# HYBRID COLLAGEN-CARBOXY-METHYLCELLULOSE/HYDROXYAPATITE COMPOSITE MATERIALS FOR BONE TISSUE REGENERATION

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*This work is devoted to the synthesis and characterisation of hybrid composite materials based on collagen (COLL), carboxymethylcellulose and hydroxyapatite (HA). The hybrid nature is assured by the presence of both 0D filling materials – HA and 1D reinforcing agent – carboxymethylcellulose (CMC). The obtained hybrid material, COLL-CMC/HA, is highly homogenous, CMC being well integrated into the COLL/HA matrix as it results from the microscopic analysis. This hybrid composite can be a proper candidate for bone grafting and support for drug delivery, especially for hard tissue repairing.*

**Keyword**: collagen, hydroxyapatite; carboxymethylcellulose; composite materials; bone graft

## 1. Introduction

Increasing needs of bone grafts lead to a higher interest for developing materials for substitute and/or repairing of bone tissue. Nowadays are known four classes of materials for bone grafts: metals and alloys, ceramics and polymers, composite and nanocomposites and tissue engineered nanocomposites [1]. So far, despite increasing efforts, bone-like materials have not been yet obtained. In terms of developing materials with improved properties (similar to natural bones) the researchers focus their attention to develop collagen / hydroxyapatite based composite materials [2-9], being known that bone is a true composite material

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mainly consisting on these components or composite materials with no or limited compositional similarity with bone [4, 10-18]. The main components used for the preparation of composite materials for bone repairing [19-32] are presented in Table 1. The interest for bone grafts is explained based on their high need, in present the need of bone being surpassed only by the need of blood [7].

*Table 1.*

**Main components of the bone grafts**

| **Polymers** | **Inorganics** |
|---|---|
| **Collagen** (**COLL**) | **Hydroxyapatite (HA)** |
| Poly – L- lactic acid PL(L)A | Different calcium phosphates |
| Poly(glycolic acid) – PGA | Calcium sulphate |
| Poly(lactic-co-glycolic) acid – PLGA | Bioglass |
| Polyethylene – PE | Silica |
| Poly(caprolactone) – PC | Aluminosilicates |
| Polyurethanes – PU | Alumina |
| Poly(3-hydroxybutyrate) | Zircona |
| Poly(3-hydroxyvalerate) | Carbon nanotube (single or multiwalled) – SWCNT and MWCNT |
| Polyphosphazene | |
| Cellulose and derivatives | Carbon fibre |
| Chitosan and chitin | |
| Polymethylmethacrylate and other acrilates | |

Due to the compositional similarity with bone, composite materials based on collagen and hydroxyapatite are intensively studied. There are two main ways to improve the properties of these materials: improving the morphology of the material on one hand, and adding adequate components on the other hand. Till now, a lot of researches were performed in the field of COLL/HA composite materials but, unfortunately, up today the mechanical properties of the bone were not obtained. Perhaps, the easiest way to improve the properties of the COLL/HA composite materials is to add a third component - X, as Table 2 presents.

*Table 2.*

**Types of COLL-HA-X ternary composites and role of the third component**

| Crt. No. | Third component - X | Role of third component | References |
|---|---|---|---|
| 1 | polyvinyl alcohol – PVA | Morphological changes of the composite materials | [9, 33] |
| 2 | collagen hydrolysate | Stronger inorganic – organic interaction | [34], |
| 3 | poly(D,L-lactic acid) | NA* | [35] |
| 4 | poly(D,L-lactic-co-glycolic acid) – PLGA | NA* | [36] |
| 5 | chitosan | Antimicrobial activity | [37] |
| 6 | magnetite | Antitumoral activity when proper electromagnetic field is applied (leading to hyperthermia) | [38] |

* not studied or data not available

## 2. Experimental

### 2.1. Materials

The collagen gel (M.W. ≈ 300,000 Da) was obtained at the National Research & Development Institute for Textiles and Leather, Collagen Department. Calf hide was used to get the collagen gel, by a special chemical technology that was purified by dialysis against water, as we previously described [39]. The pH of the collagen gel was adjusted to 7.5. Its composition is summarized in Fig. 1. Hydroxyapatite was obtained *in situ*, in the presence of the collagen gel. Sodium carboxymethylcellulose (CMC) ($M_w$ = ~90 000) was purchased from Aldrich and used without any purification. The precursors used for HA synthesis were calcium hydroxide (puriss. p.a.) and sodium phosphate monobasic monohydrate (ACS reagent), both of them purchased from Sigma-Aldrich.

### 2.2. Synthesis of COLL-CMC/HA composite material

COLL-CMC/HA composite material is obtained as presented in Fig. 1.

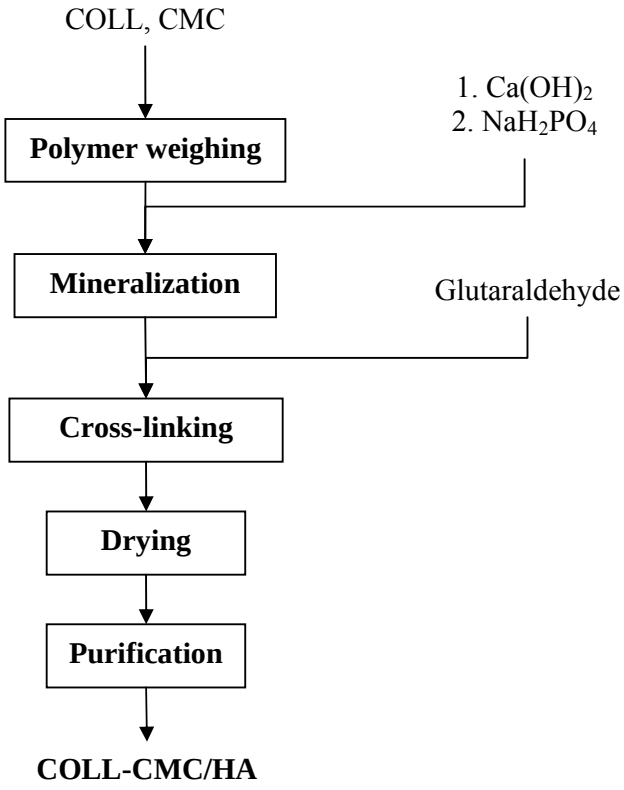



Fig. 1. Schematic representation of preparation of COLL-CMC/HA

The first step was the homogenisation of the collagen gel and carboxymethylcellulose (COLL:CMC = 2:1 (w/w)) followed by mineralization with $Ca(OH)_2$ and $NaH_2PO_4 \cdot H_2O$, as we previously presented [38, 40]. The mineralization was performed in order to obtain COLL:CMC:HA = 2:1:8 (w/w/w). Once mineralized, the sample is cross-linked with 1% (reported to the dry collagen) glutaraldehyde solution and dried at 20$^oC$ [41].

### 2.3. Methods

X-ray diffraction analysis was performed using a Shimadzu XRD 6000 diffractometer at room temperature, using Cu $K_\alpha$ radiation. The samples were scanned in the range $2\theta$ =10–70$^oC$, with a scanning rate of 2$^oC$/ min.

IR microscopy/spectroscopy was performed by using a Thermo FT-IR Nicolet iN10 MX microscope; the spectra were recorded in the wave number range of 400 – 4000 $cm^{-1}$, with a resolution of 4$cm^{-1}$. For a better identification of the peaks, the obtained spectra were resolved using a Gaussian-Lorentzian peak resolve procedure, with no baseline (for all the spectra, the baseline correction was previously done).

SEM analyses were performed on a HITACHI S2600N electron microscope with EDAX on samples covered with silver layer.

## 3. Results and discussion

After synthesis and drying, COLL-CMC/HA composite material was analyzed using X-ray diffraction (XRD), Fourier Transform – Infrared Spectroscopy and Microscopy (FTIR) and Scanning Electron Microscopy (SEM).

### 3.1. X ray diffraction

The X-ray diffraction analysis presents only the peaks for hydroxyapatite, because the bands characteristic for derivative of cellulose or collagen can not be detected.

X-ray diffractogram recorded on the COLL-CMC/HA composite material presents the characteristic bands of hydroxyapatite (Fig. 2).

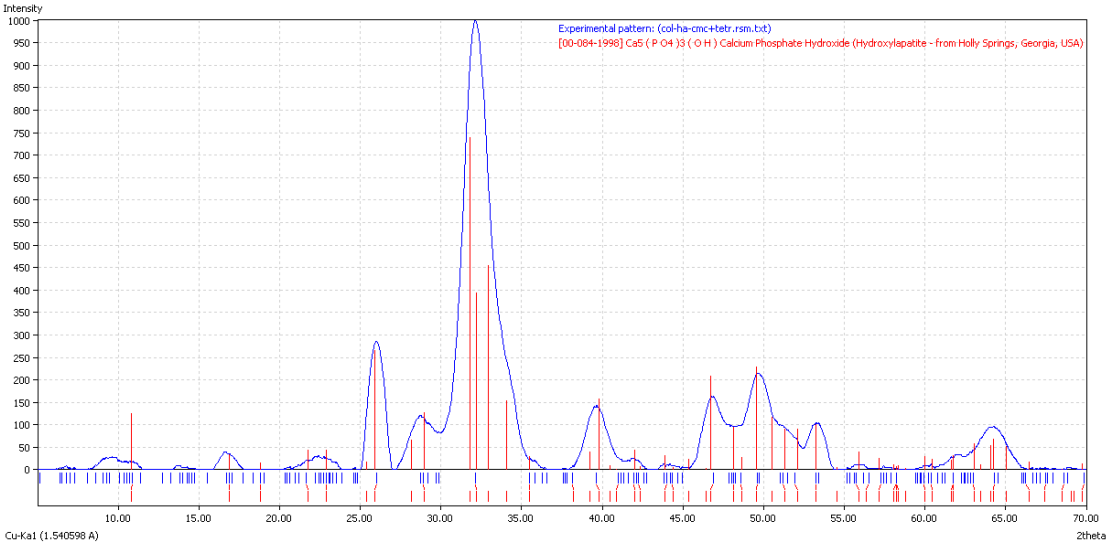



Fig. 2. XRD diffraction pattern of COLL-CMC/HA

Due to the low crystallinity of the organic phase and low levels of potential by-products, the characteristic bands of other crystalline phases are not identified beside the high content of collagen and CMC. Sodium chloride and different calcium phosphates (CaPs) can be identified as by-products. The presence of CaPs is a proof of proper synthesis conditions (especially pH and Ca:P ratio). Sodium chloride can be easily removed by washing the cross-linked composite with distilled water, as we have already presented [7].

### 3.2. FTIR spectroscopy and microscopy

FTIR spectroscopy is useful for analyzing the complex materials with many components, particularly for composite materials. For COLL-CMC/HA hybrid composite material, the three main components can be identified only after deconvolution of the spectrum, because there are a lot of bands overlapped (especially the absorption bands of HA and CMC as well as COLL and CMC) (Fig. 3 and 4). For more accurate determination, the spectrum of COLL/HA composite material was also deconvoluted. The comparison of the two deconvoluted spectra is useful to identify the contribution of CMC. Based on the shift of the position of the independent peaks obtained by deconvolution, we can conclude that CMC also interact with HA.

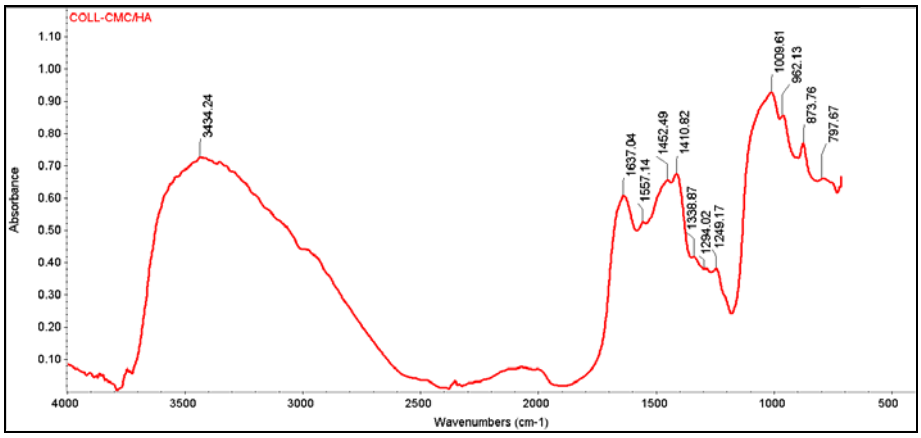



Fig. 3. FTIR spectra of COLL-CMC/HA

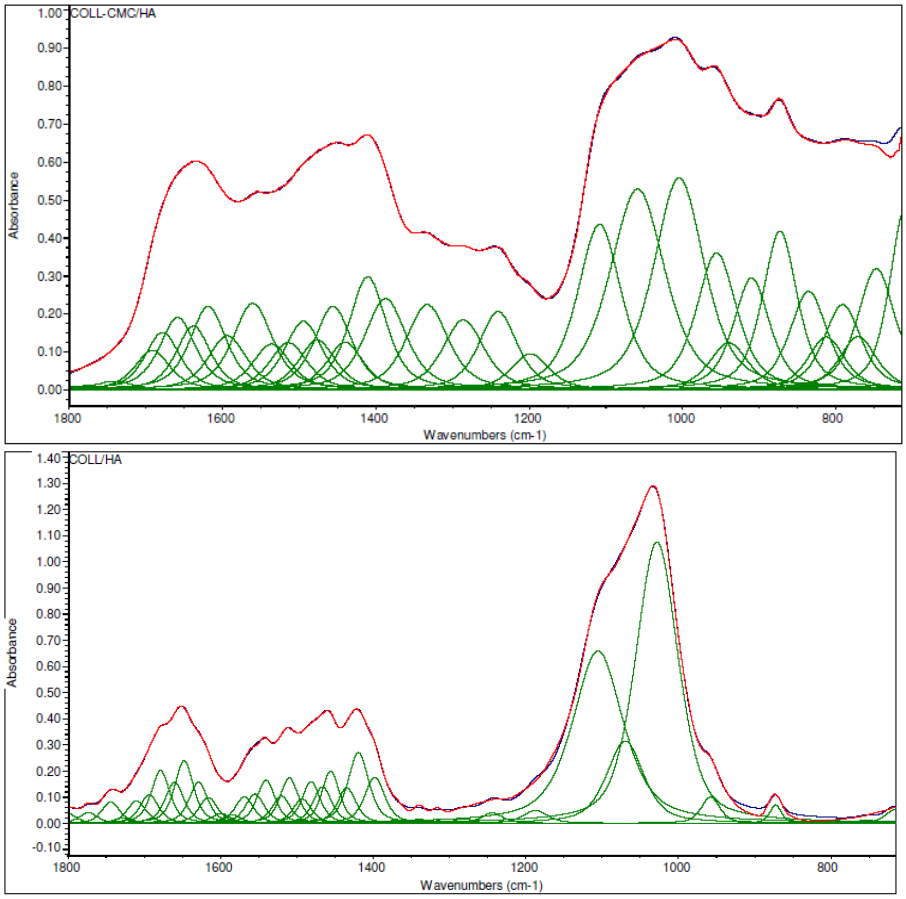
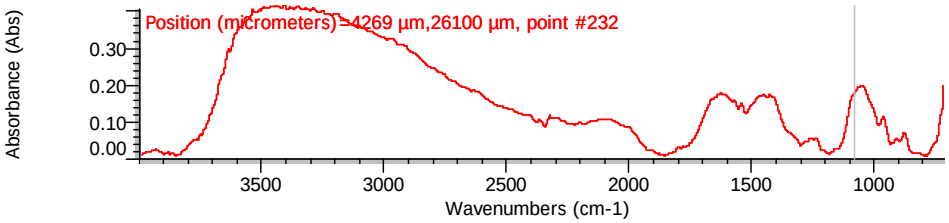


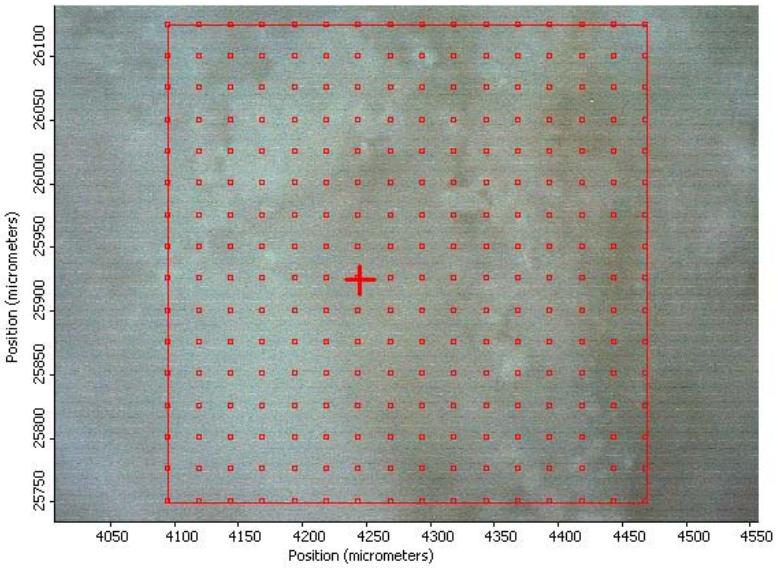

Fig. 4. Deconvoluted FTIR spectra (715-1800cm$^{-1}$) of COLL-CMC/HA and COLL/HA

FTIR microscopy was also used to characterize the hybrid composite. For this reason, the sample was fractured and the resulting surface analyzed by FTIR spectroscopy. Once obtained the microscopic information, the FTIR maps at different wavelengths were obtained. In our case, the peaks of interests were:

- 715cm$^{-1}$ for carboxymethylcellulose;
- 1655cm$^{-1}$ for collagen;
- 1033cm$^{-1}$ for hydroxyapatite.

According to FTIR maps (Fig. 5) the material is homogenous, which means that CMC microfibres are well dispersed into the COLL/HA matrix.



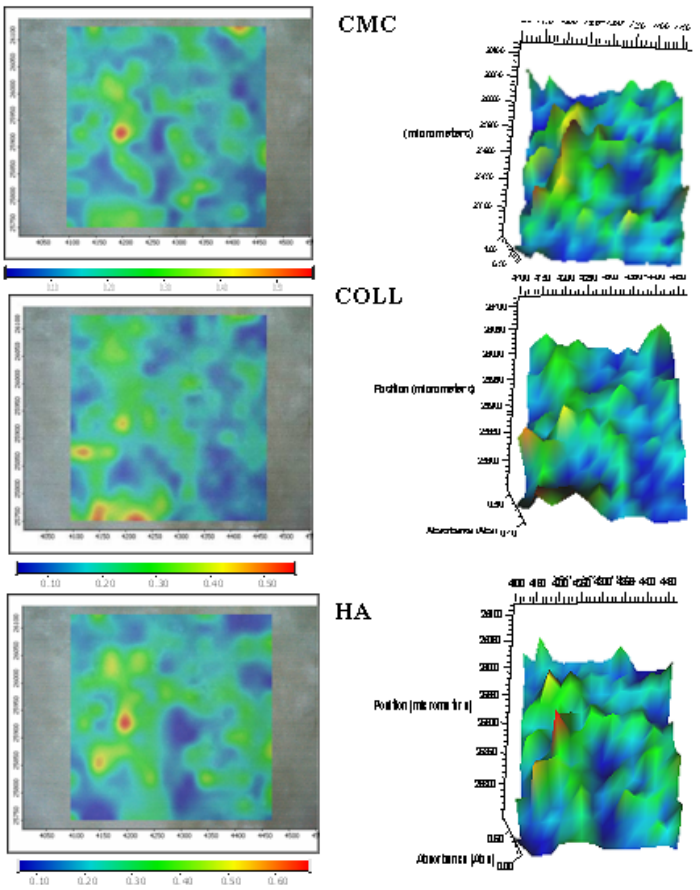



Fig. 5. FTIR images of Coll-CMC/HA

### 3.3. Scanning electron microscopy

Scanning electron microscopy was particularly useful for analyzing the microstructure of the hybrid composite obtained in the process, allowing a higher magnification than FTIR microscopy. The sample was analyzed at different magnifications from 100 to 2000x. The low content of CMC (~9.1% CMC, 18.2% COLL and 72.7%HA), as well as the good integration of CMC into the COLL/HA matrix, made difficult the identification of CMC microfibres. Only some ends of the CMC fibres could be visualized, but all these were well covered by COLL/HA matrix.

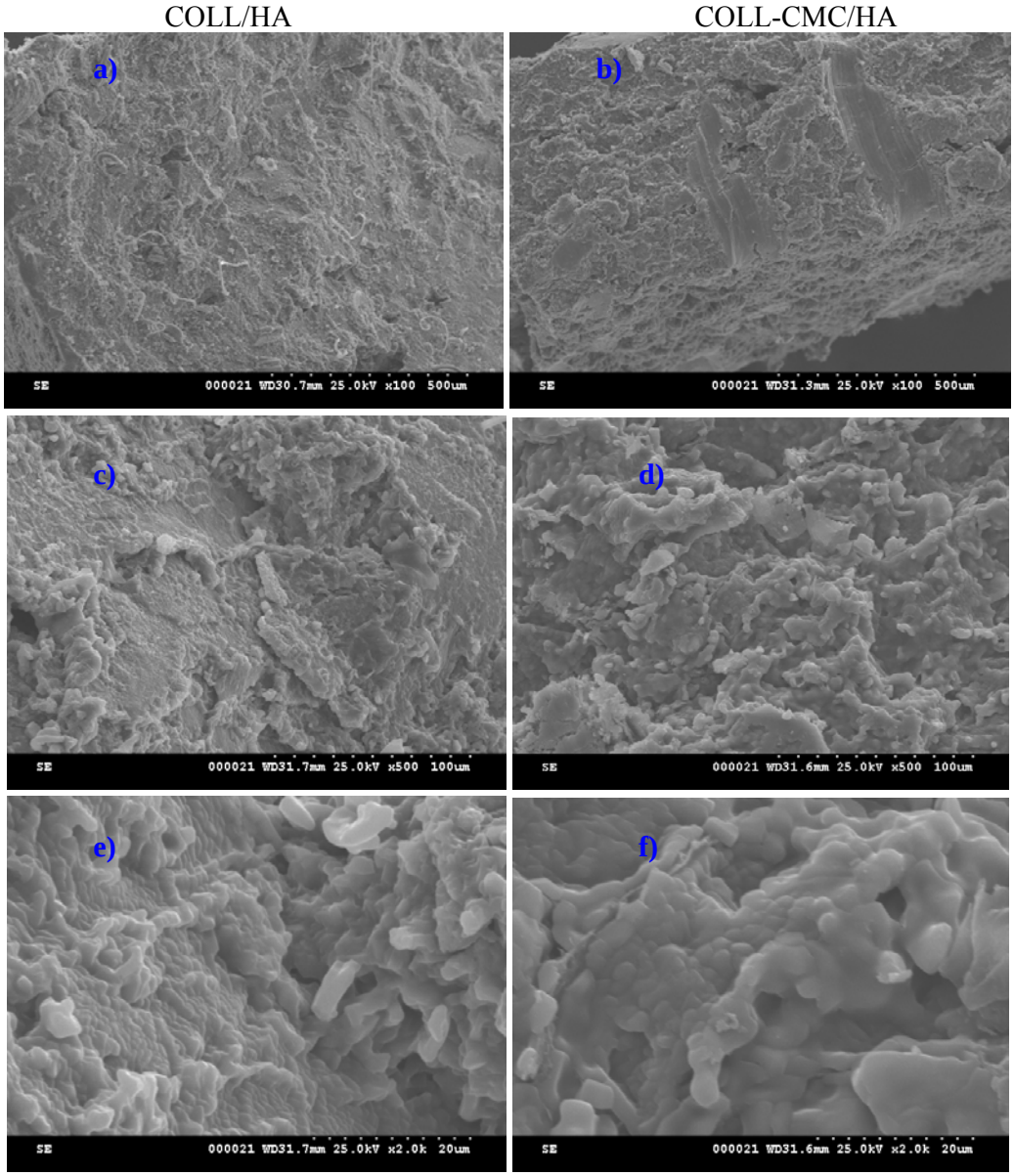



Fig. 6. SEM images of characteristic of COLL/HA (a,c,e) and COLL-CMC/HA (b,d,f) materials, at different magnifications

## 4. Conclusions

A new hybrid composite material as potential candidate for hard tissue substitution and repairing was obtained. The carboxymethylcellulose microfibers

are well embedded in the mass of mineralized polymer, being difficult to be identified by SEM in the ternary composite based on collagen, carboxymethylcellulose and hydroxyapatite. Further *in vitro* and *in vivo* tests will clarify the possibility to use this material for bone grafting and substitution, as well as for drug delivery systems, especially in the case of bone cancer and osteoporosis.

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