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Calcium phosphate fibres synthesized from a simulated body fluid

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Abstract The biomimetic coating method was used for fabricating calcium phosphate fibres for biomedical applications such as bone defect fillers. Natural cotton substrate was pre-treated with phosphorylation and a $Ca(OH)_2$ saturated solution. The pre-treated samples were then soaked in simulated body fluid (SBF) of two different concentrations, 1.5 times and 5.0 times the ion concentration of blood plasma. The cotton was then burnt out via sintering of the ceramic coating at 950°C, 1050°C, 1150°C, and 1250°C. The results demonstrated that osteoblastic cells were able to cover the entire surface cotton fibres, and the cell coverage appeared to be independent of surface roughness and Ca/P ratio of fibres.

1 Introduction

Calcium phosphate contains only non-toxic species, such as Ca, P, (H_2O, OH^-, CO_3^{2-} etc.). In contrast with fibrous materials such as glass, carbon, SiC, Si_3N_4, Al_2O_3, ZrO_2, and $K_2Ti_6O_{13}$, bioactive calcium phosphates exhibit excellent biocompatibility due to their chemical and crystallographic similarities with the mineral constituents of bone and tooth mineral [1]. Hence there is a potential opportunity for employment in biomedical applications. Pepin [2] described possible biomedical applications for HA fibres such as a cotton-like cloth form described as a bone defect filler to encourage bone growth into the defect, possibly to reinforce a hydroxyapatite matrix material resulting in a composite material. Another possible application is as scaffolds for bone ingrowth, preserving needed strength during the bone growth process.

Techniques to produce calcium phosphate fibres include pyrolysis, homogeneous precipitation, melt spinning and extrusion [1]. Iwasaki reported that fibrous HA can be prepared using pyrolysis with a reaction of ion exchange of sodium alginate (Na-Alg.) [3]. The resulting fibrous calcium phosphate was polycrystalline material with a size of about 30–40 μm in diameter. Fibrous and whisker-like material can be obtained by homogeneous precipitation from Ca^{2+} and PO_4^{3-} solutions through an aqueous system. Melt spinning and extrusion techniques produce a cotton-like product, i.e., continuous fibres. However the resulting fibres were fairly porous. The uncalcined fibres were soft to the touch. Mori et al. [4, 5] invented a fibrous product of apatite, particularly in cotton-like and non-woven fabric.

Kokubo et al. [6] performed bone bonding tests between apatite wollastonite (AW) glass-ceramic substrates in an acellular simulated body fluid (SBF). Kokubo et al., developed the bone bonding tests for the bone bonding ability between ceramics in a SBF in 1982 [7]. In 1991, it was then found that any material of any shape could receive a bioactive coating including alumina, cotton and carbon [8]. Besides a technique to improve an implant's bioactivity, or to test a material's biocompatibility; the biomimetic process using SBF can be used as an alternative coating technique to coat crystalline bone-like apatite on to most materials. For example, de Groot

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et al. [9] used the biomimetic coating technique as an alternative to the plasma spraying to produce a bioactive calcium phosphate coating on titanium and titanium alloys to aid in bone fixation once implanted.

The literature contains very few studies of biomimetic coating of natural fibrous substrates. In fact, other than the present study, the only other study of biomimetic coating of cotton is that of Mucalo et al. [10, 11]. However, Mucalo et al. [10, 11] simply looked at the coating of cotton, and did not use this technique to produce calcium phosphate fibres, as was the focus of the present study. The present study involved four aspects: (a) chemical pretreatment of cotton; (b) biomimetic coating of the pretreated cotton using SBF; (c) synthesis of calcium phosphate fibres by sintering the coated cotton; (d) cell-culturing of the calcium phosphate fibres. The present study used a similar chemical pretreatment to that of Mucalo et al. [10, 11]. However, the present study used a different SBF approach. More importantly, the present study is the first to prepare calcium phosphate fibres from coated cotton, and the first to study their properties and cellular response.

With regard to chemical pretreatment, Mucalo et al. [10, 11] found that amorphous calcium phosphate materials could be stimulated to form on cotton fibres. These fibres were chemically pretreated by phosphorylation using the urea/phosphorous acid method and soaked in a saturated $Ca(OH)_2$ for approximately one week. In 1993, bamboo was coated by Li et al. [12] and chitosan was coated by Yokogawa et al. [13] in 1997. Granja et al. [14] looked at further characterizing phosphorylated cellulose by soaking in a simulated body fluid. Neither Mucalo et al. [10, 11], Li et al. [12], Yokogawa et al. [13] nor Granja et al. [14] have heat treated the biomimetic calcium phosphate coating on the cellulose materials to either sinter the ceramic coating or burn out the combustible cellulose substrate to produce ceramic fibres.

Cotton was chosen as the substrate material in the present study as it is fibrous, combustible and an economic material for mass production. The purpose of this study was to develop a method to prepare calcium phosphate fibres for biomedical applications such as bone defect fillers. The biomimetic coating technique was applied on natural cotton using a phosphorylation and $Ca(OH)_2$ pre-treatment technique. Burnout of the cotton substrate will result in biomimetically-produced hollow calcium phosphate fibres. The coverage of osteoblast cell on fibers was used to determine the biocompatibility of the materials. The phosphorylation technique employed in this experiment will be discussed with regard to the effect it has on the cotton fibres that leads to a calcium phosphate phase deposition. Recent findings [15–18] have suggested that surface roughness may be as important for *in vivo* and *in vitro* response of orthopaedic biomaterials as surface chemistry. The effects of surface chemistry and roughness on cell/material interaction were also analyzed in this study.

2 Materials and methods

Preparation of calcium phosphate fibre manufacture

2.1 Phosphorylation treated cotton

Phosphorylation of cotton samples were carried out following the preparation reported by Inagaki et al. [19]. Cotton pieces were placed in a round bottom flask equipped with a thermometer, mechanical stirrer, condenser, and N_2 gas inlet tube. Urea dissolved in dimethyl formamide (DMF) was added to the flask and heated to 130°C, upon which phosphorous acid (H_3PO_3) was added and heated to 145°C. The reaction was allowed to reflux for thirty minutes. Cotton fibres were then washed repeatedly in distilled water and dried in an oven at 50°C.

2.2 $Ca(OH)_2$ treatment

The phosphorylated cotton was soaked (without stirring) in a saturated solution of $Ca(OH)_2$ (pH ∼11–12) in a closed screw-top glass bottle for periods of up to 8 days. The $Ca(OH)_2$ solution was renewed every 4 days. Upon completion of the soaking period the samples were subsequently filtered, rinsed thoroughly with distilled water and dried in an oven at 50°C.

2.3 Calcium phosphate deposition

1.5 times and 5 times the concentration of Simulated Body Fluid (1.5SBF, 5.0SBF) were prepared as per Table 1. SBF simulates the ionic concentration of blood plasma. The pH was measured and adjusted to pH 7.4 with tris(hydroxymethyl)aminomethane ($(CH_2OH)_3CNH_2$) and dilute hydrochloric acid (HCl). Samples of pre-treated cotton were placed in either 1.5SBF or 5.0SBF in closed screw-top glass jars and re-buffered to pH of 7.4. The glass jars were immersed in a shaking water bath at 36.5°C for two weeks. The SBF solution's were renewed every two days to maintain both pH = 7.4 and ionic concentrations. Upon completion of soaking period, samples were washed with distilled water and dried in air before further examination.

2.4 Heat treatment—cotton burnout

Coated cotton fibres were fired in air to 950°C, 1050°C, 1150°C and 1250°C at a rate of 100°C per hour with a soak time of one hour.

2.5 Cell culture of osteoblasts on sintered fibres

The sintered calcium phosphate fibres using 1.5SBF and the 5.0SBF were weighed to 3 μg and autoclaved for 45

Table 1 Table of composition of simulated body fluids

Concentration (mM)	Na^+	K^+	Ca^{2+}	Mg^{2+}	HCO_3^-	Cl^-	HPO_4^{2-}	SO_4^{2-}
Blood plasma	142.0	5.0	2.5	1.5	27.0	103.0	1.0	0.5
1.0SBF	142.0	5.0	2.5	1.5	4.2	148.0	1.0	0.5
1.5SBF	213.0	7.5	3.8	2.3	6.3	223.0	1.5	0.75
5.0SBF	710.0	25.0	12.5	7.5	21.0	740.0	5.0	2.5

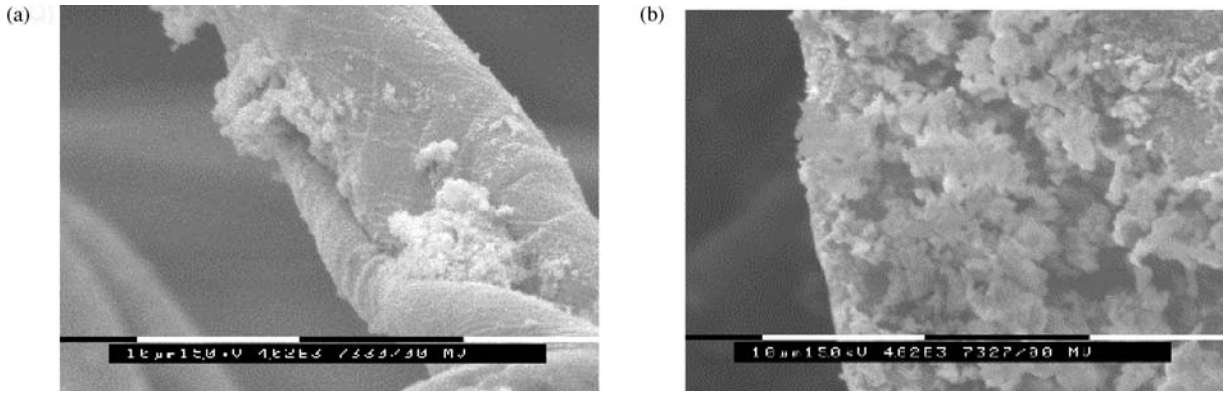

Fig. 1 SEM micrograph of (a) $Ca(OH)_2$ only pre-treated cotton soaked in 1.5SBF; and (b) phosphorylated and $Ca(OH)_2$ pre-treated cotton soaked in 1.5SBF

minutes at 125°C. Samples of both 1.5SBF and 5.0SBF groups were placed individually at the bottom of 24-well-plates and seeded with human derived MG-63 osteoblastic cell line at a density of 50000 cells per ml cell medium. Dulbecco's Modified Eagle's Medium (DMEM) with Ca^{2+} supplemented with 10% fetal bovine serum (FBS) and as ascorbate will be used as culture medium. The medium was replaced after 4 days. After one week, the medium was pipetted out of each well and the specimens were rinsed twice with Phosphate Buffer Solution (PBS). The specimens were then fixed with 2% glutaraldehyde, rinsed with PBS, dehydrated through a graded series of ethanol (50, 70, 80, 90, and 100%) for 10 min and then critical-point dried. The samples were then mounted on aluminium plates and coated with a thin layer of gold prior to scanning electron microscope (SEM) analysis.

3 Analytical methods

The sintered calcium phosphate fibres were examined in a Philips SEM 505 scanning electron microscopy (SEM) with a EDAX DX-4 EDS System (EDS) attached, operating at 7kV. A Siemans D5000 was utilised for x-ray diffraction (XRD) with a x-ray copper tube with an accelerating voltage of 40kV and a filament current of 30 mA. The angle of scan was 5–70°, with a step size of 0.1 and a step time of 320°. For surface roughness measurements a BioRad MRC600 was attached to a Zeiss axiophot in reflection confocal mode with objective lens of 50X. Surface roughness software custom designed by A./Prof Guy Cox from the Electron Microscope Unit, University of Sydney.

4 Results

4.1 Pre-treatment study

Cotton samples treated in $Ca(OH)_2$ solution and 1.5SBF both displayed evidence of a calcium phosphate phase when viewed and analyzed under SEM and EDS as seen Fig. 1. Figure 1(a) shows a fibre that did not undergo phosphorylation, while Fibre 1(b) shows a phosphorylated fibre with a high surface area of calcium phosphate coatings that covered the cotton fibre surface.

4.2 Deposition of calcium phosphate crystal in SBF

Figure 2 shows cotton that was pre-treated with phosphorylation and $Ca(OH)_2$ and soaked in 1.5SBF and 5.0SBF. Fairly uniform coverage of calcium phosphate deposition on the fibers was seen, even in non heat-treated samples. As in most cases of the 1.5SBF group, the central fibre had a good coverage of coating; however some of the cotton fibers of this group also demonstrated less extensive coating coverage. Figure 2(b) shows a very well coated cotton fibre in the foreground with all other fibres also having achieved a good coverage of deposition.

Figure 3 shows XRD analysis of the control material (the raw as-supplied cotton) in comparison with the unsintered 1.5SBF and 5.0SBF coated cotton sample. These results

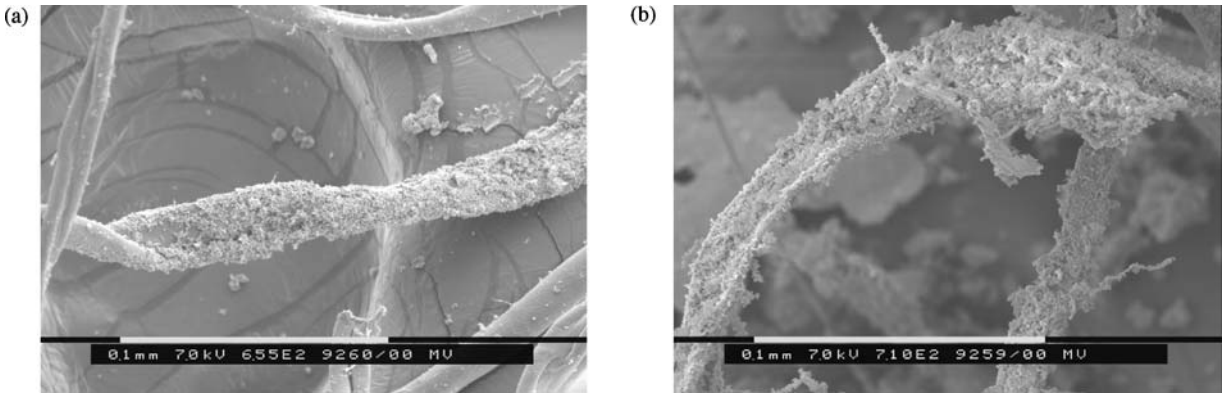

Fig. 2 (a) 1.5SBF; (b) 5.0SBF on cotton

identify the additional XRD peaks that correspond to the SBF calcium phosphate coating.

4.3 Heat treatment study

Figures 4 and 5 shows the heat treatment study on 1.5SBF and 5.0SBF at 950°C, 1050°C, 1150°C and 1250°C. Heat treatment at 950°C showed the cotton substrate was burnt out, however very little sintering of the calcium phosphate phase had occurred. Similar results with little sintering were found at 1050°C. With heat treatment at 1150°C, there was necking within the particles of the coating, indicative of the earlier stage of sintering. Heat treatment at 1250°C showed a considerable amount of sintering, with a fused structure containing some open pores. There was also a reduction in surface area at 1250°C compared with heat treatment at 950°C.

Maintenance of tubular morphology is evident from the SEM micrographs. At 950°C, the cotton has burnt out leaving a fairly thick walled (approximately 1μm) hollow fibre. Increasing the sintering temperature to 1150°C, the tubular morphology was maintained, but at 1250°C, the degree of sintering was such that the hollow fibres opened to take the form of tapes.

XRD analysis of the sintered coatings confirmed that the calcium phosphate phase was weakly crystalline apatite. This is shown in the XRD analysis for 5.0SBF sample sintered to 1050°C in Fig. 6. The main calcium phosphate phase present at the increasing heat treatment temperature was whitlockite, a dehydration product of hydroxyapatite.

EDS results show the atomic percentage of calcium versus phosphorus in Fig. 7 for (a) 1.5SBF and (b) 5.0SBF. The results support the XRD analysis that the dominant phase present was whitlockite calcium phosphate phase up to 1150°C. The Ca/P ratio increased greater with heat treatment above 1150°C.

The surface roughness of the fibres was tested with confocal optical microscopy. The roughness parameter (Ra) was measured in each case. Using the software developed in the

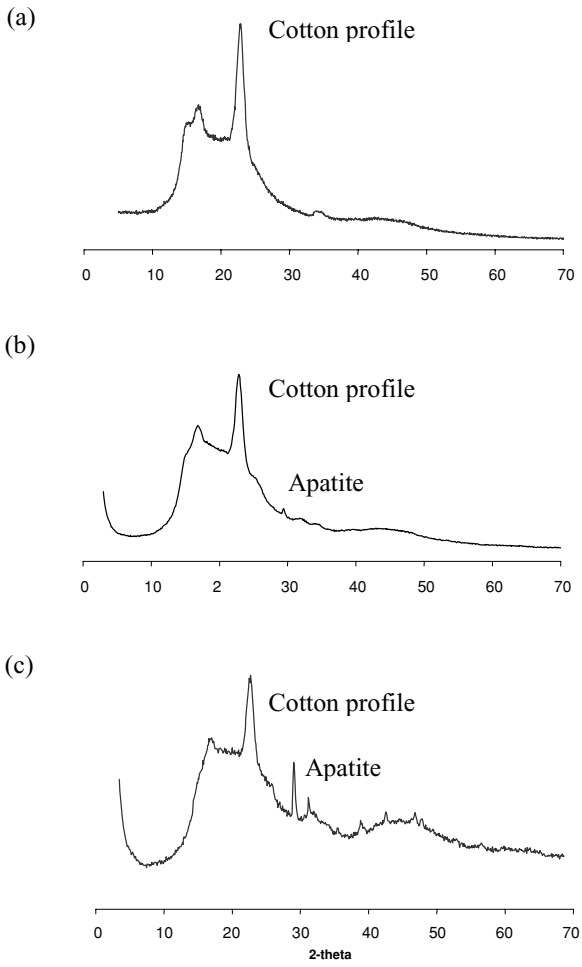

Fig. 3 XRD profiles of (a) cotton only; (b) pre-treated cotton soaked in 1.5SBF with no heat treatment; and (c) pre-treated cotton soaked in 5.0SBF with no heat treatment

Electron Microscope Unit at University of Sydney, three measurements were taken along the length of the fibre. These results are recorded below in Fig. 8 for 1.5SBF and 5.0SBF. Figure 9 shows an example of one of the three measurements taken for 5.0SBF coated cotton heat-treated at 950°C.

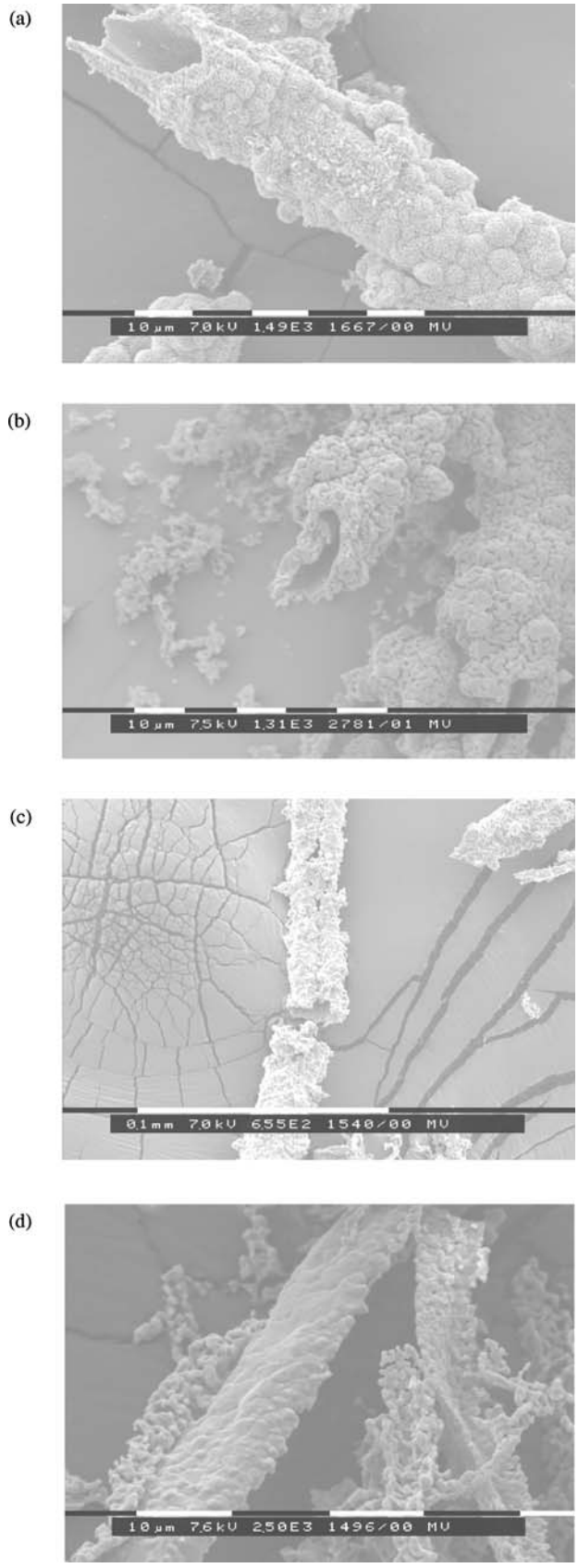

Fig. 4 Heat Treatment Study for 1.5SBF (a) 950°C; (b) 1050°C; (c) 1150°C; (d) 1250°C

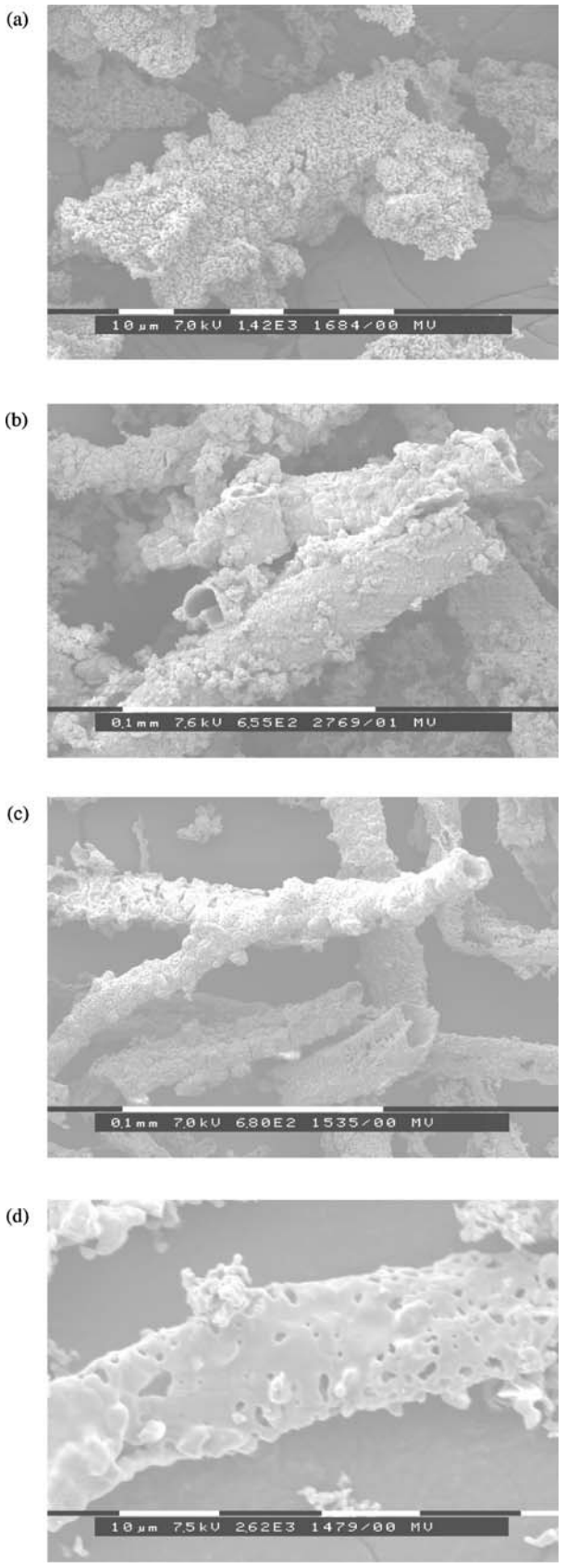

Fig. 5 Heat Treatment Study for 5.0SBF (a) 950°C; (b) 1050°C; (c) 1150°C; (d) 1250°C

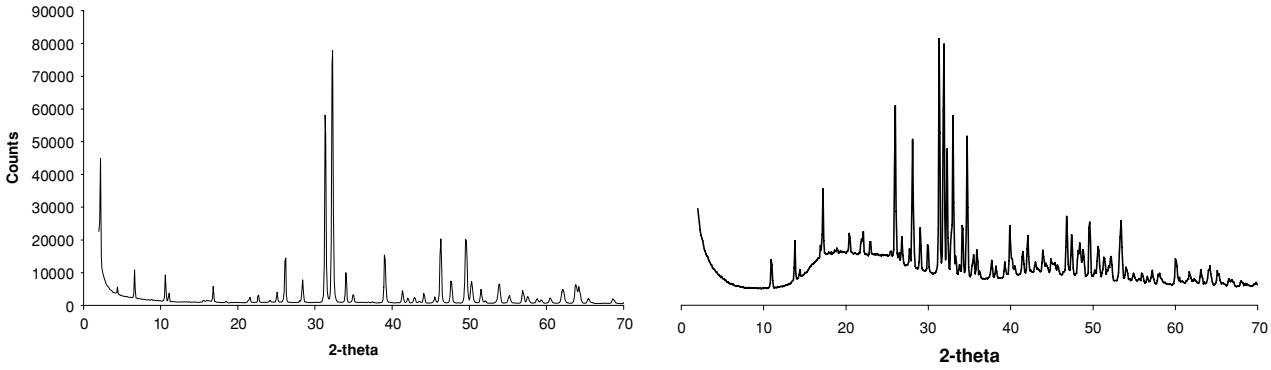

Fig. 6 XRD analysis for 5.0SBF coating sintered at 1050°C. Peaks at approximately 25.9°, 28.7°, 31.8°, 32.5°, and 34.2° in the central region correspond to hydroxyapatite. The peak at 11° indicates that DCPD, dicalcium phosphate dihydrate is also present

4.4 Cell culture study

In general, significant cell coverage was apparent for all sintering temperatures and treatment conditions. The results are present in Figs. 10 and 11. In Fig. 11(a), the osteoblast cells joined two sintered fibres. The fibre morphology appeared to affect the osteoblast cell orientation. Figure 11(b) showed the orientation of cells along the length of the fibres. In Fig. 11(c) the osteoblast cells appeared not to enter the hollow area of the fibres.

5 Discussion

5.1 Pre-treatment study

This pre-treatment study found that phosphorylation of the cotton substrate proved to be a necessary and vital step in the pre-treatment process. Figure 1(a) shows a fibre that did not undergo phosphorylation, while Fig. 1(b) shows a phosphorylated fibre with a high surface area of coating that covers the cotton fibre surface. Although coating was apparent on the fibre displayed in Fig. 1(a), the coating was only present in defective areas where heterogeneous nucleation could initiate crystal growth.

Phosphorylation was required for pretreatment of as-supplied cotton substrate and seemed to encourage greater nucleation and crystal growth than without phosphorylation pretreatment. For example, in Fig. 12 as-supplied cotton and as-supplied cotton that had been pre-treated with phosphorylation is displayed. The control, raw as-supplied cotton without phosphorylation appeared to have a smoother surface whereas the phosphorylated cotton appeared stringier within the fibre and tangled between the fibres. It is thus conjectured that this phosphorylation step achieves more than just chemical surface preparation; it may also activate the surface for further crystal growth. That is, it may increase the surface roughness and thus prepare the cotton for heterogeneous nucleation as mentioned above that the growth of coating was apparent on defective areas. This agrees with a study by Granja et al. [14] that looked at further characterizing phosphorylated cellulose by soaking in a SBF for biomedical applications; found that phosphorylation directly influenced the extent of surface mineralization.

Once the surface is activated for crystal growth, the $Ca(OH)_2$ solution seems to encourage small crystallites that lead to further calcium phosphate crystal growth. It is not clear what these small crystallites are composed of, but it is suspected that they slowly dissolve upon introduction of the

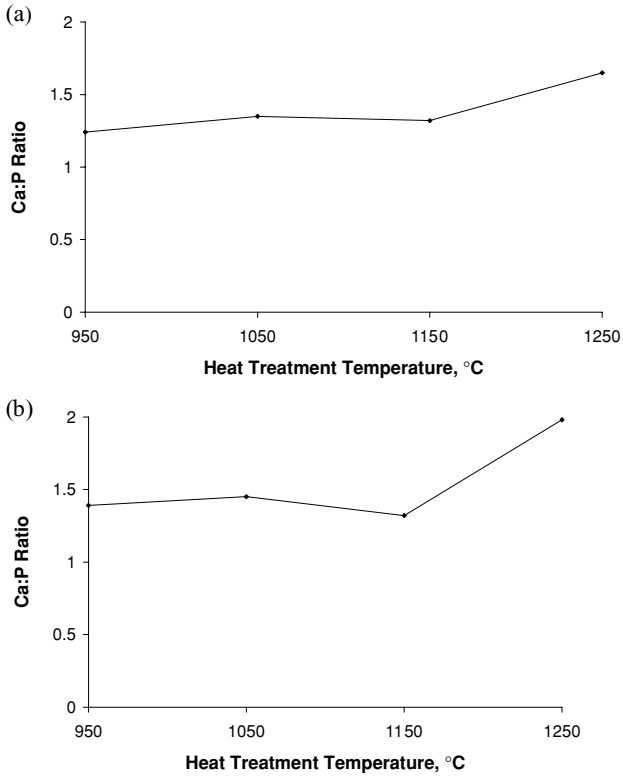

Fig. 7 EDS results for (a) 1.5SBF and (b) 5.0SBF for no heat treatment, 950, 1050, 1150, and 1250°C

cotton samples in SBF so as to elevate the Ca^{2+} ion concentration in the vicinity of the fibres and stimulate calcium phosphate formation. Mucalo et al. [11] who coated phosphorylated cotton with a calcium phosphate layer, found that amorphous calcium phosphate materials could be stimulated to form on cotton fibres. In that method the $Ca(OH)_2$ treatment was found to produce highly crystalline clusters lodged on the fibres which were confirmed by micro-FTIR to be calcium phosphate monohydrate ($CaHPO_3.H_2O$). Mucalo et al. thus stated that the clusters on the cotton may slowly dissolve upon introduction to a 1.5SBF, thus elevating the Ca^{2+} ion concentration in the vicinity of the fibres and stimulating calcium phosphate formation.

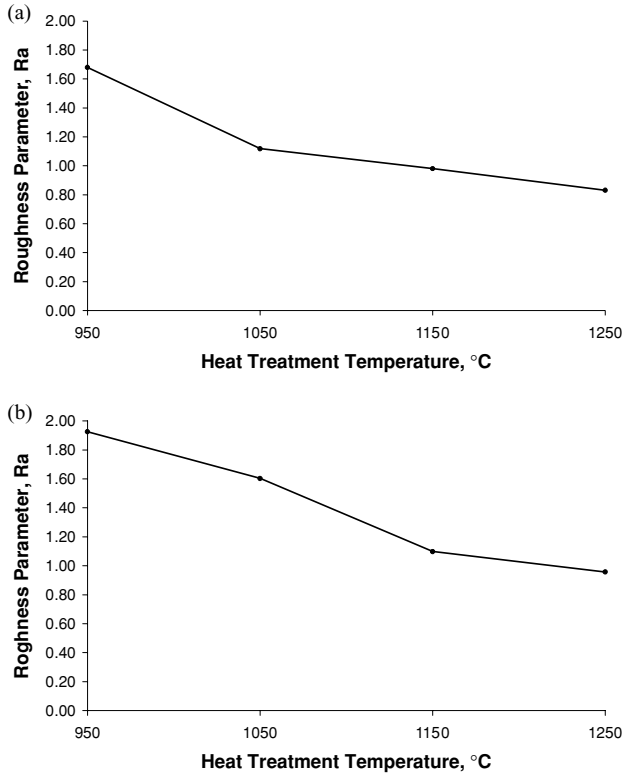

Fig. 8 Roughness parameters Ra for samples sintered at 950°C, 1050°C, 1150°C, and 1250°C; (a) 1.5SBF and (b) 5.0SBF

It is also possible that the phosphorylation technique encouraged the incorporation of phosphate groups, with lone electrons and thus increased reactivity, into the cotton material, leading to calcium phosphate crystal growth upon soaking in a SBF solution. As more than 90% of the cotton fibres are cellulose, it may be assumed that the phosphate groups were incorporated into the cellulose. Inagaki et al. [19] reported that phosphorylation of cotton fibres leads to the incorporation of phosphate groups into the material.

As phosphorylation enabled a material such as cotton to coat hydroxyapatite, it is suspected that many other fibre substrates could be coated with the aid of phosphorylation, for example bamboo. Bamboo is mainly composed of cellulose and lignin, cellulose existing in both parenchyma tissue and the bast fibres in the vascular bundles. Li et al. [12] studied phosphorylation on bamboo. After phosphorylation, it was found that phosphate groups could be incorporated with both bast fibres and the thin walled tissue giving bamboo cation exchange properties. They also suggested that some Ca^{2+} in calcification media can be absorbed on bamboo but apparently not enough to induce the precipitation of

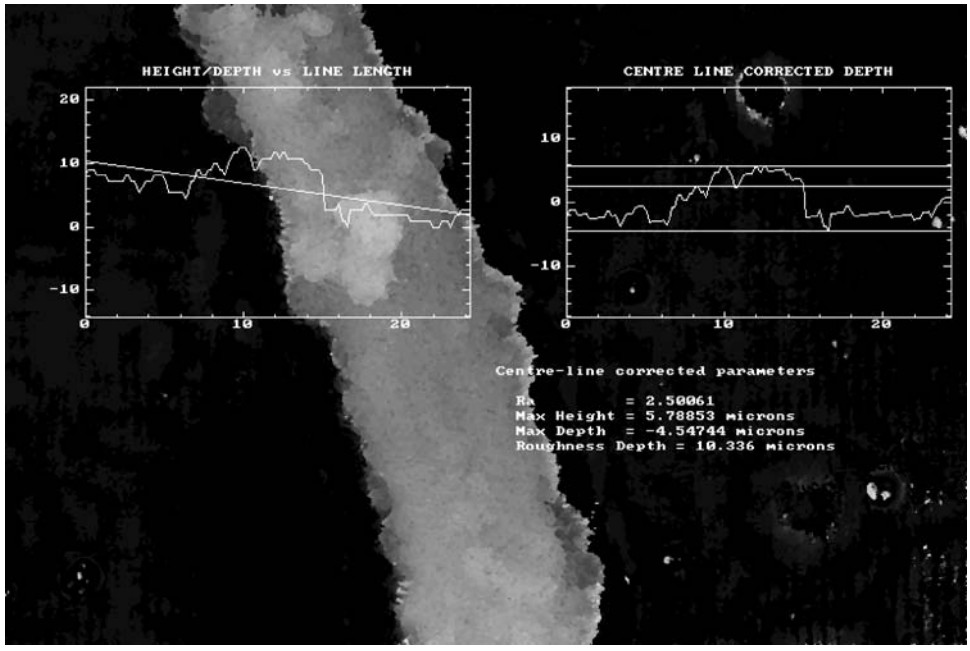

Fig. 9 Confocal microscopy result. An example of one length measurement for 5.0SBF heat treated at 950°C

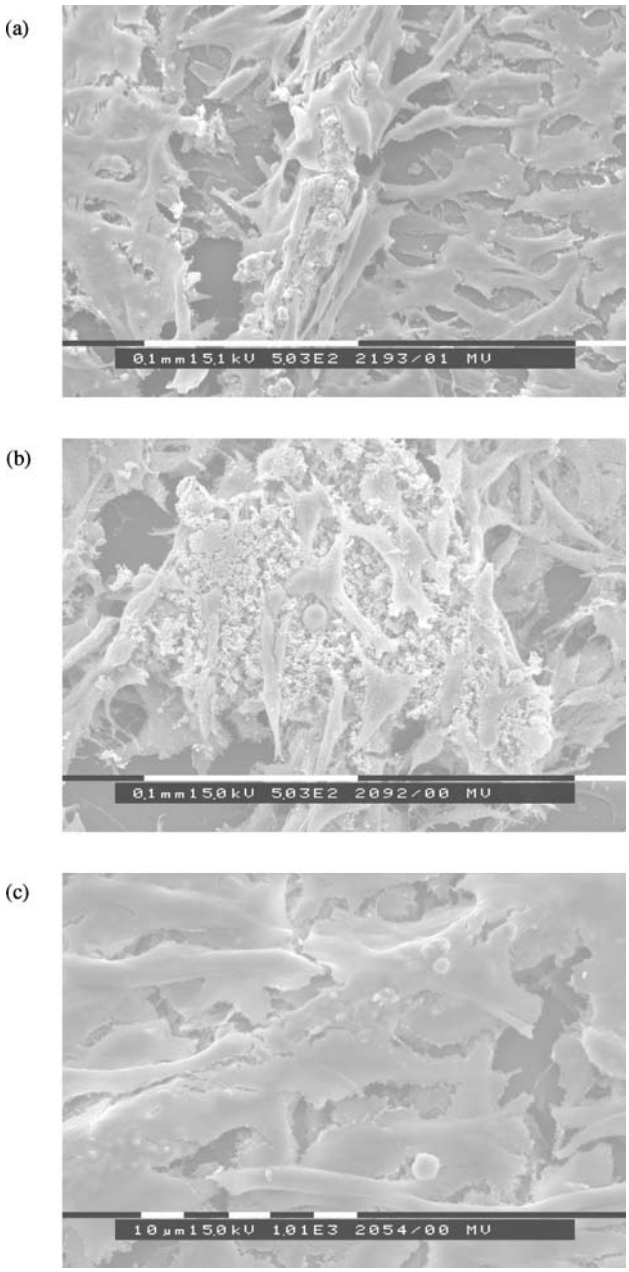

Fig. 10 Cell Attachment Study 1.5SBF (a) 950°C; (b) 1150°C; (c) 1250°C

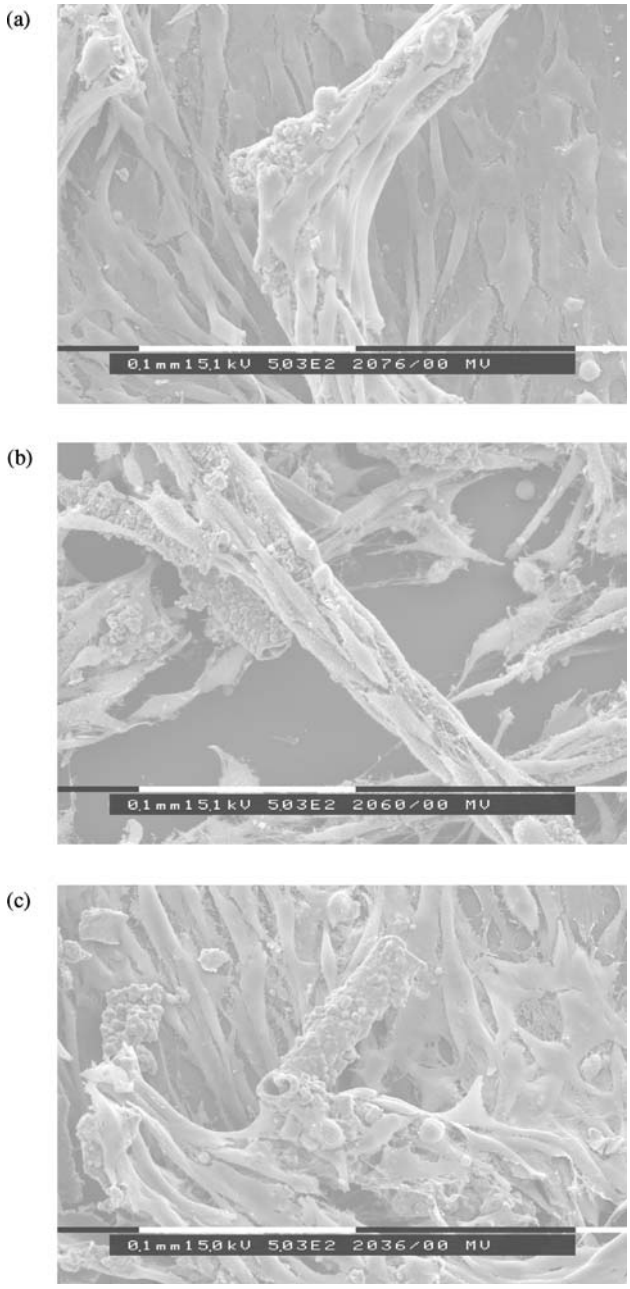

Fig. 11 Cell Attachment Study 5.0SBF (a) 950°C; (b) 1150°C; (c) 1250°C

calcium phosphate even in high calcium calcium-containing solutions [12].

The exact mechanism of this surface mineralization is not yet fully understood, but it has been assumed in this paper that phosphorylation provides some necessary surface activation of the cotton for further coating with a SBF. This pre-treatment may provide chemical stimulation; that is, precipitation and subsequent crystal growth or physical stimulation, that is, defects on the cotton substrate for heterogeneous nucleation and therefore crystal growth, or more probably a combination of both.

5.2 Calcium phosphate crystal growth in SBF

Using the phosphorylated fibrous cotton substrate a calcium phosphate layer using both 1.5SBF and 5.0SBF was achieved. As mentioned from Fig. 2 uniform coatings were achieved, however more coating was qualitatively found on the 5.0SBF treated samples. The 5.0SBF was tested in addition to the more conventionally used 1.5SBF with the assumption that the higher ion concentration would provide more available ions for coating and thus an increased rate of coating.

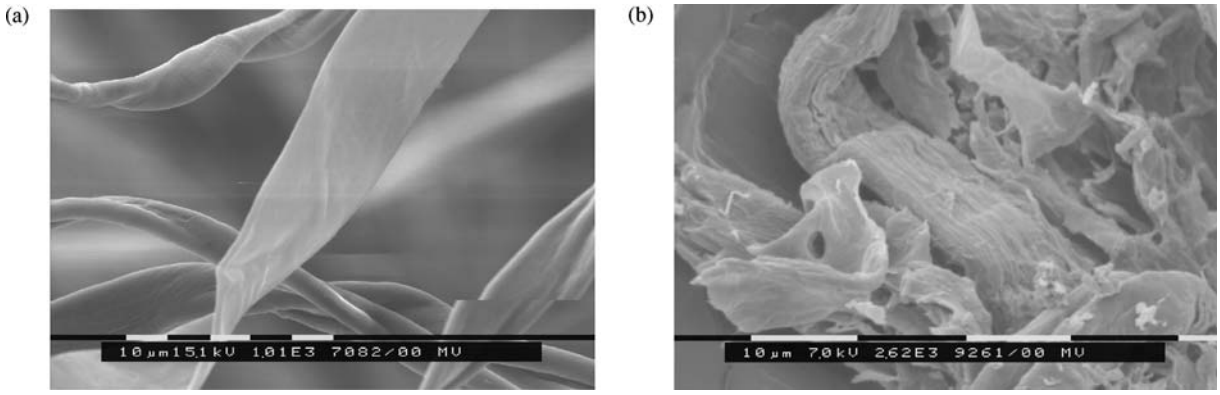

Fig. 12 (a) Control, raw as-supplied cotton with no pre-treatment; (b) Phosphorylated cotton (includes $Ca(OH)_2$ treatment). The result is a higher surface area fibre

As a more rapid coating was achieved with 5.0SBF, there was a question of coating composition. With XRD diffraction data, it was found that there was more crystalline material present as the crystal counts were more substantial and easier to detect in Fig. 3(c) for 5.0SBF compared to Fig. 3(b) for 1.5SBF. As expected, the peaks were quite broad, as is commonly found with unsintered chemically precipitated calcium apatites. They therefore were difficult to match precisely to any known calcium phosphate phase, but certainly they appeared to derive from the calcium apatite crystalline family. From SEM results, it is apparent that the crystalline peaks achieved greater intensity due to the greater amount of material present on the coated cotton, that is there was more crystalline material present or generally more coating deposited.

5.3 Heat treatment study

The sintering of the calcium phosphate coating in an ambient air atmosphere was done at a variety of temperatures to determine an appropriate sintering temperature and to burn out the combustible substrate of cotton. At 950°C, the cotton substrate was not present thus producing a hollow fibre that had not collapsed during the burn out of the cotton substrate. Very little sintering had occurred as the surface morphology of the fibre was quite rough, that is, the coating achieved a cauliflower-like crystal growth and with heat treatment to 950°C, small particles were still present, with only a small amount of necking between them. The tubular morphology was maintained with heat treatment as seen in the SEM micrographs in Figs. 4 and 5. Also as the cotton had burnt out a relatively thick walled (approximately 1 μm) hollow fibre resulted. Increasing the sintering temperature to 1050°C and 1150°C, the tubular morphology was maintained and the degree of sintering increased was necking within the particles of the coating, indicative of the earlier stage of sintering. Heat treatment at 1250°C, the degree of sintering was such that the hollow fibres started to open to take the form of tapes, while the surface showed a considerable amount of sintering, with a fused structure containing some open pores. With the increase in degree of sintering at the higher temperatures, there was also a reduction in surface area compared with heat treatment at the lower temperatures. It is therefore quite probable that the surface roughness decreased with increasing heat treatment temperature. This has been quantitatively shown in Fig. 8 where the surface roughness as measured by roughness parameter (Ra) using confocal optical microscopy generally decreased with increasing heat treatment temperature. This result supports the SEM qualitative result that the surface roughness of the fibres decreased with increasing sintering temperature.

At low sintering temperatures a microporosity or surface roughness on the fibres was evident. It is possible that the microporosity was caused by gas evolution in the burning out of the combustible substrate. By the higher temperature, the microporosity or surface roughness had smoothed with sintering of the ceramic coating, thus the microporosity could be due to the partial sintering of the coating as indicated in Figs. 4 and 5 which indicate an increasingly smooth morphology with increasing sintering temperature. Zhitomirsky [21, 22] did sintering experiments with electrophoretically deposited hydroxyapatite coatings on carbon fibres, where thermal treatment performed in an air furnace led to burning out of the combustible substrate carbon at temperatures exceeding 900°C and thus the formation of hollow calcium phosphate fibres. SEM analysis of the fibres indicated the existence of microporosity similar to the surface roughness observed in this paper. Further increase of the thermal treatment temperature brought a reduction in microporosity, however, above 1050°C, fast grain growth occurred. Zhitomirsky also suggested that the microporosity was a result of gas evolution due to the burning out of the carbon.

Figure 13(a) is an SEM micrograph of 5.0SBF heat treated sample at 1150°C. It is a high magnification micrograph showing the effect of sintering on the small calcium

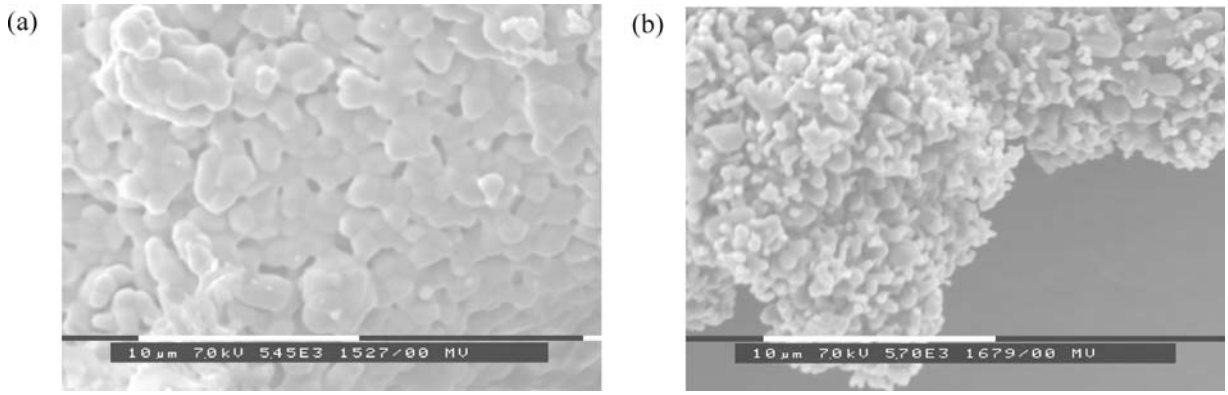

Fig. 13 SEM micrograph for 5.0SBF heat treated at 1150°C. Evidence of sintering with necking and particle agglomeration occurring

phosphate crystals. The particles have joined such that agglomeration occurred. As there is no external pressure, the porosity remained and the result was a fused porous structure. Reduction in surface area was observed with the increasing heat treatment temperature. In Fig. 13(b) 5.0SBF heat treated sample at 950°C shows a different surface morphology. With the same magnification, the particles were smaller and more numerous in the 950° heat treated sample. These small particles agglomerate at increasing temperatures to produce a smoother surface as shown in Fig. 13(a). This is an expected outcome of the sintering process.

XRD analysis of the sintered coatings confirmed that the calcium phosphate phase was weakly crystalline apatite. This is shown in the XRD analysis for 5.0SBF sample sintered to 1050°C in Fig. 6. The main calcium phosphate phase present at the increasing heat treatment temperature is whitlockite, a decomposition phase of hydroxyapatite, which was expected, in accordance with the literature [20].

EDS results show the atomic percent of calcium versus phosphorus in Fig. 7 for (a) 1.5SBF and (b) 5.0SBF. The results support the XRD analysis and again suggested a whitlockite calcium phosphate phase up to 1150°C, at which point the calcium to phosphorous ratio increased. This was possibly due to the decomposition of the hydroxyapatite phase suggesting the phosphates were volatilising at the higher temperatures.

5.4 Cell culture on fibers

Significant cell coverage was apparent for all sintering temperatures and pre-treatment conditions. The cell culture study established that osteoblasts could attach and proliferate on the surface of prepared materials and they were spread over the surface with intercellular contact. This present study found that neither the surface roughness nor small variations in the Ca/P ratio of the surface chemistry had any noticeable effect on osteoblast cell coverage within the seven days of culture period.

The purpose of the cell culture study was to test the general biocompatibility of the fibres and to see if surface morphology had any effect on cell coverage. The effect of surface morphology is obvious in all osteoblast cell and hydroxyapatite fibre interactions shown in Figs. 10 and 11. In Fig. 11(a) osteoblast cells can be seen to join two sintered fibres. If used as bone filler, these hydroxyapatite fibres could have a significant influence on the efficiency of bone repair with the hydroxyapatite fibres aiding osteoblast cell growth and attachment. In Fig. 11(b) the osteoblast cells are oriented along the length of the fibres, that is, they show longitudinal orientation. Thus the biocompatibilility test showed a very interesting and previously not published feature of the fibre morphology affecting the osteoblast cells orientation, on the hydroxyapatite fibres produced in this study via a SBF synthesis method. Also apparent in the cell culture study in Fig. 11(c) the osteoblast cells do not attempt to enter the hollow area of the fibres. It is possible that the hollow area was too narrow for cell growth. This phenomenon also has not previously been recorded. Such osteoblast cell behaviour on the hydroxyapatite fibres produced suggests they are a suitable substrate for osteoblast cells and could provide substantial benefit in cell attachment and bone ingrowth for applications as a bone filler materials and implants.

6 Conclusions

Hollow calcium phosphate fibres approximately 25 microns in diameter and 1 micron wall thickness were successfully manufactured using a surface pre-treatment, followed by growth in 1.5SBF and 5.0SBF solutions, followed by sintering. Phosphorylation was vital in the pre-treatment of cotton fibres for further calcium phosphate crystal growth from a SBF. 5.0SBF produced a thicker and more crystalline coat of greater uniformity. It is also probable that this lead to a more rapid coat compared with the 1.5SBF coating solution.

Sintering the ceramic coating at 950°C, 1050°C, 1150°C and 1250°C allowed the cotton to be burnt out and the coating to experience a range of sintering stages. As the degree of sintering increased the surface roughness was seen to decrease. XRD confirmed the presence of the hydroxyapatite and whitlockite phase. EDS demonstrated that the Ca/P ratio remained constant up to 1150°, and increased significantly above 1150°C.

The cell culture study with human derived osteoblast cells demonstrated good cell coverage for the sintering temperatures. Neither the degree of surface roughness nor the Ca/P ratio had any noticeable effect on coverage.

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