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Organic / inorganic bioactive materials Part I: Synthesis, structure and *in vitro* assessment of collagen/silicocarnotite biocoatings

Research Article

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Abstract: The silicocarnotite, as an inorganic part of the coatings, has been synthesized using a polystep sol-gel method. The chemical composition of the prepared silicocarnotite sol is described as 58.12 CaO, 29.42 P_2O_5, 12.45 SiO_2 (wt%), where Ca/P+Si = 1,67. The acid soluble type I collagen, as an organic part of the obtained coatings, was mixed with silicocarnotite powder in a weight ratio of 25:75 and 75:25 weight ratio without cross-linkage. The acidity of the obtained mixture was readjust with 25% NH_4OH to pH = 9.0. The mixture was then dried at 37°C for 12 h.

The growth of B-type carbonate containing hydroxyapatite (B-type CO_3HA) in which $CO_3^{2+} \rightarrow PO_4^{3-}$ on the surface of collagen/silicocarnotite coatings soaked in 1.5 simulated body fluid (1.5 SBF) was observed. The nucleation of B-type CO_3HA was estimated on the obtained coatings after 3 days immersion in 1.5 SBF. The negatively charged carboxylate groups from the collagen surface may be responsible for the HA deposition. This was confirmed by the "red shift" of carboxylate groups of collagen molecules in the FTIR spectra. After soaking in 1.5 SBF, the morphology of prepared coatings and HA formation was observed by SEM.

Keywords: *Collagen • Silicocarnotite • Biocoatings • In vitro bioactivity*

1. Introduction

Bone diseases and defects are one of the most significant medical conditions, which frequently require extensive use of synthetic materials. These materials need to physically and (or) biologically function in intimate contact with living tissue, but they are generally poor in bonding with the host bone. The so-called bioactive ceramics, *i.e.* Bioglass®, hydroxyapatite (HA), β-tricalcium phosphate (TCP) and apatite-wollastonite (A/W) glass-ceramics [1] are materials that induce biological tissue integration function in a tissue defect in-situ. It is known that the stages that are involved in the forming of the bone bond of bioactive glasses and glass-ceramics were summarized by Hench [2]. It is clearly recognized that for a bond with bone tissue to occur, a layer of biologically active carbonate containing HA (CO_3HA) must form. This conclusion is based on the finding that CO_3HA is the only common characteristics of all the known bioactive implant materials [3]. On the other hand, bioactivity is not an exclusive property of bioactive glasses. Hydroxyapatite and other calcium phosphates (CP) also show an excellent ability to bond with bone.

Composite materials and coatings can be defined as those materials that consist of two or more fundamentally different components that are able to act synergistically to give properties superior to those provided by other component alone. Ceramic carriers bear high mechanical loads, whereas cell benefit of the directional

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information given by the regular pattern of collagen and therefore, several research groups have evaluated some combinations of collagen and calcium phosphates [4]. In natural bone, bone apatite crystals are closely associated with collagen fibers and as a consequence of this unique architecture, bone has high torsion, tensile and compressive strength. These three mechanical characteristics have so far not been equally matched by any of the bone graft substitute materials. Nevertheless, research groups try to mimic the physiological collagen mineralization process, taking for example reconstituted collagen fibers [5,6], fabricated collagen sponges [7,8] or sheets [4,9] as templates for crystal growth in a solution rich in calcium and phosphate, or precipitation of HA within a collagen dispersion [8,10-13]. A different approach is the coating of fabricated porous ceramics with collagen [14,15], which was reported to facilitate cell attachment and invasion upon implantation [16,17]. A third option is the combination of collagen dispersions with calcium phosphates and after mixing, an application of the semi-solid or putty-like preparation is possible [18]. Further processing may include freeze-drying [19] or air drying [20,21].

In vitro studies on the mechanism of biomineralization has been documented to confirm the reproduction of CP on different bioactive ceramics [22,23]. These studies used a protein-free cellular simulated body fluid (SBF) with pH = 7.4 and ionic composition equal to those in blood plasma, indicating the compositional and structural dependence of ceramic bioactivity. For instance, glasses in the $CaO-P_2O_5-SiO_2$, $Na_2O-CaO-SiO_2$, $M_2O-SiO_2-TiO_2$ (M = Na and (or) K) and $Na_2O-CaO-Al_2O_3$ systems have been immersed in SFB [24,25]. It is known that binary glasses in the $CaO-SiO_2$, M_2O-SiO_2 and M_2O-TiO_2 systems induced CP formation, but those in the $CaO-P_2O_5$ and $M_2O-Al_2O_3$ systems were not indicative. In these fields of knowledge it is reasonable to point out that time dependent characterizations of surface composition and structure indicated that the formation of CP is preceded by the formation of hydrated silica and (or) titanium abundant on Si-OH or Ti-OH groups [26].

In recent years some authors [27-29] have prepared silicon substituted hydroxyapatites (SiHA) *via* sol-gel method using TEOS as a SiO_2 precursor for glass-ceramics materials. The synthesized materials include other crystalline phases, such as TCP and CaO. On the other hand, this kind of silica precursor can lead to different SiHA structures with different physical and chemical properties. There is extensive literature concerning the silica content in the prepared SiHA structures: 0.25 wt% [30], 0.28 wt% [31], 0.4 wt% [32-34], 0.56 wt% [31], 0.8 wt% [34-39], 1.0 wt% [30], 1.2 wt% [36,40], 1.5 wt% [38], 1.6 wt% [36], 2.0 wt% [41], 2.2 wt% [39], 4.0 wt% [38] and 4.9 wt% [39]. In these fields of studies, silicocarnotite can be defined as calcium silicophosphate with a carnotite structure. Some authors determined that the silicocarnotite structure is very close to hydroxyapatite and examined its *in vitro* bioactivity. They concluded that the introduction of silicon in HA lattice improved *in vitro* bioactive response with respect to apatites without substitutions [31]. Others have investigated that the loss of all the OH groups from HA due to SiO_4 substitution resulting in a silicocarnotite structure. They considered that silicocarnotite as a mixture of a silicon substituted dehydrated apatite and oxyapatite [42]. Ruys [28] found that silicocarnotite can be present as a impurity phase in HA structures at the lowest SiO_2 addition accompanied by increasing amount of α-TCP and β-TCP in the Ca-Si-P-O amorphous phase.

This paper will focus on the possibility of obtaining hybrid materials, containing collagen and silicocarnotite and the *in vitro* bioactivity evaluation of the synthesized coatings in a Kokubo solution (1.5 SBF).

2. Experimental

The silicocarnotite powder as the inorganic part of the coatings has been synthesized using a polystep sol-gel method. The chemical compositions of the prepared silicocarnotite sol are described as 58.12 CaO, 29.42 P_2O_5, 12.45 SiO_2 (wt%), where the molar ratio Ca/P+Si was equal to1.67.

The first step was to prepare SiO_2 sol from tetraethoxysilane (TEOS). TEOS was stirred under a mixed solvent of C_2H_5OH and H_2O with a very small amount of HCl as a catalyst in a volume ratio of $TEOS:C_2H_5OH:H_2O:HCl$ = 1:1:1:0.01. After identifying a transparent solution of the above mixture after approximately 1 hour, a mixture of calcium and phosphate sources was added under intensive stirring.

The second step was to prepare the calcium phosphate (CP) solution by mixing $Ca(OH)_2$ and H_3PO_4 at pH~10-11. This solution was added to the SiO_2 sol stirring constantly for 20 hours. The obtained sol was gelated at 120°C/12 hours and the thermal treated at 1200°C for 2 hours.

Hydrochloric acid soluble type I collagen (Fluka), as organic part of the obtained coatings, was mixed with silicocarnotite powder in 25:75 and 75:25 weight ratio without cross-linkage while stirring for 6 hours. The acidity of the obtained mixture was readjust with 25% H_4OH to pH = 9.0 and then the mixture was dried at 37°C for 12 hours.

The bioactivity of the obtained coatings were evaluated by examining the carbonate containing apatite formation on their surfaces in 1.5 SBF (pH = 7.4). The SBF solution with a 1.5 times concentration was prepared from the following reagents as follows: $(CH_2OH)_3CNH_2$ = 9.0075 g, NaCl = 11.9925 g, $NaHCO_3$ = 0.5295 g, KCl = 0.3360 g, $K_2HPO_4 \cdot 3H_2O$ = 0.3420 g, $MgCl_2 \cdot 6H_2O$ = 0.4575 g, $CaCl_2 \cdot 2H_2O$ = 0.5520 g, Na_2SO_4 = 0.1065 g, HCl (2 mol L^{-1}) = 30 mL in distilled water. Highly supersaturated and consequently unstable, the 1.5 SBF needed to be buffered at pH = 7.25 in order to prevent a homogeneous apatite formation in the solution [43,44].

The structural evolution and phase formation of the obtained hybrid materials have been studied by using XRD (Bruker D8 Advance) with CuKα radiation, FTIR (MATSON 800 FTIR) and SEM (Philips-515).

3. Results and Discussion

The XRD patterns of synthesized silica containing sample, in which the molar ratio of the components was equal to hydroxyapatite, are presented in Fig. 1.

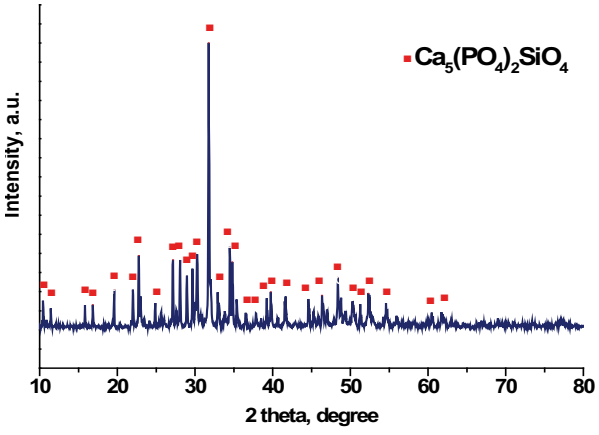

Figure 1. XRD patterns for silica containing sample, thermal treated at 1200°C / 2 h

The XRD of the obtained material with R = 1.67 shows the presence of pure $Ca_5(PO_4)_2SiO_4$ (silicocarnotite) (PDF 40-0309). The secondary phases of TCP and CaO have not been observed in the prepared materials. These phases have been detected in the sample, prepared with 3.75 wt% Si [27]. The *in vitro* bioactive future of silicocarnotite has been previously established by [31]. Infrared spectroscopy was used to study the obtained silicocarnotite after heat treatment state and to evaluate the effect of silicon substitution.

The FTIR spectra of thermal treated silicocarnotite, as an inorganic part of the prepared coatings, is shown in Fig. 2

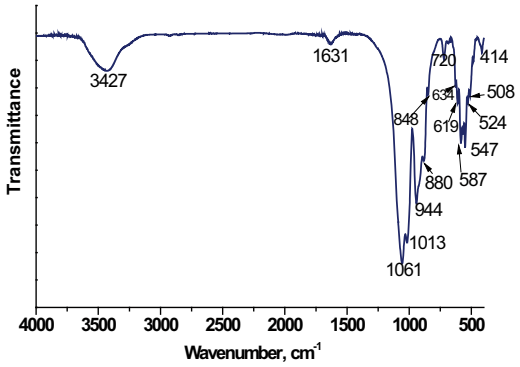

Figure 2. FTIR spectrum of silicocarnotite, heat treated at 1200°C / 2 h

The obtained FTIR spectrum is very complicated. From the literature data, the intense bands at 547, 587, 944, 1013 and 1061 cm^{-1} correspond to ν_3 and ν_1 P-O stretching vibration modes [37]. On the other hand, the bands between 1013 and 1061 cm^{-1} together with 414, 508 cm^{-1} can be assigned to the vibration of the Si-O-Si bond. The peaks centered at 587 and 619 cm^{-1} for the obtained ceramic material corresponds to the O-P-O ν_4 bending mode. The band at 3450 cm^{-1} corresponds to the stretching and vibrational modes of the hydroxyls groups, respectively. In the case of silicocarnotite an additional peak was observed at 880 cm^{-1}. Some authors have related this to the presence of silicon in HA structure [45]. We can also observe that the ν_3 stretching bands of PO_4^{3-} groups (1061 cm^{-1}) shift to higher frequencies in the presence of silica, compared with pure HA structure. We also see that in the sample, the silicon content leads to a decrease in the intensity of the band at 634 cm^{-1} that corresponds to the OH^-. This observation is consistent with the silicon substitution mechanism proposed as $SiO_4^{4-} \rightarrow PO_4^{3-}$, leading to loss of some OH^- groups to maintain the charge balance, *i.e.* the obtained silicocarnotite sample can be partially dehydroxylated [37].

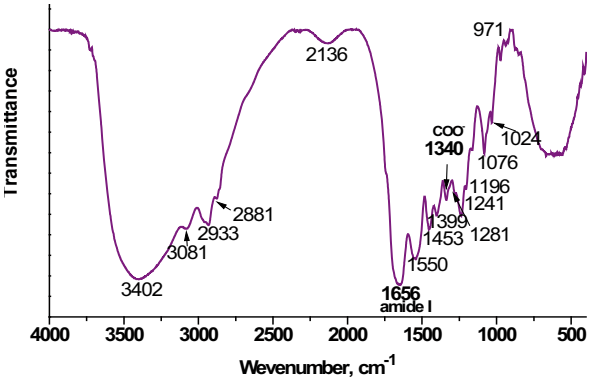

Figure 3. FTIR spectrum of pure collagen (Fluka)

Fig. 3 shows the FTIR spectrum of the collagen I, as the organic component of the obtained coatings. It is known that the amide groups of polypeptides and proteins posses a number of characteristic vibration modes of group frequencies. Especially, the amide I, II and III band region of the spectrum are directly related to the polypeptide conformation [46]. The amide I band, with a characteristic frequency in the range of 1600-1700 cm^{-1}, is mainly associated with the stretching vibrations of the carbonyl groups (C = O bond) along the polypeptide backbone [47] and is a sensitive marker of the peptide secondary structure. The amide I band in a bone spectrum is representative of the collagen content and structure. It is known that there are three are typical bands at 1650-1660 cm^{-1}, 1630-1640 cm^{-1}, and 1680-1700 cm^{-1} in the amide I region of the protein. In our case, the amide I band is centered at 1656 cm^{-1}. In the amide II region of proteins there are bands at 1540-1550 cm^{-1}, 1620-1530 cm^{-1}, and 1520-1545 cm^{-1} [48,49]. In our case, the amide II is centered at 1550 cm^{-1}. For the amide III band of protein, there are bands centered at 1270-1300 cm^{-1}, 1229-1235 cm^{-1} [48,49], and 1243-1253 cm^{-1} [48]. From Fig. 3, the amide III bands have been assigned at 1241 and 1281 cm^{-1}. Amide B is centered at 3081 cm^{-1}, as described previously in [47]. The two bands visible at 1399 and 1340 cm^{-1}, can be assigned to the presence of COOH and COO^{-} in the spectrum of pure gelatin and collagen [50].

Fig. 4 shows the IR spectra of obtained collagen-silicocarnotite coatings, before immersion in 1.5 SBF.

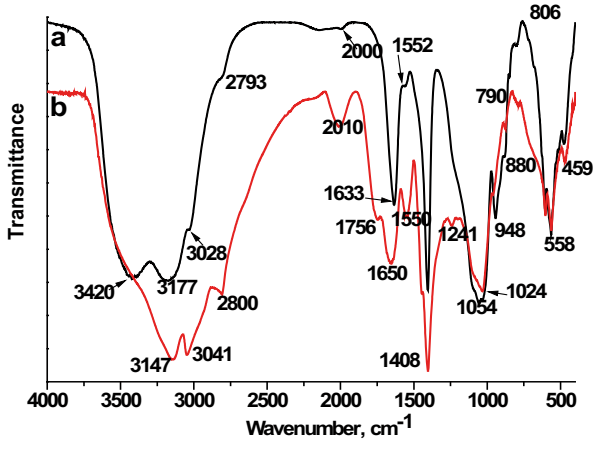

Figure 4. FTIR spectra of collagen/silicocarnotite coatings, before immersion in 1.5SBF with different quantity of collagen and silicocarnotite: 25:75 wt% (a) and 75:25 wt% (b)

As can be seen in Fig. 4, there is a big difference between pure collagen (Fig. 3), pure silicocarnotitite (Fig. 2) and the obtained coatings. There was a ν_1 PO_4^{3-} mode at 948 cm^{-1}, ν_2 PO_4^{3-} modes at 459 cm^{-1}, and a ν_4 PO_4^{3-} mode at 558 cm^{-1} as characteristic for the inorganic part of the coatings. The bands centered at 1024 and 1054 cm^{-1} were assigned to the presence of ν_3 PO_4^{3-} modes and to the vibration of the Si-O-Si bonds of silcocarnotite. Furthermore, the presence of a $\nu_2CO_3^{2-}$ mode, centered at ~880 cm^{-1} indicates that the synthesized coatings were similar to the natural bone [51-55]. On the basis of these results, we interpreted that we had prepared biocoating materials.

The obtained FTIR spectra (Fig. 4a) shows that the amide B band does not show a conformation change of the secondary structure of collagen matrix in the process of hybrid preparation. The amide II band in the obtained coatings is centered at ~ 3034 cm^{-1}. This absorption maximum is 76 cm^{-1} much higher than the first overtone (2×1555 cm^{-1}) of the amide II band frequency for the prepared coatings, *i.e.* amide II does not undergo perceptible changes. Some authors describe, the amide B band undergoing a strong modification in the presence of cross-linkage, *i.e.* glutaraldehyde [56,57].

In the FTIR spectra, amide I band is strong (1633 and 1650 cm^{-1}), the amide II band, centered at ~1555 cm^{-1} is weak and amide III (1241 cm^{-1}) is moderate [56]. It implied that inorganic crystals were embedded into the collagen matrix and blocked the vibration of organic groups, such as carboxyl and carbonyl groups [58]. This argument is in agreement with Zhang *et al.* [59]. On one hand, as shown in the Figs. 4a,b the amide I bands were changed and they shifted to lower wavenumber from 1656 cm^{-1} for pure collagen (Fig. 3) to 1633 cm^{-1} (curve a) and 1650 cm^{-1} (curve b) for the synthesized coatings. This change could be assigned to the formation of hydrogen bonds between collagen and the rehydrated silanol groups from the silicocarnotite. A second point is that the amide I band in a spectrum of bone is representative of the collagen content and extremely important for bone mineralization [60]. Other authors note that the "red shift" of amide I caused a covalent bond formation with Ca^{2+} of hydroxyapatite crystals [50]. Very similar results could be found in the case of preparing of bioactive coatings between collagen and HA, prepared *via* simultaneous titration method, cross-linked by glutaraldehyde [56]. The amide II bands of collagen/silicocarnotite coatings are centered at ~1555 cm^{-1} without the presence of others minor bands at ~1533 and ~1521 cm^{-1}. It was difficult to analyze amide II band in the fields of conformation future, as it has a complex nature with is vibration source, which arises primarily from of combination of the N-H banding coupled to the C-N stretching vibrations of the peptide linkages [48]. We observed that in the sample collagen/silicocarnotite (25:75 wt%) the amide II bands shifts only 2 cm^{-1}, which

can be attributed to a little conformational change in collagen, as a result of method for the preparation of the samples. Independently, the intensity of amide II increases with increasing of the collagen content in the coatings (Fig. 4, curve b).

The obtained FTIR data (Fig. 4) allows us to evaluate the interfacial bond between collagen and silicocarnotite in the prepared coatings. From the O-P-O bending modes (ν_4 PO_4^{3-}) and P-O stretching modes (ν_1, ν_2, ν_3 PO_4^{3-}) as shown in the same figure, it is considered that the presence of the ν_3 PO_4^{3-} is the indicator of the amount of inorganic part of the coatings. As it is known, the presence of ν_1 and ν_2 PO_4^{3-} reflects on the rate of crystallinity of the obtained glass-ceramics. From the spectra, there are two kinds of P-O-H bending mode, centered at 1241 and 790 cm^{-1} (curve b) and 806 cm^{-1} (curve a), respectively. The presence of the 1241 cm^{-1} mode reflects the organic coordination of ceramic phase with collagen, but the presence of two other modes centered at 790 and 806 cm^{-1} are specific for the samples prepared at higher temperatures. This fact was ascertained for hydroxyapatite/gelatin composites, prepared *via* co-precipitation method at different temperatures from Chang *et al*. [61]. In our case, the presence of the obtained modes could be assigned to the presence of two processes: the partial denaturation of collagen due to the use of NH_4OH for the preparation of the coatings, and partial dissolution of mixed silicocarnotite with acid soluble collagen for 6 hours.

The obtained FTIR data are in a good correspondence with XRD for the obtained coatings before immersion in 1.5 SBF (Fig. 5).

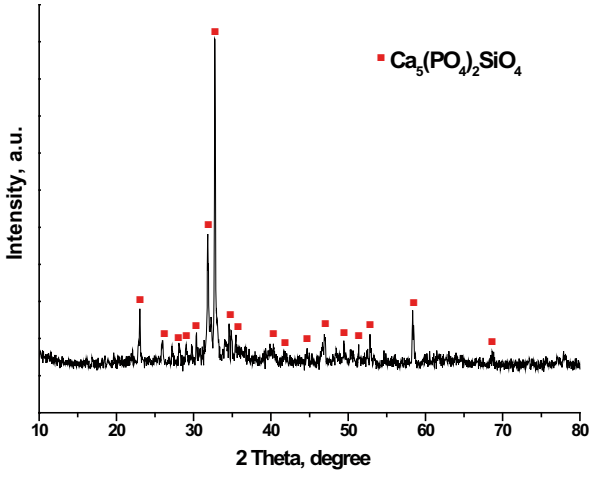

Figure 5. XRD of collagen/silicocarnotite (25:75 wt%) before immersion in 1.5SBF

The XRD patterns of the silicocarnotite (Fig. 1) and for the collagen-silicocarnotite coating before immersion in 1.5 SBF (Fig. 5) are with different intensity. Some peaks of silicocarnotite in the obtained coating (Fig. 5) in the range of 10-23 (2θ) are absent. The other peaks in the range 35-60 (2θ) are with slightly decreased intensity. This fact suggests the presence of the processes of "self-organizing" between collagen and silicocarnotite in the obtained coatings. In conclusion, we can see that the collagen/silicocarnotite coatings can provide defined peaks with less width in spite of the presence of an amorphous phase, which is suggests the association of a crystalline silicocarnotite to an amorphous collagen. Similar results were observed with the HA/collagen and HAβTCP/collagen composites [62].

The FTIR spectra of prepared collagen-silicocarnotite coatings after immersion in 1.5 SBF are given in Fig. 6.

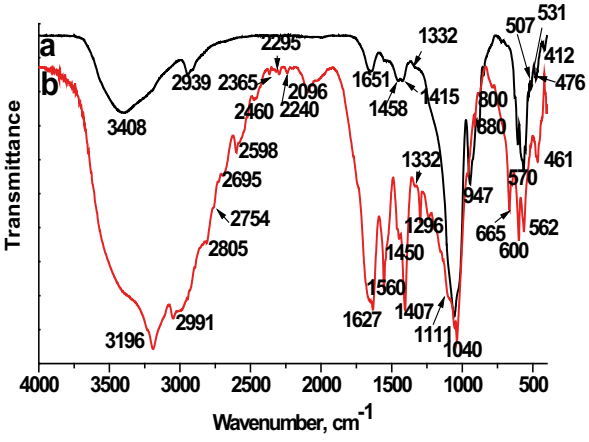

Figure 6. FTIR spectra of collagen/silicocarnotite coatings, after immersion in 1.5SBF with different quantity of collagen and silicocarnotite: 25:75 wt.% (a) and 75:25 wt.% (b)

As can be seen, the spectra contained various bands from respective PO_4^{3-} groups at 1111 cm^{-1} (for ν_3 PO_4^{3-}), 947 cm^{-1} (for ν_1 PO_4^{3-}), 507, 562 and 600 cm^{-1} (for ν_4 PO_4^{3-}), 412, 461 and 476 cm^{-1} (for ν_2 PO_4^{3-}), 800 and 880 cm^{-1} (for SiO_4^{4-} and $\nu_2CO_3^{2-}$) and OH^- groups at 3408 and 3196 cm^{-1} of HA and SiHA, which are in agreement with previously published data [52-56,63].

The 1340 cm^{-1} band in collagen (Fig. 3) does not simply represent the carboxyl group, but is one of a number of bands in the range 1400-1260 cm^{-1}, which are attributed to the presence of type I collagen in biological tissue [64]. The band at 1340 cm^{-1} in collagen is predominantly attributed to the so-called wagging vibration of propylene side chains [46]. Other peptides, such as gelatin, also correspond to the band at 1339 cm^{-1} [50]. As shown in Fig. 6 we confirm the "red shift" of this band for collagen/silicocarnotite coatings

with different amount of components. The band at 1332 cm^{-1} in the spectra of the two samples can be shown in a wagging vibration through the covalent bond formation with Ca^{2+} of partial dissolution in 1.5 SBF silicocarnotite crystals, embedded in the collagen matrix, after soaking with 1.5 SBF. It is known that the carboxylic groups of collagen are in ionic form in that medium [1], which is appropriate for the binding sites with Ca^{2+} [50,56]. As can be seen, the asymmetric stretching vibration of the mentioned above carboxylate anions (COO^{-}), observed at pure collagen at 1340 cm^{-1} (Fig. 3), shifted to lower wavenumber in the case of the coating (Figs. 6a,b). This means that the two carboxylic groups from the protein chain of collagen dissociate and become two carboxylic anions leading to the formation of a new chemical bond with Ca^{2+}. The amount of "red shift" is determined by the preparation conditions, such as pH, temperature and concentration [50]. In our case the "red shift" it is not influenced by the concentration of organic and inorganic parts of the obtained coatings. Our opinion is in a good agreement with the literature [46,50,56]. In the same figure, the presence of the absorption band at 1415, 1458 cm^{-1} (curve a) and 1407 cm^{-1} (curve b) indicated the formation of carbonate containing apatite with B-type substitution, named as B-CO_3HA in which CO_3^{2-} is substituted with PO_4^{3-} in the prepared collagen/silicocarnotite coatings [65]. This substitution leads to the distortion of a crystallographic lattice of the product B-CO_3HA [66]. Finally, we concluded that SBF could be re-arranging the collagen from the obtained coatings, because it contains TRIS, and the partially dissolved silicocarnotite from 3 days of soaking in 1.5SBF. On the other hand, the supersaturation of the SBF with Ca^{2+} from the partially dissolved silicocarnotite can lead to the formation of bioapatite (B-CO_3HA) on the surface of the prepared coatings. If the Ca^{2+} meets with the PO_4^{3-} it will make hydroxyapatite crystals by a homogeneous reaction. If the COO^{-} from collagen reacts with the Ca^{2+}, the nucleation of hydroxyapatite formation will be initiated from these active sites as a heterogeneous reaction. Afterwards PO_4^{3-} will accumulate at the calcium complexes and grow to the critical size of nucleation.

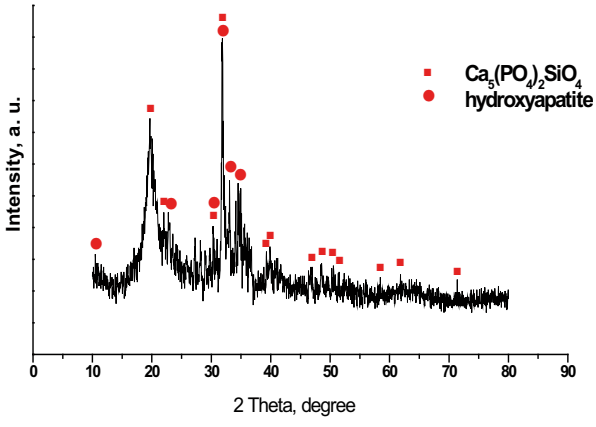

Figure 7. XRD of collagen-silicocarnotite (25:75 wt%) after 3 days immersion in 1.5SBF

The XRD diffraction patterns of the obtained coating after 3 days immersion in 1.5 SBF are given in Fig. 7

The depicted XRD detects the presence of a collagen amorphous halo with great intensity at 20 (2 θ), silicocarnotite and the main peaks of hydroxyapatite after immersion of the obtained coating in 1.5 SBF for 3 days.

The SEM images of the pure collagen (Fig. 8a), silicocarnotite (Fig. 8b), collagen/silicocarnotite coatings before (Figs. 8c,d) and after soaking in 1.5 SBF (Figs. 8e,f) are markedly different.

The SEM of the pure collagen (Fig. 8a) can be characterized by an amorphous structure with disordered fibrils and areas with irregular and scattered pores. It is worth to point out that their connectivity can play an important role in the new bone growth as described in reference [62]. Silicocarnotite (Fig. 8b) has a plate-like morphology, which is characteristic for silicon substituted hydroxyapatite structures as denoted in reference [67]. From depicted SEM image (Fig. 8b) it can also be seen that the prepared sample is non-densificated silicocarnotite. The preparation of these structures could be assigned to the presence of silica and with the temperature of thermal treatment of the obtained dried silicocarnotite gels. In accordance with other authors [68], we concluded that the densification of the obtained silicocarnotite decreases with increasing the silicon content. Therefore, the SEM image is distinguished from those published by Pilard and coworkers [69], which are prepared fully dense silicon substituted (Si_xHA) ceramics with x<0.5 wt.%, without the decomposition of initial silicon substituted apatite. When silicocarnotite was mixed with collagen in 75:25 wt.%, SEM image (Fig. 8c) displays that the silicocarnotite did not undergo any significant morphological change during the hybrid preparation. It can be seen that collagen fully covered silicocarnotite surface. When the quantity of collagen in the obtained hybrid materials increased to 75 wt.%, silicocarnotite was embedded in denaturated collagen matrix during hybrid preparation (Fig. 8d). On the basis of the obtained FTIR data and the presented at in a larger magnification view (Figs. 8e,f), it could be concluded that hydroxyapatite phase was formed on the surface of synthesized hybrid materials. On the other hand, it can be observed accumulation of HA on the surface of collagen fibrils, which was in good correspondence to the data, obtained by other authors working in the field of biomimetic mineralization of collagen fibrils [46,62,65,70-72].

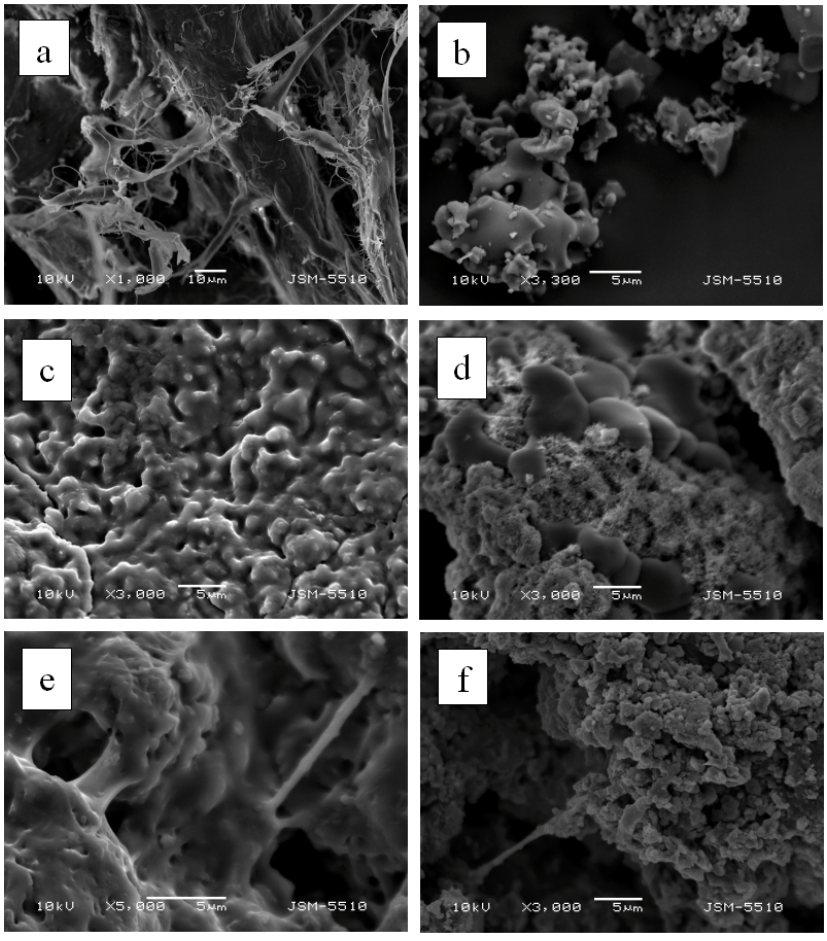

Figure 8. SEM images for pure collagen (a), silicocarnotite (b), silicocarnotite/collagen 75:25 wt% (c), silicocarnotite/collagen 25:75 wt% before SBF (d), silicocarnotite /collagen 75:25 wt% (e), silicocarnotite /collagen 25:75 wt% after immersion in SBF for 3 days (f)

4. Conclusions

The purposes of this article are to prepare and evaluate the *in vitro* bioactivity of hybrid coatings between collagen and silicocarnotite glass-ceramics in Kokubo solution (1.5 SBF). The silicocarnotite bioactive ceramics were synthesized *via* polystep sol-gel route with Ca/P+Si molar ratio 1.67. The hydrochloric acid soluble type I collagen was mixed with silicocarnotite powder in a 25:75 and 75:25 weight ratio without binding agents. The acidity of the obtained mixture was readjust with 25% NH_4OH to pH = 9.0. The mixture was then dried at 37°C for 12 hours. The X-ray diffraction of the obtained coatings depicts the presence of amorphous hallo from collagen and the crystalline phases from the silicocarnotite glass-ceramic. The FTIR depicts, the "red shift" of the strong amide I band at ~1660 cm^{-1} (for pure collagen) to ~1650 cm^{-1} (for the coatings), can also be assigned to the presence of chemical bond in the coated materials. The FTIR observations after the *in vitro* test proved that the carbonate containing hydroxyapatite (CO_3HA) can be formed on the surface of the synthesized coatings. The negatively charged carboxylate groups of collagen surface may be responsible for deposition of HA. This fact was confirmed by the "red shift" of carboxylate groups of collagen molecules in the FTIR spectra. The CO_3HA consisted of B-type CO_3^{2-} ions ($CO_3^{2+} \rightarrow PO_4^{3-}$). The SEM micrographs depicted different forms of HA particles that has precipitated on the surface after soaking in 1.5 SBF.

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