COLLAGEN-FIBROIN-HYDROXYAPATITE SCAFFOLDS FOR BONE TISSUE ENGINEERING

ALINA TEODORA BIICIN[1], ELENA DĂNILĂ[2], MĂDĂLINA GEORGIANA ALBU KAYA[2], CĂTĂLIN ZAHARIA[3], ŞTEFANIA MARIN[2], IOAN CRISTESCU[4]

[1]*University Politehnica Bucharest, Faculty of Medical Engineering, 1-7 Gheorghe Polizu St., Bucharest, Romania*

[2]*INCDTP - Division Leather and Footwear Research Institute, 93 Ion Minulescu St., 031215, Bucharest, Romania, *albu_mada@yahoo.com (corresponding author)*

[3]*University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Science and Engineering of Polymers Department, 1-7 Gheorghe Polizu St., Bucharest, Romania*

[4]*"Carol Davila" University of Medicine and Pharmacy*

Bone regeneration is a serious problem nowadays because of the increased number of people suffering from infections, arthritis and bond loss. The aim of the present work was to develop and characterize collagen – fibroin - hydroxyapatite matrices for hard tissue regeneration. In our study we wish to develop biomaterials which mimic bone composition and prevent allergic or toxic effects. The composites matrices obtained by freeze drying were characterized by FT-IR analysis, water uptake capacity and optic microscopy. The results obtained from analyses confirmed that collagen – fibroin - hydroxyapatite matrices exhibit proper characteristics for bone mineralization.

Keywords: collagen hydroxyapatite, fibroin, bone regeneration

INTRODUCTION

Bone problems can appear from different reasons such as medications, bacteria or unstable lifestyle. Bone regeneration approaches require a biocompatible material such as a scaffold to support cell proliferations as well as to deliver drugs needed for a proper recuperation.

Among natural polymers, collagen is one of the most common proteins in mammals so it can be successfully used as a biomaterial for medical application, because it has excellent biocompatibility, permeability, hydrophilicity and it is stable *in vivo* (Mederle *et al.*, 2016). Collagen scaffolds could also be used for tissue regeneration being proper for cell deposition and proliferation (Marin *et al.*, 2014). In order to obtain mechanical strength and elasticity comparable to those of extracellular matrix collagen gels are crosslinked (Aziz *et al*., 2005).

Hyroxyapatite (HAP) is a natural mineral found in bones that is responsible for mechanical properties of hard tissue (Kobayashi *et al*., 2001). Hyroxyapatite, as a scaffold, can improve, enhance sinterability and densification which may improve fracture toughness. Hydroxyapatite can be obtained by different methods such as hot pressing, isostatic hot pressing and slip casting, tape casting or injection molding (Rodriguez *et al.*, 2001). Among all methods the most advantages is gel casting.

Silk fibroin (SF) has outstanding properties including biocompatibility, water vapor permeability, biodegradability and little inflammatory reaction. Generally, fibroin is isolated from silkworm cocoons and purified from the sericin content. The studies showed that fibroin maintain the adhesion and proliferation of the fibroblasts and it helps the regeneration of the bone. The regenerative proprieties of fibroin were improved by using hydroxyapatite. Moisenovich *et al.* introduced nano-hydroxiapatyte and collagen to enhance their compatibility, maintain the adhesion and proliferation of fibroblasts (Moisenovich *et al*., 2014).

Collagen and HAP are the most important composites studied for bone tissue. Azami *et al.* prepared the nanocomposite trough a freeze-drying technique. The scaffolds are well defined with interconnected pours. Cells exhibited good proliferation which indicates a high level of biocompatibility (Azami *et al.*, 2006).

Fibroin caught the attention in tissue engineering field because of its outstanding biocompatibility, biodegradability and minimal inflammatory reaction. (Pascu *et al.*, 2014).

The aim of this paper was to develop and characterize collagen – fibroin - hydroxyapatite scaffolds in order to be used in medical applications such as bone mineralization and regeneration.

MATERIALS AND METHODS

Materials

The type I fibrillar collagen gel having a concentration of 2.85% (w/w) was extracted from calf hide using technology currently available at the Research-Development Textile Leather National Institute Division Leather and Footwear Research Institute – Collagen Department (Albu, 2011). The hydroxyapatite nano-powder was purchased from Sigma Aldrich, the fibroin was obtained in the Science and Engineering of Polymers Department laboratory, from University Politehnica of Bucharest (Zaharia, 2016).

Preparation of Collagen Scaffolds

The concentration of each collagen gel was adjusted at 1% and 7.2-7.4 pH using 1M sodium hydroxide (the pH of the physiological medium). 2% fibroin was added to collagen gel and 70% HAP (w/w), then the collagen gels were cross-linked with 0.05% glutaraldehyde (GA)(reported to collagen dry substance) as Table 1 presents

Table 1. Composition and name of collagen gels

Code of gels	Col, %	SF, %	HAP, %	GA, %
Coll	1	0	0	0.05
Coll-SF	1	2	0	0.05
Coll-HAP	1	0	70	0.05
Coll-SF-HAP	1	2	70	0.05

The collagen gels were freeze-dried using Delta 2-24 LSC (Martin Christ, Germany) lyophilizer and spongious forms was obtained. All the samples were characterized by FT-IR analysis, water absorption and optic microscopy.

FTIR-ATR Analysis

FT-IR spectral measurements were recorded by spectrophotometer Jasco FT/IR-4200. All the spectra were recorded at the following parameters: spectral range 4000-600 cm^{-1}, resolution 4 cm^{-1} with 30 acquisitions per each sample.

Water Absorption

In order to determine the water absorption, the scaffolds were first immersed in water and at well defined time intervals; the samples were withdrawn and weighted. The water absorption was calculated using the following equation:

$$\% \text{ Water up-take} = (Wt - Wd)/Wd \text{ g/g} \quad (1),$$

where Wt denotes the weight of the swollen samples at immersion time t, and Wd denotes the weight of the dry samples. All the samples were studied in triplicate.

Optical Microscopy Study

All images were captured with a Leica Stereomicroscope S8AP0 model and 20-160x magnification capacity. For better evaluation of the samples, a 20x magnification and incident external cold light were used.

RESULTS AND DISCUSSION

After lyophilization the 3D porous collagen sponges based on collagen, fibroin and hydroxyapatite, were obtained, with the appearance presented in Figure 1.

Figure 1. A-Coll, B-Coll-SF, C-Coll-HAP, D-Coll-SF-HAP

The results of FT-IR spectra are presented in Figure 2.

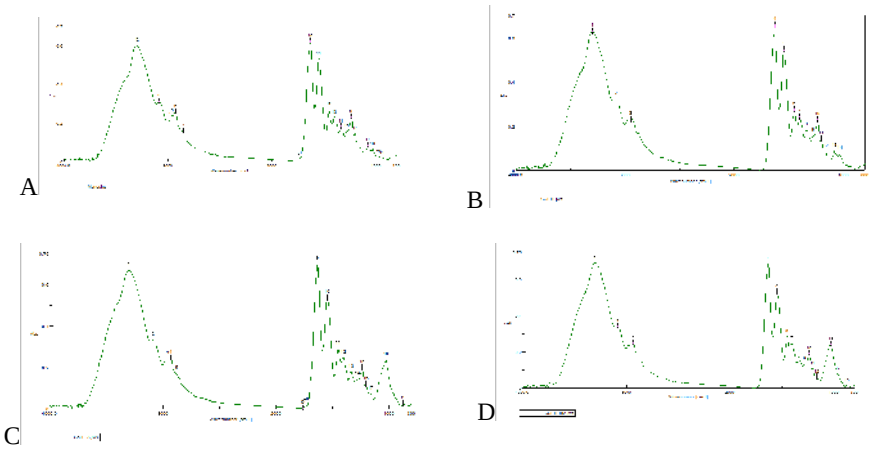

Figure 2. FT-IR spectra of spongious matrices:
A-Coll, B-Coll-SF, C-Coll-HAP, D-Coll-SF-HAP

The FTIR spectra of collagen with and without silk fibroin and hydroxyapatite matrices are represented in Figure 2. The spectrum of collagen matrix (Fig. 2A) exhibited typical amide bands of proteins i.e. 3303 cm^{-1} and 2924 cm^{-1} for amide A and B respectively, 1630 cm^{-1} was ascribed to amide I (C=O stretching), 1544 cm^{-1} to amide II (N-H deformation) and 1238 cm^{-1} to amide III (N-H deformation) (Albu, 2011). When silk fibroin was added only amide B shifted to 2950 cm^{-1}, maybe because its small amount. More changes were visible in matrix Collagen-Fibroin-hydroxypatite, such as the 1030 and 961 cm^{-1} bands corresponded to v3 and v1 mode vibration of $PO4^{3}$.

Figure 3 presents the water up-take during 72 hours for the studied samples.

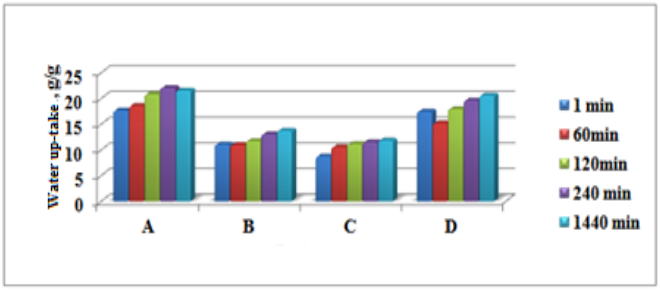

Figure 3. Water up-take for collagen scaffolds:
A-Coll, B-Coll-SF, C-Coll-HAP, D-Coll-SF-HAP

From Figure 3 can be observed that the reference collagen sample (Coll) absorbs the heights amount of water, namely up to 20g/g in 72 hours. Fibroin addition leads to a lower absorption capacity, Coll-SF sample absorbing 15 g/g, less than the blank sample,

which indicates density increasing. Hydroxyapatite addition contribute to density increasing, sample Coll-HAP having a water absorption capacity of 10 g/g. The last sample, containing collagen, fibroin and hydroxyapatite presented a high water absorption capacity due to the structure formed, porous structure favoring the uptake of water.

The optical microscopy results are presented in the Figure 4.

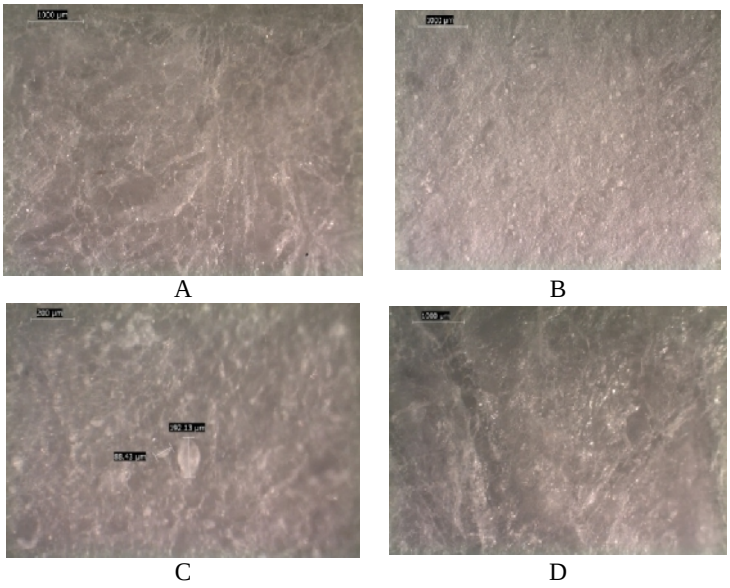

Figure 4. Optical microscopy for obtained matrices:
A-Coll, B-Coll-SF, C-Coll-HAP, D-Coll-SF-HAP

From optical microscopy images it can be notice the porous structure of obtained samples. Sample Coll and Coll-SF-HAP present a porous structure with interconnected pores, associated with collagen structure, while sample Coll-SF and Coll-HAP show a denser structure, with lower pore size that result from addition of fibroin and hydroxyapatite. Obtained results after optical microscopy analysis are in agreement with result obtained following water up-take analysis. Water absorption capacity is correlated with samples structure and pores morphology.

CONCLUSIONS

Type I collagen with silk fibroin and hydroxyapatite and their combinations were used in order to obtain matrices for bone tissue regeneration. The combination between components were highlighted by FT-IR spectra changes when fibroin and hydroxyapatyte was added. The matrices absorbed between 20 and 10 g/g water, being less absorbable the one with fibroin. The most compact samples were the one with hydroxyapatite. The results were proved by optical microscopy images and showed that collagen-silk fibroin-hydroxyapatite are potentially novel candidates as scaffolds for bone tissue engineering applications.

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