In–vitro calcium phosphate growth over functionalized cotton fibers

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Biomimetic growth of calcium phosphate compound on cotton sheets treated with tetraethoxy silane and soaked in simulated body fluid solution was studied using scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX), micro-Fourier transform infrared spectroscopy (FTIR) and X-ray diffractometry (XRD). Micro-FTIR and EDAX results show that silicon was coupled to the cotton fiber when cotton was treated with tetra-ethoxy silane (TEOS) at 125 °C for 1 h. Calcium phosphate nucleation started to occur on the surface of TEOS-treated cotton fibers upon immersion in 1.5 × SBF (simulated body fluid solution) within 3 days and after 20 days, all the fiber surfaces were found covered with a thick and porous coating of calcium phosphate. The Ca and P determined by inductively coupled plasma spectroscopy (ICP) analysis revealed that the Ca/P ratio as well as the amount of calcium phosphate coating depends on the soaking time in SBF solution.

1. Introduction

Even though a number of metallic, ceramic and polymeric implants are being used for various hard tissue prosthetic applications, research on new or improved biomaterials which can integrate with natural bone more closely is still an area of active interest [1]. One of the essential prerequisites for any implant material to integrate with natural bone is its ability to make strong calcium phosphate interfacial bonds with the natural tissue. Synthetic hydroxyapatite bioceramics have been extensively studied for their ability to make strong bonds with natural bone at their surfaces under *in-vivo* conditions. Recent 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 calcium phosphate nucleation under *in-vitro* and *in-vivo* conditions [2, 3]. Phosphorylated polymers immersed in simulated body fluid (SBF) environment were found to initiate epitaxial growth of hydroxyapatite over their surfaces owing to their functional and stereochemical properties [4]. On understanding the significance of the surface functionalization for biomimetic growth, the authors have studied growth of calcium phosphate compound over various biopolymer materials such as cotton, chitin etc. [5, 6]. Similarly, Ti-OH and Si-OH terminal groups in titanium and Bioglass implants, respectively, were shown to help in calcium phosphate nucleation through formation of a primary amorphous calcium phosphate layer over their surfaces [7, 8]. A number of reports on the biomimetic deposition of hydroxyapatite over various bioglasses, sol–gel derived silica glass etc. are now available and the role of silanol (Si-OH) radicals on nucleation and growth of apatite layer from an SBF solution is also well documented [9, 10].

In the present work, silicon alkoxide has been used as a Si coupling agent for cotton fibers which are later found to be highly susceptible to calcium phosphate growth upon immersion in SBF solution. Results of the calcium phosphate growth on such surface fuctionalized cotton immersed for different times in 1.5 × SBF solution is described. Characterization methods such as scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX), Fourier transform infrared spectroscopy (FTIR), X-ray diffractometry (XRD) and inductively coupled plasma spectroscopy (ICP) have been used for studying the nature and growth of calcium phosphate material.

2. Experimental procedure

2.1. Materials

Ordinary cotton wipes (Ceigal, 100% cotton, 4-ply) supplied by Chiyoda Co. Ltd., were used as raw material. All chemicals were supplied by Wako Pure Chemical Company and were used without further purification. The growth medium, 1.5 × SBF solution, was prepared as reported elsewhere [11]. This was prepared by adding 15 ml of each of 2.74 mol l^{-1} NaCl, 0.06 mol l^{-1} KCl, 0.05 mol l^{-1} $CaCl_2$, 0.03 mol l^{-1} $MgCl_2$,

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0.0895 mol l^{-1} $NaHCO_3$, 0.02 mol l^{-1} K_2HPO_4 and 0.01 mol l^{-1} of Na_2SO_4 to a 200 ml volumetric flask along with 25 ml each of 0.4 mol l^{-1} tris-hydroxy-methylmethane-amine and 0.36 mol l^{-1} of HCl. The pH of the solution was adjusted to 7.3 by adding few drops of HCl and the remainder of the volume was made up with distilled water.

2.2. Tetra-ethoxy silane (TEOS) treated cotton fibers

Twenty 2 × 2 cm size cotton sheets were cut form the original cotton and put in a round bottomed flask fitted with a condenser, magnetic stirrer and nitrogen gas inlet. Care was taken to make sure that the flask was thoroughly dried. About 50 ml of tetra-ethoxy saline (TEOS, 99.9%) was then carefully added. The flask was placed inside an oil bath kept at 125 ℃ to heat the contents. The heating was continued for 1 h. After cooling the flask, cotton sheets were filtered out and washed with 100% ethyl alcohol. The excess alcohol from the cottons sheets was removed by pressing the sheets inside thick cotton wads.

2.3. SBF treatment

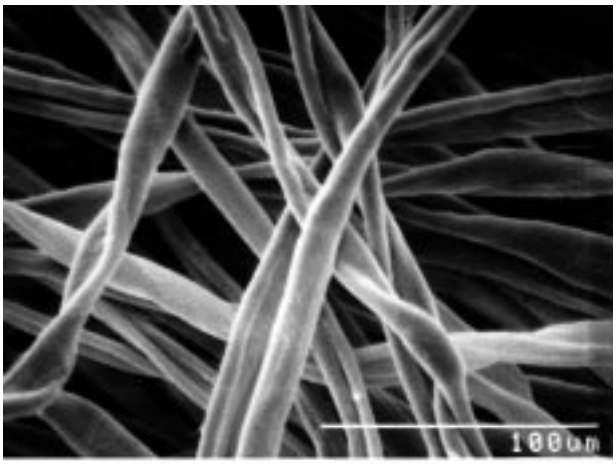
The TEOS treated, alcohol washed cotton sheets were then kept immersed in 50 ml of 1.5 × SBF solution in plastic jars. The jars were closed with air-tight lids and kept in an air oven at 36.5 ℃. The 1.5 × SBF solution was replaced each day. After immersion for various periods, the cotton sheets were removed from the SBF solution, washed with distilled water, and then dried at 60 ℃ in an air oven.

2.4. Characterization

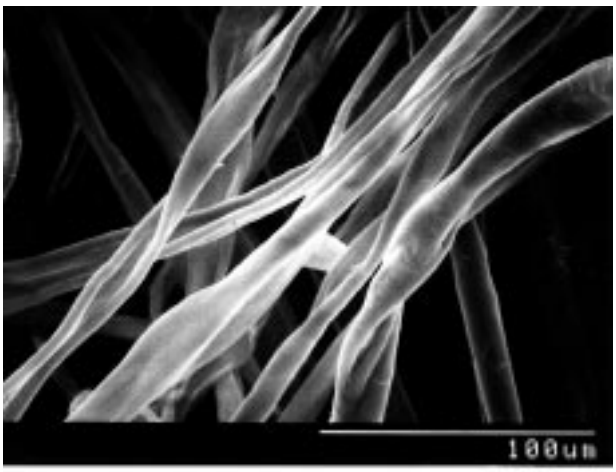
SEM and EDAX analysis were carried out using a Hitachi S- 530 SEM and a Horiba EMAX 2200 X-ray micro analyzer. Micro-FTIR measurements were performed in a Jasco Micro-FTIR Jansen Fourier transform infrared spectrometer. The fiber samples were encased in 1 mm transparent KBr pellets in order to view under the micro-FTIR microscope. Si content of the TEOS-treated cotton sheets and P and Ca content of the SBF treated cotton were determined using a Nippon Jarrell-Ash ICAP-1000S ICP–Auger electron spectroscopy (AES) instrument. Thin-film X-ray diffraction spectrum was recorded using a Philips XRD spectrometer using CuKα radiation at 50 kV and 100 mA.

3. Results

Fig. 1a and b show the SEM pictures of the fibers of cotton sheets before and after treatment with TEOS. Morphologically, there is not much difference in the fiber surface characteristics. The EDAX spectrum of the TEOS-treated fiber is provided in Fig. 2. A strong Si signal was observed all over the fiber surfaces. The Au peak due to the surface coating can also be seen. The amount of silicon was estimated by ICP analysis and it was 0.03–0.05 wt% of the fiber. The weights of the cotton sheets, before and after TEOS treatment were also



(a)



(b)

Figure 1 Scanning electron micrograph of cotton fibers (a) before and (b) after TEOS treatment.

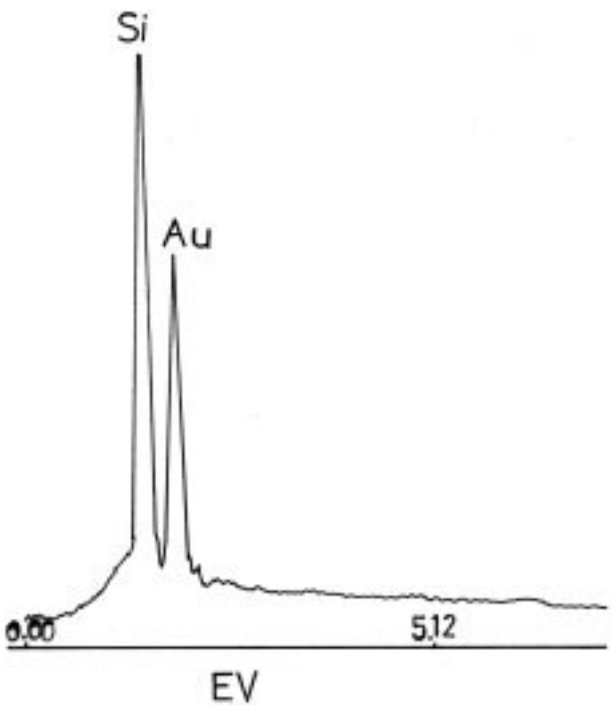


Figure 2 EDAX pattern of TEOS treated cotton fiber.

measured and no significant increase in weight could be observed for the TEOS-treated cotton, revealing that the actual amount of Si incorporated in to the fiber is too small to be detected by the 4-digit electronic balance.

The FTIR spectrum of cotton sheets before and after TEOS treatment are shown in Fig. 3a and b. Both spectra show the characteristic peaks for the cellulose. But the additional or new peaks in Fig. 3b clearly show the reaction between cellulose and TEOS. The new peaks at 470 and 801 cm^{-1} are due to the Si–O–C bond. There is considerable reduction in the intensity of hydroxyl peaks in the region 3600–2500 in the case of TEOS treated cotton due to the reaction between the terminal OH group of cellulose and the $Si\text{-}OC_2H_5$. Fig. 4a–d show the SEM pictures of TEOS treated cotton fibers after immersion in SBF for 3, 7, 14 and 21 d, respectively. Nucleation of an initial calcium phosphate layer started to occur all over the fiber surface within 3 days. Later, growth of secondary particles in the form of spherical clusters had occurred over this initial layer. This secondary growth is a random phenomenon and as the aging time increases, the fiber became completely covered with such nodular clusters of calcium phosphate (Fig. 4d). The morphology of the sample immersed for 30 days was also the same as that of the 21-day sample. Each spherical particle appeared as porous at high magnification (Fig. 4e). The set of EDAX patterns of the SBF-treated samples as a function of their aging time is provided in Fig. 5. The silicon peak disappears as the time of immersion increases. It is clear from the SEM and EDAX results that the fibers have become covered with nodular clusters of calcium phosphate. It is also clear from the patterns that the intensity of Ca peak increases, compared to P peak, as a function of aging time in SBF solution. The Ca/P values of the coatings and percentage increase in the weight of cotton samples immersed in SBF solution as a function of soaking time, obtained by ICP measurements are provided in Fig. 6. The Ca/P value of the coating formed after 3 d is close to 1.3, while the value for the sample immersed for 21 d is 1.6. The Ca/P does not increase appreciably further with increase in soaking time. The micro-FTIR spectra of samples immersed in SBF solution for different periods are provided in Fig. 7. Like in the previous spectra for pure cotton and Si treated cotton (Fig. 3a and b), the major peaks for the cellulose are retained for samples soaked for less than 7 d. The PO_4 peaks corresponding to the stretch (1060 cm^{-1}) and bend (600 cm^{-1}) emerge progressively as the main and predominant ones as the soaking time increases. The Si peaks at 801 cm^{-1} and 511 cm^{-1} became absent from the 7-d sample onwards. The peak observed at 2400 cm^{-1} for the sample soaked for 3 d could be due to the HPO_4. The absorption peak at 1440 cm^{-1} is due to the C–O stretch arising out of the carbonate ions substituted in apatite. Fig. 8 shows the thin-film XRD patterns of the cotton samples immersed for various time periods in SBF. The broad peaks at 2θ value in the range 32 and 26 are due to hydroxyapatite and its intensity increases with an increase in soaking time. The pattern is quite broad, which indicates the submicron nature of the apatite microcrystals in the coating. The peaks around 23, 15 and 17 are due to the cellulose substrate and the broad peak around 12 is due to the tape used for sticking the samples.

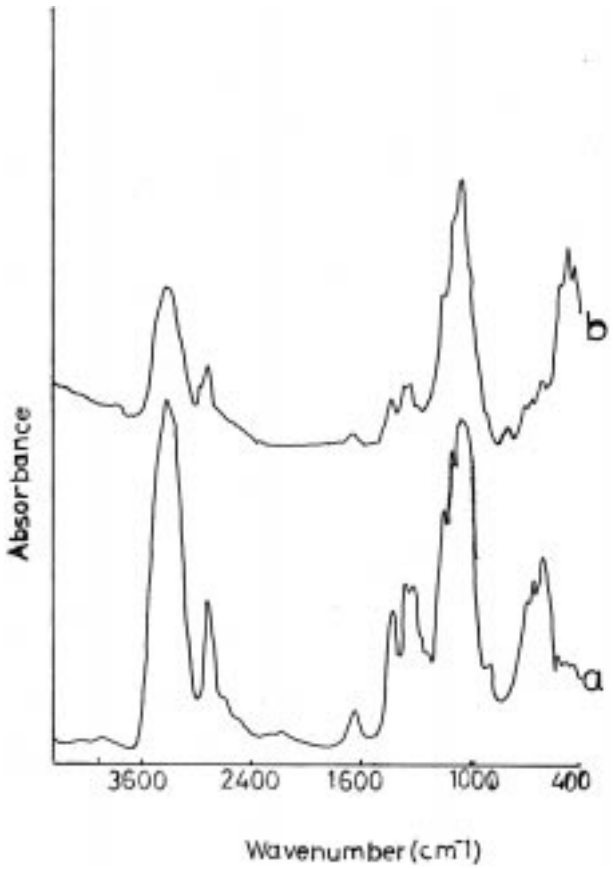


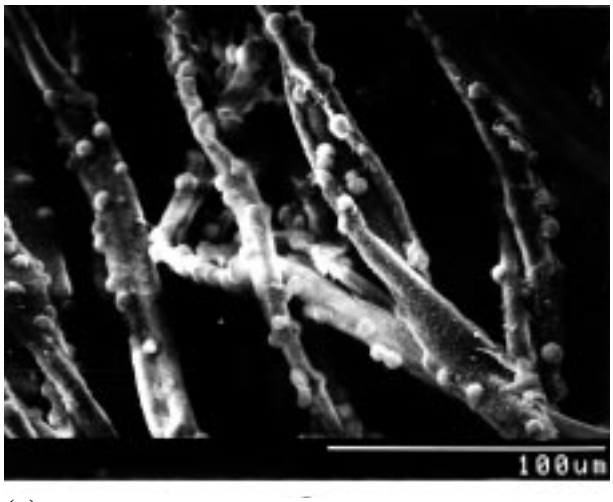
Figure 3 Micro-FTIR spectrum of cotton sample (**a**) before and (**b**) TEOS treatment.

4. Discussion

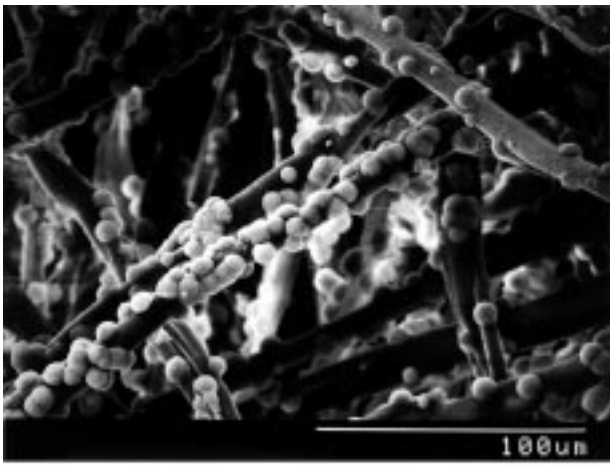
A number of bioglasses as well as sol–gel derived silica glasses have shown the characteristic hydroxyapatite growth over their surfaces upon immersion in simulated body solutions. The basic unit responsible for such direct calcium phosphate growth is studied as silanol group over their surfaces. In the present study, the silanol groups were attached over the surface of cotton fibers by treating the fibers with a silicon alkoxide, silicon tetraethoxide (TEOS). The FTIR results show the presence of Si-O bonds in the fibers. The quantitative estimation by ICP revealed that total amount of silicon, compared to the weight of cotton, is very small. This may be due to the fact that silica is restricted to the one reacted with the hydroxyl groups of cellulose.

During the immersion in SBF solution, an initial calcium phosphate layer was deposited over the entire fiber surface. Later, secondary nucleation of a characteristic spheroidal type formation of particles started to grow over this initial layer. The initial layer is having a Ca/P ratio comparable to that of octacalcium phosphate (OCP) phase and which might have acted as the precursor phase for the secondary growth. The surface became completely covered by thick coating after 20 d and the phase is identified as carbonate-substituted apatite.

Compared to the morphology of calcium phosphate derived through other biomimetic processes, these



(a)



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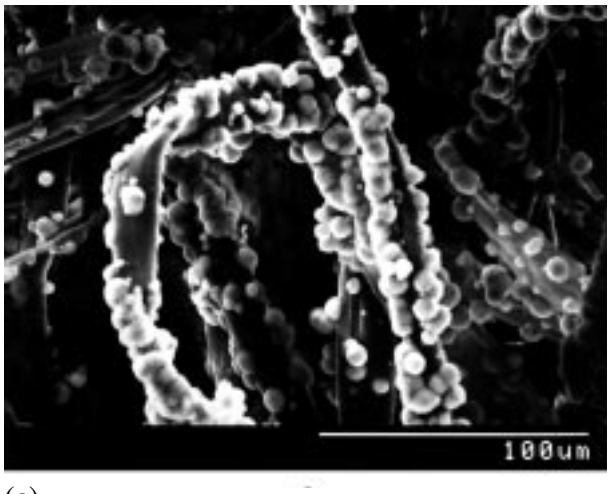

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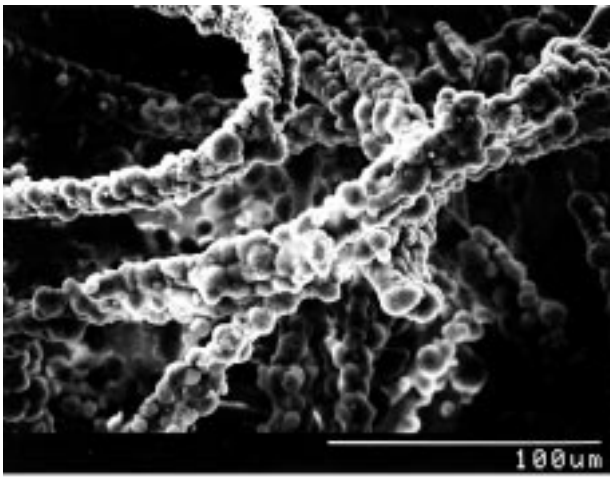
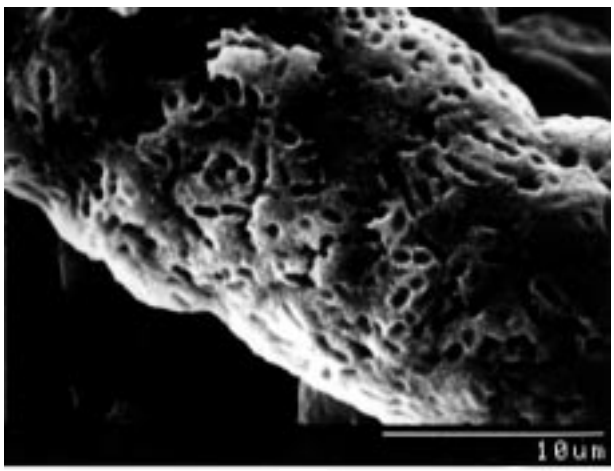


(d)



(e)

Figure 4 Scanning electron micrograph of the surface of TEOS treated cotton fibers after immersing in 1.5 × SBF solution for different periods of time (a) 3 d (b) 7 d (c) 14 d (d) 21 d (e) sample d at higher magnification.

coatings are morphologically different. It is clear that the basic chemistry of the substrate determines the actual morphology and kinetics of growth of calcium phosphate compound. For example, each spheroid appears as a porous one in the present experiment. Once the initial layer has been formed, further growth of calcium phosphate obeys a linear relation with respect to the time of immersion in SBF as shown in the quantitative estimation chart in Fig. 6.

5. Conclusions

Cotton sheets subjected to silicon coupling and subsequent immersion in SBF solution resulted in a thick calcium phosphate coating. The silanol groups coupled to the hydroxy group of the cellulose are believed to be responsible for making the cotton surface susceptible to biomimetic deposition of hydroxyapatite type coating. This process is a promising route for the surface functionalization of substrates intended for various

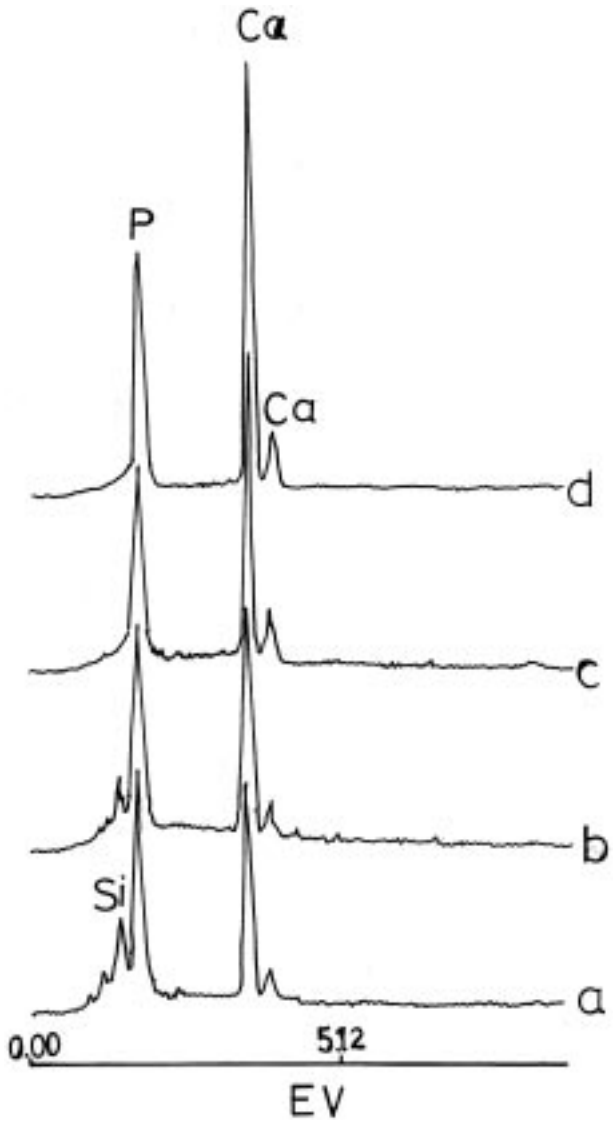


Figure 5 EDAX patterns of TEOS treated samples immersed in SBF solution for various periods: (a) 3 d; (b) 7 d; (c) 14 d; (d) 21 d.

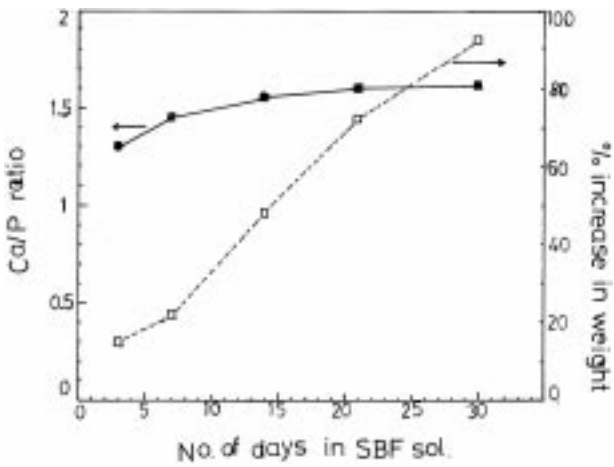


Figure 6 Plot of Ca/P values of the calcium phosphate coating and the corresponding percentage increase in weight of the TEOS treated cotton samples as a function of soaking time in SBF solution.

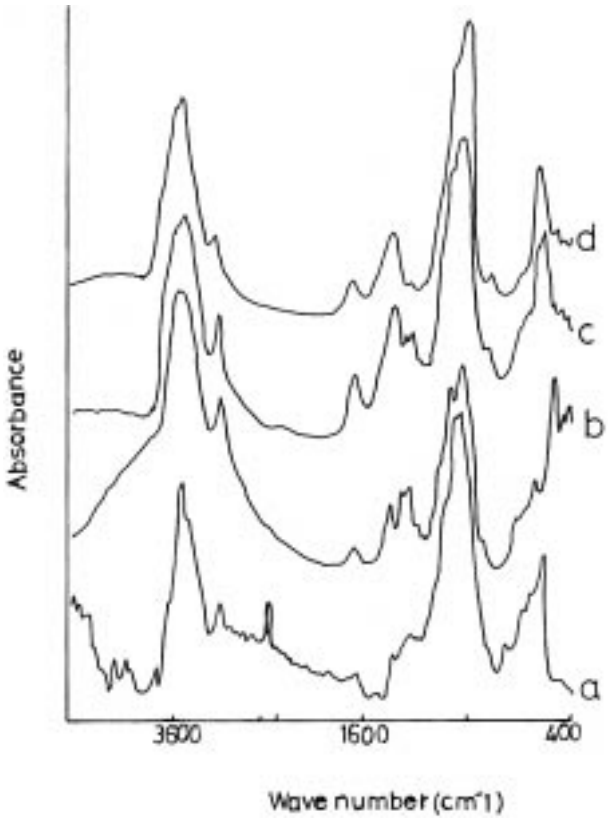


Figure 7 Micro-FTIR spectrum of TEOS treated cotton samples immersed in SBF for various time periods: (a) 3 d; (b) 7 d; (c) 14 d; (d) 21 d.

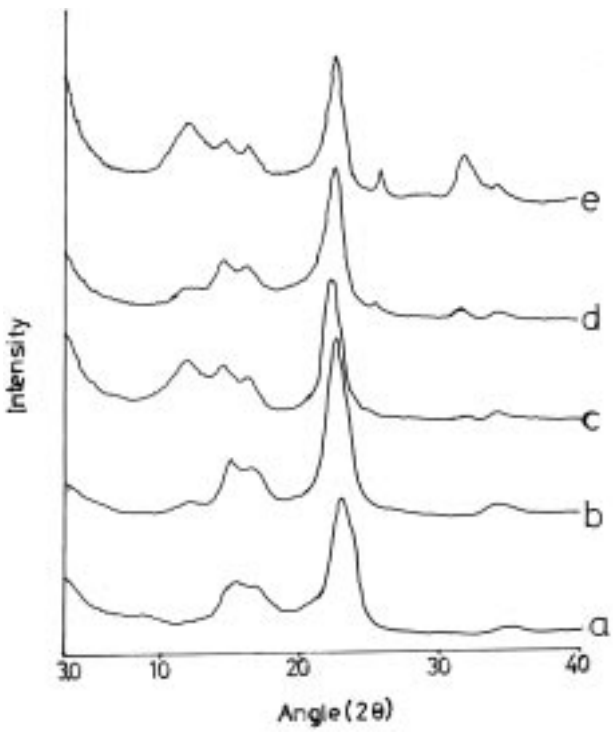


Figure 8 Thin-film XRD patterns of (a) cotton sample (b) after treating with TEOS and after immersing in SBF solution for (c) 3 d (d) 7 d and (e) 30 d.

biomimetic procedures. The coating consisted of spheroidal formations and they are porous in nature. Work is undergoing on the efficacy of this process with other biopolymer substrates.

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