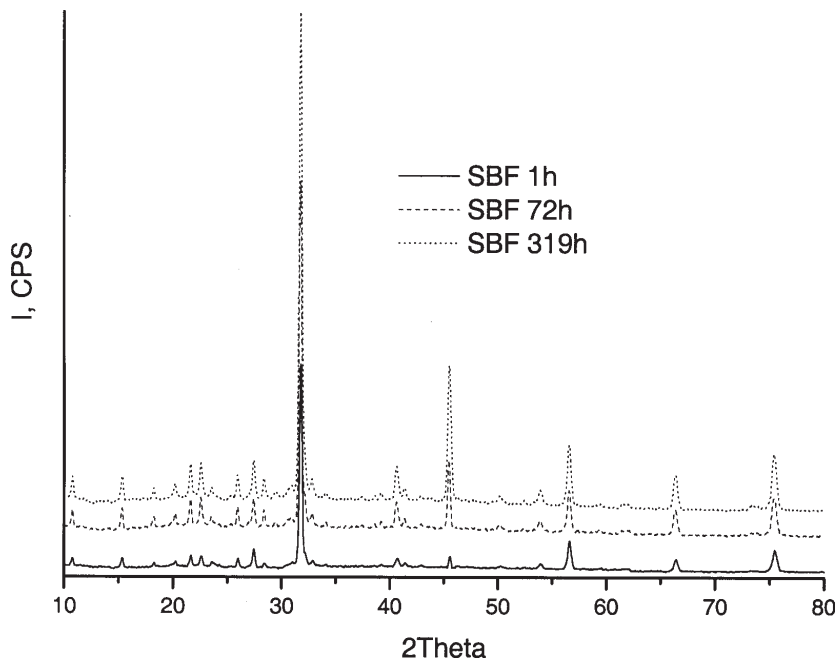


Fig. 1. XRD pattern of COLL/HA composites obtained by COLL matrix immersion in SBF, for 1h, 72h and 319h

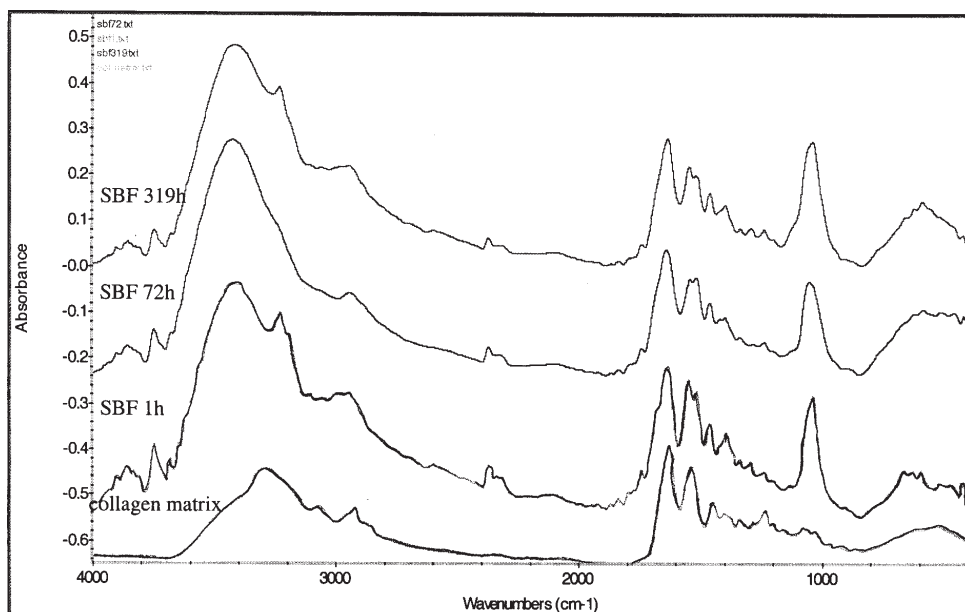


Fig. 2. FTIR spectra of the composite collagen matrix and composite materials obtained by immersion of collagen matrices in SBF for 1h, 72h and 319h

In order to assure biomimetic conditions, the working temperature was set up at 37°C.

The composite materials were lyophilized and analyzed by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and complex thermal analysis (DTA-TG).

X-ray diffraction analysis was performed using a Shimadzu XRD 6000 diffractometer at room temperature. In all cases the Cu K α radiation from a Cu X-ray tube was used. The samples were scanned in the Bragg angle, 2 θ range of 10–70 grd at a scan rate of 2 grd/min. All samples were pressed to a thin film before analysis.

SEM images were obtained with a HITACHI S2600N with an EDAX probe. All samples were covered with a silver layer prior to imaging.

IR spectroscopic measurements were performed using a Shimadzu FTIR 8400 spectrophotometer. The spectra were recorded over the wavenumber range of 400–4000cm⁻¹ with a resolution of 2cm⁻¹.

Differential thermal analyses (DTA) coupled with thermogravimetric analyses (TGA) were performed in air with a Shimadzu DTG-TA-50H analyzer at a heating rate of 10°C.

Results and discussion

The composite materials were characterized from qualitative and quantitative point of view.

X-Ray diffraction - XRD

XRD analyses of the three composite are given in figure 1. Unfortunately, the HA main peak is overlapped on the NaCl main peak. In order to be able to monitor, semi-quantitatively, the evolution of the NaCl content, in all three cases we report all the peaks' intensity as percent towards the peak from 2 θ =31.76; the evolution of the NaCl content being the same as the intensity ratio. It is worthy to note the evolution of the HA/NaCl ratio, with the increasing immersion time. As resulted from the XRD patterns, the amount of NaCl increases with the immersion time increasing.

Fourier transform infrared spectroscopy – FTIR

IR spectroscopy is a useful tool to analyze the composite material especially because XRD gave information only about the crystalline phases. The main peaks of collagen are: amide I (C=O) at 1630; amide II (N-H) at 1550; amide III (C-N) at 1240 while the pyrrolidine ring vibrations appear at ~ 1450 cm⁻¹. The main peaks of hydroxyapatite,

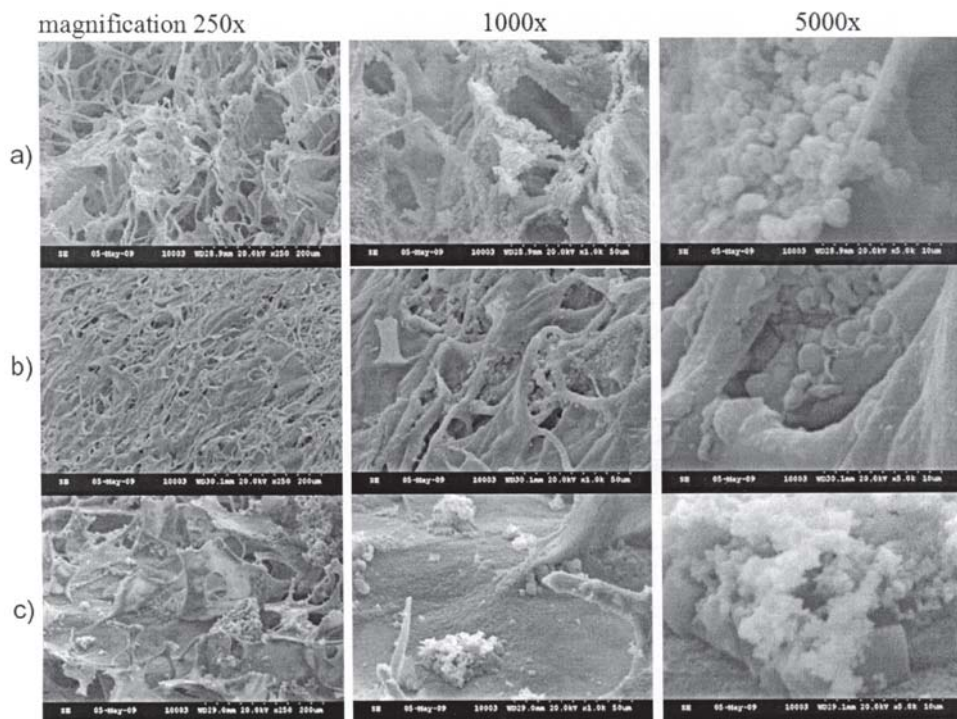


Fig. 3. SEM images of mineralized matrices, at different magnification: 0.25, 1 and 5kx for each composite: a) immersion time 1h; b) immersion time 72h and c) immersion time 319h

corresponding to phosphate groups are: 1030, 601 and 563 cm^{-1} . The large band from 3000 – 3600 cm^{-1} is due to the associated hydroxyl groups from collagen, hydroxyapatite and water.

Analyzing the spectra of collagen matrix and COLL/HA composite materials shown in figure 2 it can be clearly visualize the collagen mineralization by the increasing of phosphate group peaks (especially the peak from $\sim 1030 \text{ cm}^{-1}$).

Scanning electron microscopy – SEM

SEM images (fig. 3) are very suggestive in the study of the mineralization of collagen matrices. The composite morphology is mainly influenced by immersion time. Also it can be seen that the morphology varies very much and, with the increase of immersion time, the composite became more compact, due to the increase of the deposited mineral phase. This variation of the morphology can be well visualized especially at low magnification (250 and 1000 x). At a higher magnification (5000x) it can be seen that the morphology of the composites obtained by immersion for 1 and respectively 72h are very similar but, the composite obtained for 319h immersion time the mineral morphology is different due to the sodium chloride deposition onto the matrix. The sodium chloride can be identified based on its special cubic morphology (fig. 3c – 5000x).

The ATD (fig. 4) curves of the composite sample obtained by immersion in SBF for 1h show the main thermal effects, which occur upon the thermal treatment. The endothermic effect between 30 – 125 $^{\circ}\text{C}$ which is due to the water evaporation; the weak endothermic effects between 125-250 and 250-380 $^{\circ}\text{C}$ accompanied by mass loss which are due to the collagen denaturation and the exothermic effect, accompanied by mass loss between 200 and 600 $^{\circ}\text{C}$ which is due to collagen burn.

Based on the recorded TGA data the content of hydroxyapatite of each composite was determined and plott against the immersion time (fig. 5). It can be seen that the kinetic curve has two distinct zones. The first zone corresponds to a high mineralization rate while the second zone is assigned to a low mineralization rate. In fact, the kinetic curve shape is due to two oposed processes:

deposition and dissolution. From this point of view, it can be conclude that, in the first zone the rate of deposition is much greater than the rate of dissolution while in the second

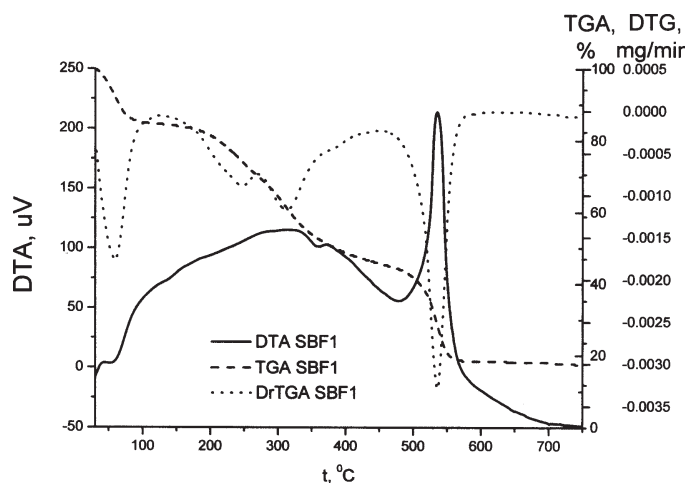


Fig. 4. DTA-TG curve recorded for the collagen/HA composite material obtained by collagen mineralization by SBF immersion method for 1 h

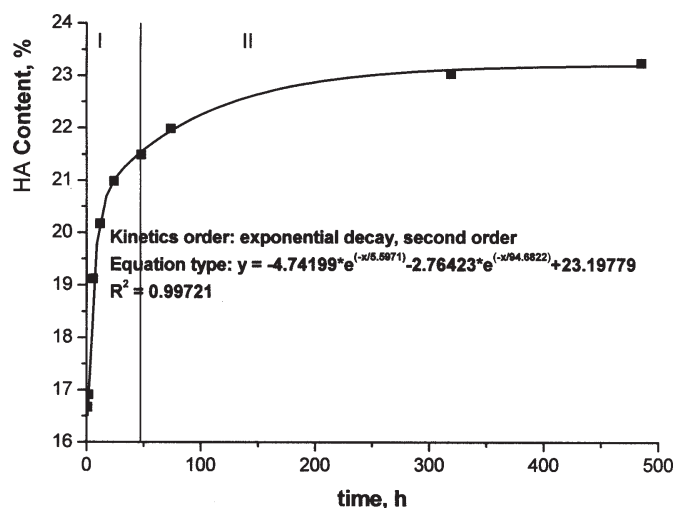


Fig. 5. Kinetic curve of HA deposition on collagen matrices by SBF immersion

zone the rate of deposition and dissolution exhibit the same magnitude.

In addition, it is important to remark that sodium chloride crystals appear only in those composite materials, which were obtained by SBF immersion for more than 48h. The mineralization kinetic was determined using Origin 7.5 software, the best correlation coefficient being obtained by fitting the experimental data with a predefined second order, exponential decay.

Conclusions

This work was focused on the synthesis and characterization of three kind of COLL/HA composite materials. The synthesis method was the well studied SBF immersion technique.

The novelty of this work was the study of the morpho-compositional changes of the material versus the immersion time. Thus the increasing immersion time induces an increasing amount of HA deposition, the NaCl appears in the slow region of mineralization and the materials became more compact.

In many respects, the mineralization of the collagen matrices by SBF immersion method may be compared with the natural osteosynthesis. Two major similarities between the natural osteosynthesis and the biomimetic synthesis described by us are: in both cases, the preformed collagenous support is mineralized in two successive steps, which consist in a rapid mineralization of the collagen followed by a slow mineralization accompanied by the mineral phase reorganization.

Based on the thermogravimetric data, the kinetic of the hydroxyapatite deposition by SBF immersion method was determined, the correlation factor being very good ($R^2=0.9972$).

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