Hydroxyapatite growth on cotton fibers modified chemically

J.L. VARELA CASELIS*, E. REYES CERVANTES, G. LANDETA CORTÉS, R. AGUSTÍN SERRANO,
E. RUBIO ROSAS

Centro Universitario de Vinculación y Transferencia de Tecnología, Benemérita Universidad Autónoma de Puebla,
Prolongación de la 24 Sur y Av. San Claudio. Col. San Manuel. Ciudad Universitaria. C.P. 72570, Puebla, Puebla. México

We have prepared carboxymethyl cellulose fibers (CMC) by chemically modifying cotton cellulose with monochloroacetic acid and calcium chloride solution. This modification favored the growth of hydroxyapatite (HAP) on the surface of the CMC fibers in contact with simulated body fluid solutions (SBF). After soaking in SBF for periods of 7, 14 and 21 days, formation of HAP was observed. Analysis by scanning electron microscopy and X-ray diffraction showed that crystallinity, crystallite size, and growth of HAP increased with the soaking time. The amount of HAP deposited on CMC fibers increased greatly after 21 days of immersion in the SBF, while the substrate surface was totally covered with hemispherical aggregates with the size of the order of 2 microns. Elemental analysis showed the presence of calcium and phosphate, with calcium/phosphate atomic ratio of 1.54. Fourier transform infrared spectroscopy bands confirmed the presence of HAP. The results suggest that cotton modified by calcium treatment has a nucleating ability and can accelerate the nucleation of HAP crystals.

Keywords: *hydroxyapatite; biomimetics; simulated body fluid*

© Wrocław University of Technology.

1. Introduction

Hydroxyapatite (HAP), due to similar composition to the mineral component of bones, is the most commonly used calcium phosphate in implant production. Moreover, it exhibits valuable properties as a biomaterial, such as biocompatibility, bioactivity, osteoconductivity, direct bonding with bones, etc. However, its mechanical properties are poor. Basically, its fracture toughness is very low, severely restricting its field of application in orthopaedics [1–3]. For this reason many efforts have been made to develop composite materials based on HAP on polymers such as polylactic acid, collagen, chitosan, polyethylene, etc. [4–6].

In recent years, the synthesis of organic-inorganic composite materials using biomimetic processes for the application to artificial bones or scaffolds for tissue engineering has been developing rapidly [4]. As the crystallochemical specificity of inorganic solids simulates biological processes

like self-assembly and formation of peculiar crystal morphology, the elucidation of biomineralization processes would offer valuable insights, especially into biomimetic materials chemistry.

The deposition of HAP on polymeric substrates is important not only for fundamental research concerning biomineralization but also for practical applications such as the design and development of biomaterials [7–9].

Studies in the area of biomimetic chemistry have contributed significantly towards the basic understanding on some of the physical and chemical characteristics of various material surfaces, which can also give rise to, or stimulate, phosphate nucleation under in-vitro and in-vivo conditions [10, 11]. In this subject, functionalized polymers with high polar terminal groups, such as carboxyl and hydroxyl, immersed in SBF environment, were found to initiate epitaxial growth of HAP over their surfaces owing to their functional and stereochemical properties [12].

Cellulose has attracted considerable attention for bone tissue scaffold application, as one of

*E-mail: jvarelacaselis@yahoo.com

the most abundant biodegradable and biocompatible natural polymers [13–15]. A large amount of hydroxyl groups on the surface of cellulose has been proved to effectively promote the synthesis of nanoparticles [16]. However, a great amount of hydroxyl groups of cellulose results in a weak apatite nucleation ability in SBF [17, 18]. In this regard, COOH functional group present in CMC, has been proven to effectively induce the formation of apatite crystal [19]. Based on this, CMC was selected as supporting scaffolds due to following reasons: it degrades in the body to non-harmful and non-toxic compounds; negligible foreign body and inflammatory response reactions in vitro and in vivo applications [20–23]; stable and good mechanical properties in physiological environments during in vitro and in vivo aging; abundant reactive hydroxyl groups on the macromolecular chains. Furthermore, it has been used in various fields such as nutrition.

The CMC scaffolds have been investigated by various researchers for possible medical applications [24], HAP has grown on phosphorylated cellulose [25, 26], functionalized bacterial cellulose [27], functionalized cotton [28, 29] and composites based on CMC [30]. Although the preparation of CMC scaffolds has been well reported, there are only few studies in the literature related to the preparation of HAP-CMC composites scaffolds using natural cotton fibers, chemically modified with COOH groups and calcified.

In the present study cotton fibers were chosen as the substrate material, as it is abundant and an economic material for mass production, CMC fibers were prepared by chemical reaction of cotton cellulose fibers with monochloroacetic acid. The fibers were soaked in saturated calcium chloride (CaCl_2) solution, which led to the enrichment of calcium on the surface, after that the fibers were used as a scaffold for the nucleation and growth of HAP using a modification of an established biomimetic method. The chemical modification technique employed in this experiment will be discussed with regard to the effect it has on the cotton fibers that leads to a calcium phosphate phase deposition.

2. Experimental

2.1. CMC preparation by chemical modification of cotton fibers

All chemicals used in this study were supplied by Sigma-Aldrich and used without further purification. Chemical modification of cotton fibers was carried out as follows: cotton fibers (5 g), isopropanol (500 ml) and 30 wt.% sodium hydroxide solution (70 ml), were added into a round bottom flask equipped with a thermometer, mechanical stirrer, condenser, and all the ingredients were heated to the boiling temperature for 1 h, then 2.8 ml of monochloroacetic acid (78 wt.%) dissolved in isopropanol was added to the reaction mixture dropwise. The reaction was allowed to reflux for 4 h. The CMC fibers obtained were washed repeatedly in distilled water and dried in an oven at 80 °C.

2.2. CaCl_2 treatment

The CMC fibers obtained in the above step were soaked in a saturated solution of CaCl_2 and the reaction was allowed to reflux at the boiling temperature for 2 h. After completion of the soaking period, the fibers obtained in this step were washed repeatedly in distilled water and dried in an oven at 80 °C. This material was named CMC-Ca.

2.3. Calcium phosphate deposition

SBF was prepared by dissolving NaCl (0.143 M), K_2HPO_4 (0.9 mM) and CaCl_2 (0.0015 mM) in distilled water. The solution was buffered at pH 7.4 with tris (hydroxymethyl) aminomethane ($(\text{CH}_2\text{OH})_3\text{CNH}_2$) and 1M hydrochloric acid (HCl) at 60 °C. Samples of pre-treated cotton were soaked in SBF solution at 60 °C for 7, 14 and 21 days. The SBF solution was renewed every day to maintain both pH = 7.4 and ionic concentrations. Upon completion of soaking period, the samples were washed with distilled water and dried at 60 °C. The samples were examined using a JEOL JSM59000-LV scanning electron microscopy (SEM) with an OXFORD X-ray microanalyzer attached. All samples were sputter-coated with gold before SEM observation.

Fourier transform infrared spectroscopy (FT-IR) spectra were recorded using samples encased in a transparent KBr matrix on a Nexus 670. The solid phases were analyzed by X-ray diffraction (SIEMENS D-5000).

3. Results and discussion

Fig. 1a shows a SEM image of cotton fibers before the test. Approximately 20 μm in diameter, smooth and circular fibers are randomly distributed. An energy dispersive X-ray spectroscopy (EDS) spectrum of the fibers surface demonstrates that they contain C and O (Fig. 1b). After treatment with CaCl_2 , the CMC-Ca fibers are compact and circular, their surface displays particles (Fig. 1c); the EDS spectrum of the fibers surface demonstrates that they contain C, O and Ca (Fig. 1d).

Fig. 1. SEM images of fibers: (a) SEM image of cotton fibers, (b) EDS of cotton, (c) SEM image of CMC-Ca fibers, (d) EDS of CMC-Ca fibers.

The CMC-Ca fibers were cultured in SBF to test the HAP growth on them. After 7 days in SBF, only few particles were found on their surfaces (Fig. 2a). More bigger particles were found on the fibers after 14 days, so that the fibers were partially covered (Fig. 2b). The particle size increased further on the 21st day (Fig. 2c) and displayed a special morphology, i.e. the fiber was wrapped by HAP. An EDS spectrum of these particles demonstrates that they contain calcium and phosphorous

elements (Fig. 2d). EDS analyses of the samples after 21 days of immersion in SBF were made at different areas of the samples and were averaged. The EDS averaged results of the analysis are as follows: carbon 25.86 %, oxygen 30.32 %, sodium 1.49 %, phosphorus 13.62 %, chlorine 1.62 %, calcium 27.09 %, with a calcium/phosphate atomic ratio of 1.54, which is very close to the ratio of 1.67 expected for stoichiometric HAP.

Fig. 2. SEM micrographs of CMC-Ca fibers after soaking in SBF at different times: (a) 7 days, (b) 14 days, (c) 21 days, (d) EDS of CMC-Ca fibers after soaking for 21 days in SBF.

Fig. 3 shows magnified images of the microstructure of the material after 14 days in SBF, where it is possible to observe the substrate and the presence of uniformly distributed hemispherical structures (Fig. 3a). This image indicates the existence of favorable nucleation sites, with a low surface energy, which allows calcium phosphate mineralization. SEM micrographs with higher magnification reveal that the hemispherical structures have a size of the order of 2 micron (Fig. 3b) and are composed of platelike structures (Fig. 3c).

In order to illustrate the further growth of hydroxyapatite and the chemical interaction between HAP and CMC-Ca fibers, FT-IR spectroscopy measurements were carried out on cotton fibers. The CMC-Ca fibers before and after soaking in SBF solution and commercial HAP are shown in Fig. 4.

Fig. 3. SEM micrographs of the CMC-Ca fibers soaked in SBF solution for 14 days: (a) microstructure of the deposited material on the fiber, (b) magnified image of hemispherical structures, (c) magnified image of platelike structures.

The infrared spectrum of the cotton fibers (Fig. 4a) shows the basic vibration modes of cellulose: the broad peak at $3500 - 3100 \text{ cm}^{-1}$ corresponding to stretching vibrations of hydroxyl groups, the peaks at $2900 - 2850 \text{ cm}^{-1}$ indicating the absorption region of the symmetrical stretching C-H of polysaccharides, and the peaks at $1136 - 1070 \text{ cm}^{-1}$ corresponding to C-O bond. Fig. 4b shows the spectrum of CMC-Ca fibers. Compared with the spectrum of cotton fibers, the peak at 3337 cm^{-1} , corresponding to stretching vibrations of hydroxyl group of cellulose, is shifted to low wavenumbers and becomes the strong broad adsorption band. It indicates that the hydroxyl groups had a strong interaction with C-H of monochloroacetic acid. At the same time, the symmetrical and asymmetrical stretching mode of $(\text{COO})^-$ ions were detected at 1587 and 1421 cm^{-1} , respectively. After the nucleation of HAP crystals on the CMC-Ca fibers (Fig. 4c), the symmetrical stretching modes of $(\text{COO})^-$ ion appeared at 1604 cm^{-1} and the shift was 17 cm^{-1} . It suggests that there were chemical interactions between the $(\text{COO})^-$ groups in the CMC-Ca molecules and the nucleated HAP crystals. The 3570 cm^{-1} absorption band corresponds to the stretching vibration of the OH^- groups that are present in the HAP structure and the absorption bands at 1036 and 962 cm^{-1} are attributed to the stretching mode of $(\text{PO}_4)^{3-}$ ion. A weak peak, which denotes the stretching mode of the $(\text{CO}_3)^{2-}$

ion, is also observed at 866 cm^{-1} . These observations mean that the HAP crystals were formed on the surface of CMC-Ca fibers. Finally, the spectrum presented in Fig. 4d corresponds to commercial HAP, showing absorption bands at 1036 , 962 , 605 and 565 cm^{-1} related to vibrational modes of $(\text{PO}_4)^{3-}$. It is possible to observe a great correspondence between the spectra (c) and (d).

Fig. 4. FT-IR spectra of (a) cotton fibers; (b) CMC-Ca fibers; (c) CMC-Ca fibers, after soaking in SBF solution for 21 days; (d) commercial HAP.

Fig. 5. X-ray diffraction patterns of the sample after soaking in SBF solution: 14 days, 21 days.

The X-ray diffraction (XRD) patterns of the CMC fibers after soaking in SBF are presented in Fig. 5. The pattern shows characteristic peaks at 2θ of 12.1 , 21.5 , and 23.5° for the crystallographic planes (101), (110) and (002) of cellulose

diffraction, respectively. The sharp peak shape indicates that the crystallinity of cellulose is high. The XRD patterns of the fibers after 14 and 21 days of immersion in SBF show crystalline characteristics (Fig. 5a and 5b). However, after 14 days, not all diffraction planes in the 2 theta range can be clearly identified. Approximate values at 34°, 46.7°, 49.5°, and 53.14° correspond to diffraction planes (202), (222), (213) and (004) of the HAP, respectively, due to the low intensity of the diffraction lines. Better defined peaks with higher intensity on XRD patterns were found after 21 days. We attribute this result to a higher amount of material deposited on the fibers. The samples show a positive correlation with the HAP card of the JCPDS (9-432).

The HAP was grown on cotton fibers using a modification of the method described by Kokubo *et al.* [8, 31], in which CMC-Ca fibers obtained from cotton fibers were immersed in a plasma-like SBF. The morphological characteristics of the apatite deposited during 7, 14 and 21 days were determined by SEM analysis and it could be observed that the main differences between the obtained samples were the particle size and the amount of material grown on the substrate surface. It was observed that the largest amount of HAP was obtained after 21 days of immersion in SBF. Previous reports [31–33] indicated that this kind of morphological conformation in the HAP coating was compatible with the hard tissue human ensemble, which indicates that the material obtained can be used as a biomaterial.

4. Conclusions

This research has shown that hydroxyapatite nucleation in SBF solution can be made on cotton fibers chemically modified by calcium chloride pretreatment. The results therefore suggest that the cotton modified by the calcium treatment has a nucleating ability and can accelerate the nucleation of HAP crystals. The deduction was confirmed by FT-IR and XRD.

Finally, using a modified biomimetic processes, a direct nucleation of HAP on CMC-Ca fibers was performed to set up a CMC-hydroxyapatite crystals

composite as a potential new particularly attractive material for bone repair and reconstruction.

References

- [1] SÁENZ A., MONTERO M.L., MONDRAGÓN G., RODRÍGUEZ V., CASTAÑO V.M., *Mater. Res. Innov.*, 7 (2003), 68.
- [2] RODRÍGUEZ V., BRAGADO G.A.C., CASTAÑO V.M., *Mater. Manuf. Process.*, 18 (2003), 67.
- [3] LAHIRI D., GHOSH S., AGARWAL A., *Mat. Sci. Eng. C-Mater.*, 32 (2012), 1727.
- [4] SUN F., ZHOU H., LEE J., *Acta Biomater.*, 7 (2011), 3813.
- [5] LIANG O.K., JEI C.R., YI T.F., YU L.P., WEI H.S., YI C.S., *J. Mech. Behav. Biomed.*, 4 (2001), 618.
- [6] NIETO V.M., NAVARRO C.H., MORENO K.J., MORQUECHO A.A., VALDEZ A.C., MIRANDA S.G., HERNÁNDEZ J.F.L., *Prog. Org. Coat.*, 76 (2013), 204.
- [7] JUAN Z.L., SHENG F.X., GUO L.H., JIN Q.D., ZHANG L., LING Y.X., ZHAI C.F., *Mater. Lett.*, 58 (2004), 719.
- [8] KOKUBO T., KUSHITANI H., OHTSUKI C., SAKKA S., YAMAMURO T., *J. Mater. Sci.-Mater. M.*, 4 (1993), 1.
- [9] SATO K., KOGURE T., KUMAGAI Y., TANAKA J., *J. Colloid Interf. Sci.*, 240 (2001), 133.
- [10] VARELA J.L., GUTIÉRREZ C., RUBIO E., *Mater. Res. Innov.*, 17 (2013), 278.
- [11] STUPP S.I., BRAUN P.V., *Science*, 277 (1997), 1242.
- [12] DALAS E., KALLITSIS J.K., KOUTSOUKOS P.G., *Langmuir*, 7 (1991), 1822.
- [13] ZAKHAROV N.A., EZHOVA Z.A., KOVAL E.M., KALINNIKOV V.T., CHALYKH A.E., *Inorg. Mater.*, 41 (2005), 509.
- [14] RISBUD M.V., BHONDE R.R., *J. Biomed. Mater. Res. A*, 54 (2001), 436.
- [15] CZAJA W.K., YOUNG D.J., KAWECKI M., BROWN R.M., *Biomacromolecules*, 8 (2007), 1.
- [16] ZIMMERMANN K.A., LEBLANC J.M., SHEETS K.T., FOX R.W., GATENHOLM P., *Mat. Sci. Eng. C-Mater.*, 31 (2011), 43.
- [17] WAN Y.Z., HUANG Y., YUAN C.D., RAMAN S., ZHU Y., JIANG H.J., HE F., GAO C., *Mat. Sci. Eng. C-Mater.*, 4 (2007), 855.
- [18] GRANDE C.J., TORRES F.G., GOMEZ C.M., BAÑO M.C., *Acta Biomater.*, 5 (2009), 1605.
- [19] JIANG L., LI Y., ZHANG L., WANG X., *Mat. Sci. Eng. C-Mater.*, 29 (2009), 193.
- [20] BACKDAHL H., HELENIUS G., BODIN A., NANNMARK U., JOHANSSON B.R., RISBERG B., GATENHOLM P., *Biomaterials*, 27 (2006), 2141.
- [21] KLEMM D., SCHUMANN D., UDHARDT U., MARSCH S., *Prog. Polym. Sci.*, 26 (2001), 1561.
- [22] MARTSON M., VILJANTO J., HURME T., LAIPPALA P., SAUKKO P., *Biomaterials*, 20 (1999), 1989.
- [23] RODRIGUEZ K., RENNECKAR S., GATENHOLM P., *ACS Appl. Mater. Inter.*, 3 (2011), 681.
- [24] JIANG L., LI Y., WANG X., ZHANG L., WEN J., GONG M., *Carbohydr. Polym.*, 74 (2008), 680.

- [25] KOLOS E.C., RUYS A.J., ROHANIZADEH R., MUIR M.M., ROGER G., *J. Mater. Sci.-Mater. M.*, 17 (2006), 1179.
- [26] LI K., WANG J., LIU X., XIONG X., LIU H., *Carbohydr. Polym.*, 90 (2012), 1573.
- [27] YIN N., CHEN S., OUYANG Y., TANG L., YANG J., WANG H., *Prog. Nat. Sci.*, 21 (2001), 472.
- [28] VARMA H.K., YOKOGAWA YESPINOSA F.F., KAWAMOTO Y., NISHIZAWA K., NAGATA F., KAMEYAMA T., *J. Mater. Sci.-Mater. M.*, 10 (1999), 395.
- [29] XING, S. GE, HUANG B., BO Y., ZHANG D., ZHENG Z., *Mater. Res. Bull.*, 47 (2012), 1572.
- [30] HE M., CHANG C., PENG N., ZHANG L., *Carbohydr. Polym.*, 87 (2012), 2512.
- [31] KOKUBO T., KUSHITANI H., SAKKA S., KITSUGI T., YAMAMURO T., *J. Biomed. Mater. Res. A*, 24 (1990), 721.
- [32] ABE Y., KOKUBO T., YAMAMURO T., *J. Mater. Sci.-Mater. M.*, 1 (1990), 233.
- [33] ZHANG Y., HE P., XU X., LI J., *Thin Solid Films*, 468 (2004), 273.

Received 2014-05-10

Accepted 2014-05-19