RAPID BIOMIMETIC CALCIUM PHOSPHATE COATING ON METALS, BIOCERAMICS AND BIOPOLYMERS AT ROOM TEMPERATURE WITH 10xSBF

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ABSTRACT

This paper reports the utilization of high ionic strength (>1100 mM) calcium phosphate solutions in depositing 20-65 μm-thick, bonelike apatitic calcium phosphate on Ti6Al4V within 2 to 6 hours, at room temperature. The solution used here multiplied the concentrations of calcium and phosphate ions in human plasma or synthetic/simulated body fluid (SBF) by a factor of ten. The solutions did not contain any buffering agents, such as Tris or Hepes. With these solutions there was no CO_2 bubbling required. The carbonate content (8 wt%) and Ca/P molar ratio (1.57) of the coated calcium phosphates qualified them as bonelike. The same solutions were successfully used in coating macroporous β-TCP (β-tricalcium phosphate, β-$Ca_3(PO_4)_2$) cylinders and macroporous collagen membranes with apatitic calcium phosphate.

INTRODUCTION

SBF (synthetic/simulated body fluid) solutions are shown [1-3] to induce apatitic calcium phosphate formation on metals, ceramics or polymers (with proper surface treatments) soaked in them. SBF solutions, in close resemblance to the Hanks Balanced Salt Solution (HBSS) [4], are prepared with the aim of simulating the ion concentrations present in the human plasma. It is noted that physiological HBSS solutions are also able to induce apatite formation on titanium [5]. To mimic human plasma, SBF solutions are prepared to have relatively low calcium and phosphate ion concentrations, namely, 2.5 mM and 1.0 mM, respectively [6]. Furthermore, to mimic human plasma, pH value of SBF solutions was adjusted to the physiological value of 7.4 by using organic buffers, such as Tris [3] or Hepes [7]. These compounds are not present in human plasma. The buffering agent Tris present in conventional SBF formulations, for instance, is reported [8] to form soluble complexes with several cations, including Ca^{2+}, which further reduces the concentration of free Ca^{2+} ions available for coating. Hepes, on the other hand, is rather unstable and easily loses a certain fraction of carbonate ions [9]. The hydrogencarbonate ion (HCO_3^-) concentration in SBF solutions was kept between 4.2 mM (equal to that of HBSS) and 27 mM [7, 9, 10].

Having their ionic compositions more or less similar to that of human blood plasma, HBSS or SBF formulations have only a limited power with respect to the precipitation of apatitic calcium phosphates. As a direct consequence, nucleation and precipitation of calcium phosphates from HBSS or SBF solutions are rather slow [11]. To get total surface coverage of a 10 x 10 x 1 mm titanium or titanium alloy substrate immersed into a 1.5 or 2 x SBF solution, one typically needs to wait for 2 to 3 weeks, with frequent (at every 36 to 48 hours) replenishment of the solution [12]. The broad motivation in this work is to enhance the kinetics of coating deposition.

In order to achieve the above objective, Barrere *et al*. [13-17] have developed 5xSBF-like solution recipes (with pH values close to 5.8), which did not employ any buffering agent, such as Tris or Hepes. In these studies [13-17], coating was achieved by employing two different solutions (solutions A and B as they referred), and pH was adjusted by continuous bubbling of CO_2 gas into the reaction chamber. A coating thickness of about 30 μm was achieved only after 6 h of immersion, which did not increase much even after 48 hours of further soaking, stirring and constant CO_2 bubbling at 50°C [13]. Moreover, they also introduced additional intermediate steps. These included [13] immersing the metal strips in the first 5xSBF solution (to seed the surface with calcium phosphate

nuclei) for 24 h at 37°C, followed by another soaking in their second 5xSBF solution (to form the actual coat layers by a so-called growth process) for 6 to 48 h at 50°C [13]. Dorozhkin *et al*. [18] modified this CO_2-bubbling technique, by using two different "4xSBF" solutions instead. These additional intermediate steps and second solution treatments add extra time and oppose the advantage gained by the enhanced kinetics.

There is yet another concern over the above-mentioned CO_2-bubbling technique. Bubbling of CO_2 (with the sole purpose of maintaining the solution pH at around neutral values, through concentrated SBF-like solutions) results in calcium phosphate coatings with significantly increased carbonate ion concentrations. A quantitative evidence for this phenomenon was provided by Dorozhkina *et al*. [11]. Dorozhkina *et al*. [11] reported the CO_3^{2-} weight percentages of 19, 26 and 33 (in the resultant calcium phosphates) for 2, 4 and 8xSBF solutions, respectively, when CO_2 bubbling was used for pH regulation at 37°C. They also noted that the same samples with such high carbonate concentrations also exhibited Ca/P molar ratios of 1.8, 1.9 and 2.3, respectively. For comparison's sake, human bones contain 7.4% CO_3^{2-}, 34.8% Ca^{2+}, 46.6% PO_4^{3-}, 0.72% Mg^{2+} and around 10.5% H_2O, by weight [11]. The Ca/P molar ratio of human bones is around 1.75 [19, 20]. Therefore, a calcium phosphate-based material with a Ca/P molar ratio of 2.3 and a carbonate content of about 33 wt% can not be regarded as a "bonelike" substance.

The aim of this paper is to present the preparation of a new acidic solution, which contains 10 times the calcium and phosphate ion concentrations of human blood plasma. Such a solution should enhance the kinetics of coating formation even more. Furthermore, it is preferred that other than the surface treatment step, not too many intermediate steps are involved. The only step that is needed is to add $NaHCO_3$ into the solution to raise its pH to around 6.5. The resultant solution is able to coat Ti6Al4V strips, macroporous β-TCP cylinders and porous collagen membranes (at RT, 22±1°C) rapidly, in as little as 2 hours. It was hereby shown that it is not necessary to use those so-called biomimetic conditions (i.e., 37°C and pH 7.4) for the coating purposes.

EXPERIMENTAL PROCEDURE

Preparation of Ti6Al4V Strips

Sheets of Ti6Al4V (Grade 5 ASTM B265) were cut into rectangular strips with typical dimensions of 10 x 10 x 0.20 mm and first abraded manually with a 1200-grit SiC paper. Strips were then cleaned with acetone (15 min), ethanol (15 min) and deionized water (rinsing), followed by etching each strip in 150 mL of a 5 M KOH solution at 60°C for 24 h, in a sealed glass bottle. Thoroughly rinsed (w/deionized water) strips were finally heat-treated at 600°C for 1 h in clean Al_2O_3 boats, with heating and cooling rates of 3°C/min.

Preparation of Macroporous β-TCP Cylinders

Macroporous, trabecular hydroxyapatite (of bovine origin, Endobon®, Merck Biomaterials GmbH, Darmstadt, Germany) cylinders were used to form macroporous β-TCP samples. One such cylinder (approx. 3 cm in height and 1 cm diameter, weighing 3.5 g) was placed into a 100 mL-capacity glass media bottle containing 90 mL deionized water and 18.0 g $(NH_4)_2HPO_4$, and the bottle was sealed prior to placing it into a 60°C oven. The cylinders were removed from the media bottles after 4 h at 60°C, and kept in a drying oven at 60°C (the cylinder was not washed with water) in unused Al_2O_3 dishes for 12 h. The dried samples (in the same Al_2O_3 dishes) were finally heated to 1225°±3°C at the heating rate of 5°C/min, soaked at this temperature for 12 h and cooled back to the room temperature at the rate of 2°C/min to obtain β-TCP cylinders.

Porous Collagen Membranes

Approximately three millimeter-thick collagen membranes or sponges (i.e., Matrix Collagen Sponge™) were obtained from Collagen Matrix, Inc. (Franklin Lakes, NJ, USA), and used as-received after cutting those into 1 x 1 x 0.3 cm coupons. These sponges were consisting of purified Type I

collagen (which in turn is rich in glycine, proline, and hydroxyproline) in its native triple helical structure.

Coating solutions

Solution preparation recipe (for a total aqueous volume of 2 L) is given in Table 1. The chemicals given in Table 1 are added, in the order written, to 1900 mL of deionized water in a glass beaker of 3.5 L-capacity. Before the addition of the next chemical, the previous one was completely dissolved in deionized water. After all the reagents were dissolved at RT, the solution volume was completed to 2 L by adding proper amount of deionized water. This extremely stable (against the formation of any visible calcium phosphate precipitates) stock solution of pH value of 4.35-4.40 can be stored at RT, in a capped glass bottle, for more than a year without precipitation.

Coating of Samples

Just prior to coating a Ti6Al4V strip, a 200 mL portion of the above-mentioned stock solution was placed into a 250 mL-capacity glass beaker, and a proper amount of $NaHCO_3$ powder was added to raise the hydrogencarbonate ion (HCO_3^-) concentration to 10 mM, under vigorous stirring. Following the rapid dissolution of the $NaHCO_3$, the pH of the clear solution automatically rose to 6.50 at RT. This solution (with an ionic strength of 1137.5 mM) was then transferred to a 250 mL-capacity glass bottle, which contained the Ti6Al4V strip inside, tightly capped and kept at RT for 2 to 6 hours during *in situ* coating. For coating the macroporous β-TCP cylinders and the porous collagen membranes, the same procedure was repeated in 250 mL-capacity glass media bottles strictly in the same manner as described for the Ti6Al4V strips.

Table I Stock solution preparation recipe, for a total volume of 2 L

Reagent	Order	Amount (g)	Concentration (mM)
NaCl	1	116.8860	1000
KCl	2	0.7456	5
$CaCl_2 \cdot 2H_2O$	3	7.3508	25
$MgCl_2 \cdot 6H_2O$	4	2.0330	5
NaH_2PO_4	5	2.3996	10

Sample Characterization

After the experiments were over, the samples were taken out of the solutions and rinsed respectively with an ample supply of deionized water and 80% ethanol solutions, followed by drying in air in a well-circulated fume hood for 24 h. Samples were characterized by XRD (D8 Advance, Bruker AXS, Karlsruhe, Germany; operated with Cu K_α radiation at 40 kV and 40 mA), FTIR (SpectrumOne, Perkin-Elmer, MA), SEM-EDXS (Hitachi S-4700 in the secondary electron mode, acceleration voltage 5-15 kV), and ICP-AES (Thermo Jarrell Ash, Model 61E, Woburn, MA). Gold sputtering was employed to make the coating surfaces conductive for the SEM investigations. In order to measure the thickness of the coat layers, the metallic strips were tilted by 45 degrees and studied by SEM.

RESULTS AND DISCUSSION

The chemical and thermal treatment of Ti6Al4V strips prior to the coating runs were mainly performed according to the previously published methods [6, 23, 24]. However, in our modification to the alkali treatment procedure, we have used 5 M KOH solution instead of 5 M NaOH. Figure 1a showed the surface of 5 M KOH + 600°C treated Ti6Al4V. The aggregated rosettes seen on the surface (Fig. 1a) belong to a potassium titanate phase of a possible composition of $K_2Ti_5O_{11}$. It should be

pointed out that this tentative formula is only based on the quantitative SEM-EDXS analyses performed on the rosettes seen in Fig. 1a. A phase of similar stoichiometry (i.e., $Na_2Ti_5O_{11}$) was also observed in case of using 5 M NaOH+600°C-treatment [24]. The surface of the alkali- and heat-treated strips also contained rutile (TiO_2), as seen in the XRD chart of Fig. 1b. The peak positions (labeled with "1") for the potassium titanate phase of the XRD chart in Fig. 1b match well with those reported previously, for sodium titanate, by Kim *et al*. [24]. Masaki *et al*. [25] recently reported the complete conversion of Ti metal powders into $KTiO_2(OH)$, upon soaking the metal powders in a concentrated (>35 M), hot (150°C) bath of KOH. Masaki *et al*. [25] also noted that this new phase transformed at 528°C into $K_2Ti_2O_5$, when heated in air. On the other hand, Yuan *et al*. [26] reported that TiO_2 powders heated in an 8 M KOH solution first formed $K_2Ti_{18}O_{17}$ nanowires, which would then decompose into $K_2Ti_6O_{13}$ and TiO_2 upon calcination in air at 600°C.

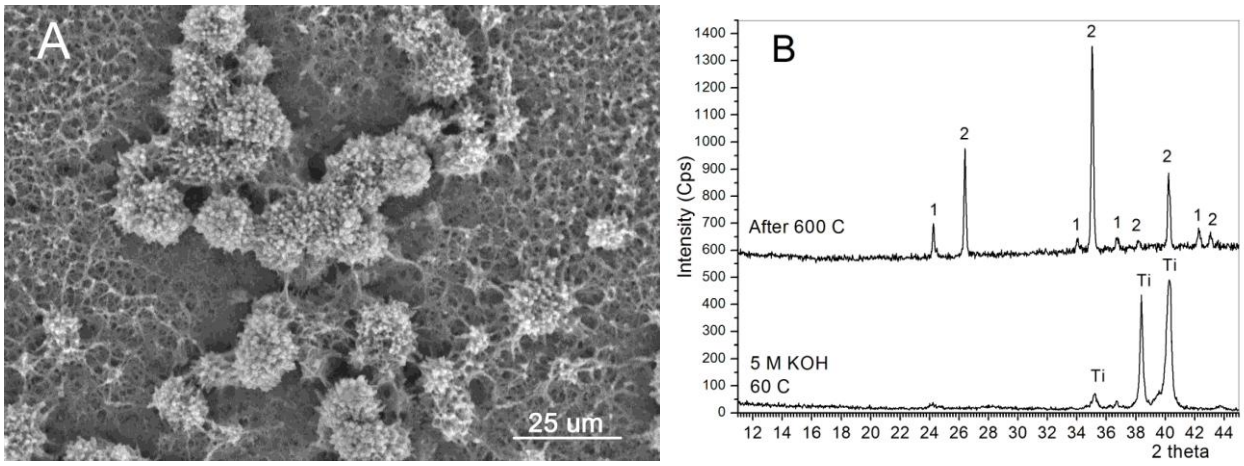

Figure 1 (a) Surface of 5 M KOH + 600°C-treated Ti6Al4V strips prior to coating, (b) XRD data of 5M KOH- (*trace-a*) and 5M KOH+600°C-treated Ti6Al4V strips; *phase 1: potassium titanate, phase 2: rutile, TiO_2.*

K^+ ions originating from the potassium titanates formed on the surfaces of KOH- and heat-treated Ti6Al4V strips, when exposed to the coating solution, were released into the solution in exchange of H_3O^+ ions, and eventually resulting in the formation of a Ti-OH layer. Ca^{2+} ions from the coating solution were then incorporated in this basic layer and act as embryonic sites for the nucleation of carbonated apatitic calcium phosphates [23]. The coating solution described above was not stable against precipitation (at RT) after the addition of $NaHCO_3$ to raise its pH to the vicinity of 6.5. The rise of pH in these solutions was quite monotonical (Fig. 2a). pH versus time curve depicted in Figure 2a was obtained after adding 1.68 g $NaHCO_3$ (i.e., 10 mM HCO_3^-), in powder form, to the solution (of pH 4.4 at 22°C) given in Table I. The stability against homogeneous precipitation only lasted from 5 to 10 minutes at RT, following the addition of $NaHCO_3$. After that period, solutions containing the metal strips slowly started displaying turbidity (from 10 minutes to the end of the first hour), and by the end of 2 hours the solution turned opaque. The colloidal calcium phosphate nuclei formed in the solution stay suspended, and could only be separated from the mother liquor by centrifugal filtration (>3000 rpm). However, it is interesting to note that the solution pH at the end of 2 hours of soaking period stayed the same or slightly increased to around 6.57 or 6.58. This slight increase in pH was ascribed to the release of CO_2 [14]. A pH decrease would have been encountered during the formation of colloidal precipitates due to H^+ release, but such a pH drop is not always observed [14, 27]. To perform a run with 6 hours of total soaking time, the coating solution for the same strip was replenished twice with a new transparent solution (of pH = 6.5) at the end of each 2-hours segment (see the XRD data of Fig. 2b below). The start of precipitation indicated the stage where the solution reached supersaturation.

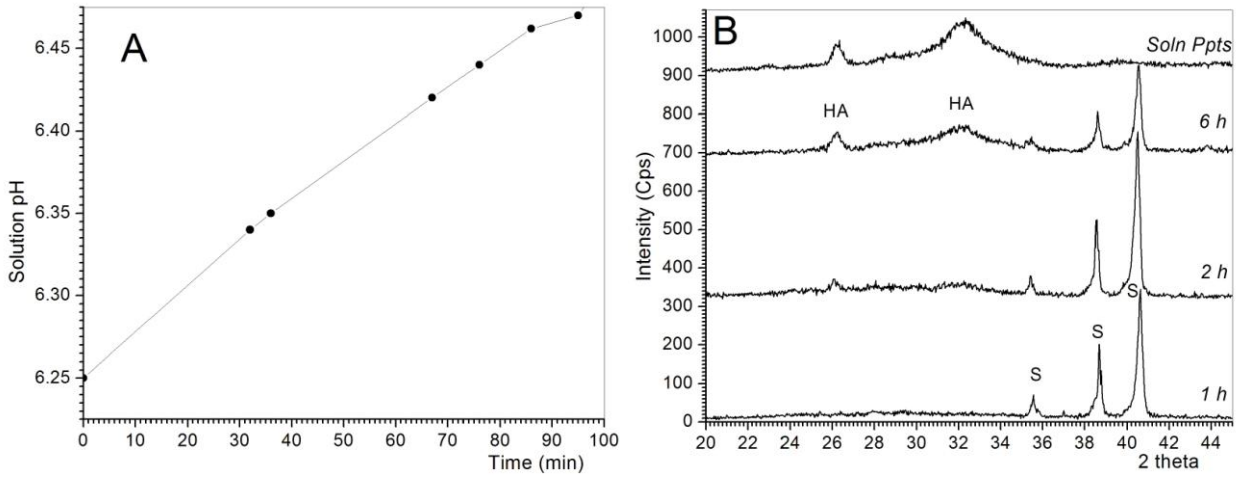

Figure 2 (a) pH *vs* time curve of solution given in Table I immediately after the addition of 1.68 g $NaHCO_3$ (*at 22°C*), (b) XRD data of coated Ti6Al4V strips as a function of soaking time (S: peaks of substrate) and those of colloidal calcium phosphate precipitates

It must be noted that the extremely simple solution recipe and the robust coating process described in this study utilized a HCO_3^- concentration of only 10 mM. This number is significantly lower than those used in "5xSBF + continuous CO_2-bubbling" method [13-18], mainly because in the latter, the coating needed to be continued for at least 72 h at 37° or 50°C, under continuous bubbling of CO_2 [13]. Due to this long time of deposition, attention must be paid there to ensure that coating deposition is linear. Increased carbonate concentration in a coating solution would result in calcium phosphate-like solid deposits with unacceptably high levels (25 to 30 wt%) of carbonate ions [11].

The coating process reported in this paper, on the contrary, does not require any attention. The inexpensive and stable solution given in Table I is simply prepared, then 10 mM $NaHCO_3$ is added to it at once in powder form, the solution and sample to be coated is placed in a sealed, undisturbed glass container, and after keeping it at RT for 2 to 6 hours, the sample is *in situ* coated by an apatitic calcium phosphate layer. Table II shows the deposition rate (measured in terms of coating thickness) as a function of immersion/soaking time.

Table II Coating thickness as a function of soaking time at RT in 10xSBF

Soak time (*h*)	Coating thickness (μm)
1	13 ± 2
2	22 ± 2
4	46 ± 4
6	68 ± 5

Such a linear and enhanced coating rate has never been achieved before by using either 1.5xSBF or 5xSBF solutions. With the use of 5xSBF solutions under constant CO_2 bubbling, the maximum coating thickness attained was around 35 μm after 3 days (i.e., 72 hours) of deposition [13-17].

Figure 3 depicts the SEM photomicrographs of coated surfaces of Ti6Al4V strips as a function of coating time (1 to 6 h; Figs. 3a through 3d) at RT. By using 10xSBF solutions described here, the surface of the KOH- and 600°C-treated surface of Ti6Al4V strips are rapidly covered within the first hour of immersion (Fig. 3a) with a smooth, nano-textured calcium phosphate layer of about 13 μm-

thick. By the end of the second hour in solution, coating develops to a thickness of about 22 μm, however, the attachment of calcium phosphate globules onto that initially-formed smooth surface becomes more enhanced (Fig. 3b). Such globules of apatitic calcium phosphate were quite similar to the previously reported results relevant to biomimetic SBF coating, excepting that biomimetic conditions (i.e., pH 7.4 and 37°C) were not met in our study. SEM micrograph given in Figure 3f is supplied for comparison purposes only. It was recorded from a Ti6Al4V strip soaked in *Tas*-SBF (a Tris-buffered SBF of pH 7.4, with a HCO_3^- concentration equal to 27 mM [10]) for 2 weeks at 37°C. A conventional SBF solution (i.e., 1.5x*Tas*-SBF) can only coat a 20 μm-thick layer of apatitic calcium phosphate after two weeks of soaking at 37°C, while the 10 SBF reported here achieves this in only 2 hours at RT. High-magnification photomicrographs of Figures 3c and 3d showed that the globules actually consisted of petal-like nanoclusters.

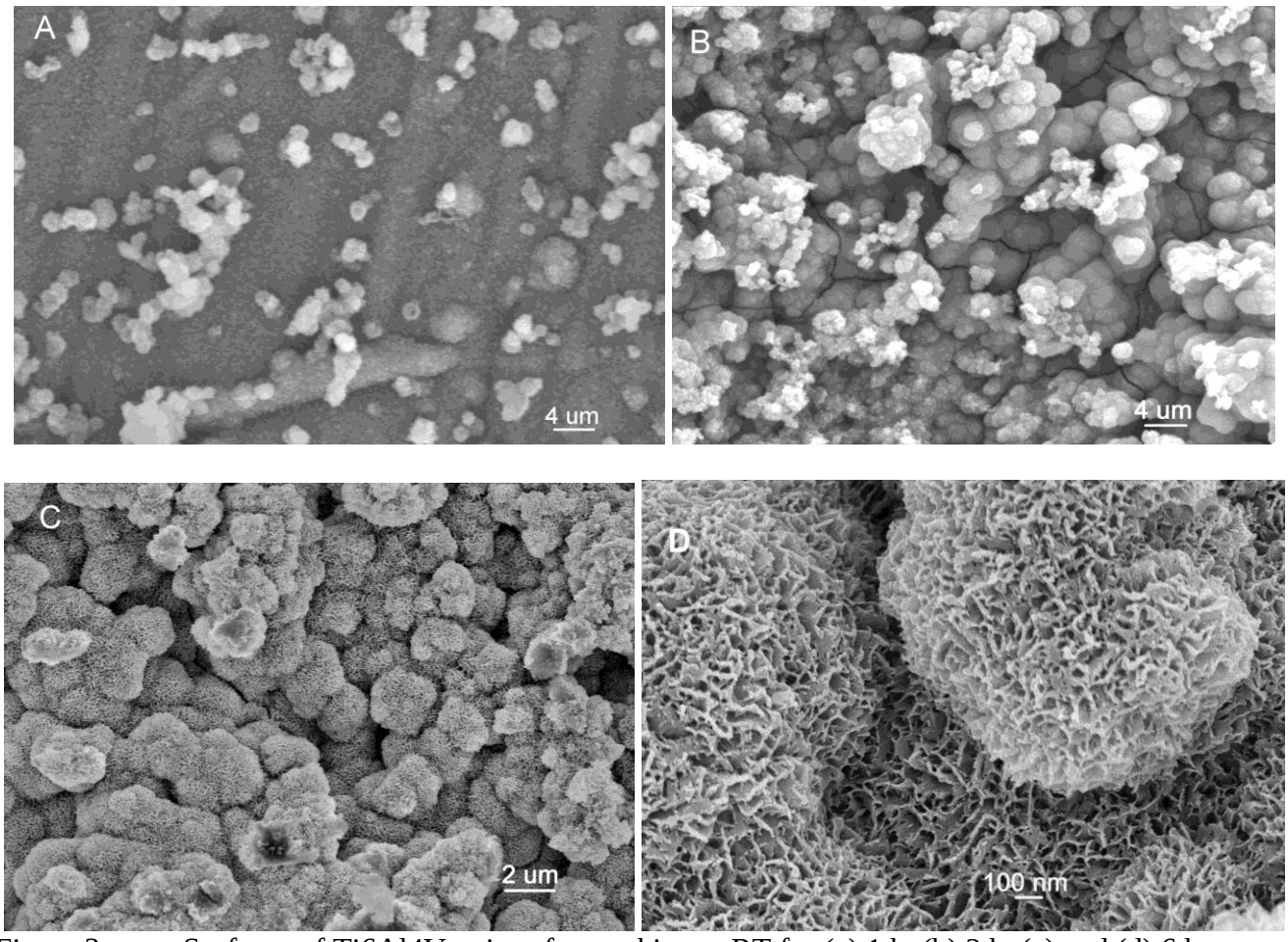

Figure 3 Surfaces of Ti6Al4V strips after soaking at RT for (a) 1 h, (b) 2 h, (c) and (d) 6 h

The FTIR data of the solution precipitates (chart not shown) indicated the nano-crystalline apatitic calcium phosphates formed in the solution, with characteristic IR bands of the O–H stretching and bending of H_2O at 3440 and 1649 cm^{-1}. Presence of carbonate groups was confirmed by the bands at 1490-1420 and 875 cm^{-1}. PO_4 bands were recorded at 570 and 603 (ν_4), 962 (ν_1), 1045 and 1096 (ν_3) cm^{-1} [10]. It is important to note that neither the precipitates themselves nor the coating layer (on Ti6Al4V strips) contained $CaCO_3$ (calcite) [28].

If the sole aim of a process is to coat titanium or titanium alloy surfaces with a carbonated apatitic calcium phosphate layer, then there is no need to maintain the pH value of a coating solution exactly at the physiologic value of 7.4. This point has been successfully confirmed in the work of

Barrere *et al*. [13-17, 29]. One only needs to be aware of the delicate balance between the solution pH, HCO_3^- ion concentration and temperature in determining which phases will be soluble or not under a specific set of those conditions [30]. On the other hand, the presence of TRIS or HEPES (added for the sole purpose of fixing the solution pH at around 7.4) in an SBF formulation simply retards the coating process to the level that in order to obtain a decent surface coverage one needs to wait for 1 or 3 weeks [6-8, 24].

Fast coating solutions, sometimes named as supersaturated calcification solutions (SCS) are not new either; for instance, the pioneering work of Wen *et al*. [31] showed that even in a TRIS-buffered SCS solution it would be possible to form 16 μm-thick calcium phosphate coat layers in after 16 hours of immersion. Choi *et al*. [32] reported the room temperature coating (about 10 μm-thick in 24 hours) of nickel-titanium alloy surfaces by a simple SCS solution, which was not even buffered at the physiologic pH. The present paper corroborates these previous findings and reports further improvements.

It is known that an amorphous calcium phosphate (ACP) precursor is always present during the precipitation of apatitic calcium phosphates from the highly supersaturated solutions, such as the one used here [33]. Posner, *et al*. [34] proposed that the process of ACP formation in solution involved the formation first of $Ca_9(PO_4)_6$ clusters which then aggregated randomly to produce the larger spherical particles or globules (as seen in Figs. 2d and 4), with the intercluster space filled with water. Such clusters (with a diameter of about 9.5 Angstrom [33]), we believe, are the transient solution precursors to the formation of carbonated globules with the stoichiometry of a calcium-deficient hydroxyapatite, namely, $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$, where x might be converging to 1 [14].

Ca/P molar ratio of the coat layers (after scraping small portions of the coatings off of the Ti6Al4V strips) was measured by ICP-AES analysis. The samples collected were carefully ground into a fine powder, followed by dissolving those in a concentrated acid solution prior to the ICP-AES runs. Ca/P molar ratio in these samples turned out to be 1.57 ± 0.05. Carbonate content was found to be less than 10 wt% (i. e., $8.2 \pm 0.3\%$). This means that the deposited material consists of "carbonated, calcium-deficient, poorly crystallized hydroxyapatite." This is how DeGroot and Kokubo [35] defined, back in 1994, the material coated on a titanium substrate immersed in a conventional SBF solution as "bonelike." From this viewpoint, the present coatings can be classified as bonelike.

Onuma *et al*. [36] have demonstrated, by using dynamic light scattering, the presence of calcium phosphate clusters from 0.7 to 1.0 nm in size in clear simulated body fluids. They reported that calcium phosphate clusters were present in SBF even when there was no precipitation. This was true after 5 months of storage at RT. The solution coating procedure described here probably triggered the hexagonal packing [36] of those nanoclusters to form apatitic calcium phosphates, just within the first 5 to 10 minutes, following the introduction of $NaHCO_3$ to an otherwise acidic calcium phosphate solution. Since these nanoclusters are always present even in a conventional ionic strength SBF, the insertion of a suitable alkali- and heat-treated Ti6Al4V surface into such a solution immediately starts the coating process, as explained above. This is how the dense-looking under coat layer is formed (Figs. 3a, 3b, and 3f) in less than an hour, provided that the solution is concentrated and supplying enough Ca and HPO_4 ions to the metal-solution interface. What is achieved here, with this new solution in less than an hour, can only be done with a conventional SBF in about a week. On the other hand, the colloidal precipitates (as a result of the hexagonal packing of the invisible nanoclusters [36]) of 10 SBF solution are formed by a homogeneous nucleation process. The presence of these precipitates within the solution, possibly, further accelerates the coarsening of the newly deposited calcium phosphate globules. Conventional, Tris or Hepes-buffered SBF solutions (1.5 SBF) are able to form those precipitates by the end of 2^{nd} or 3^{rd} day of soaking at 37°C. Since the Ca/P molar ratio of all SBF solutions (including the one presented here) are 2.50, they are not stable against hydroxyapatite precipitation when the solution pH is higher than 6.4 [10, 11].

Figure 4 showed the XRD data of macroporous bioceramic cylinders (initially HA, finally β-TCP with a low HA content), as a function of increasing calcination time from 900° to 1225°C. The samples shown in this figure were prepared in the manner described in the Experimental chapter, the only difference among them is the temperature of calcination. Figure 5a depicted the macroporous nature of the $(NH_4)_2HPO_4$-treated and 1225°C-heated bovine cylinders. The procedure used here to transform the HA-based macroporous bovine cylinders to β-TCP was simply adapted from the procedure described by Bauer [37]. It is important to note that the 10xSBF solution of this study was able to significantly cover the available bioceramic surfaces in a time as short as 6 h.

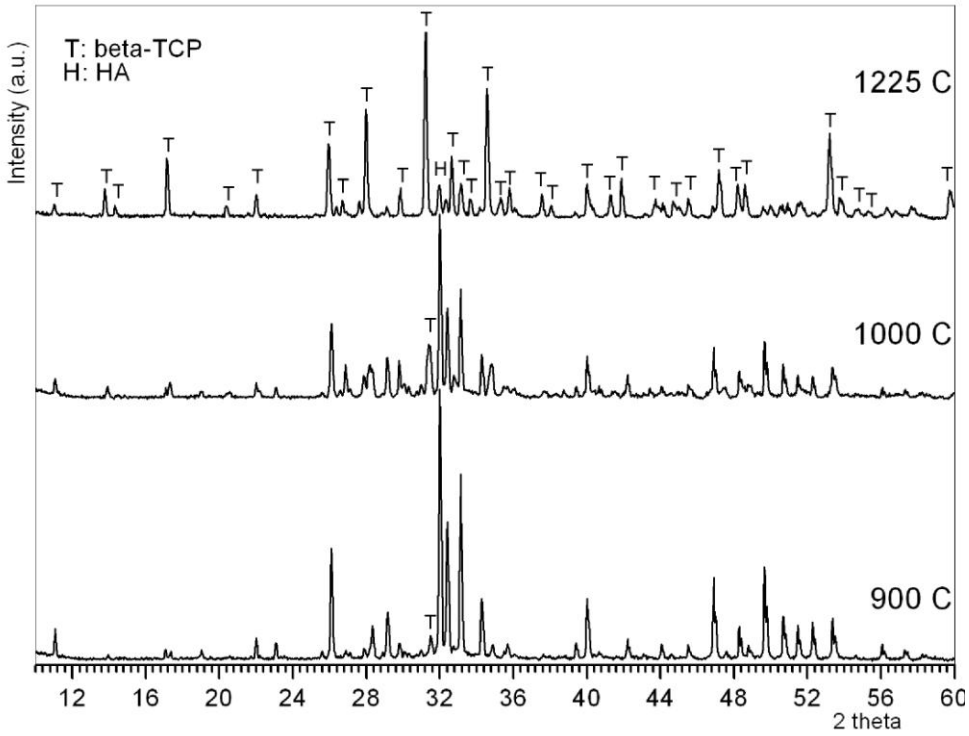

Figure 4 XRD traces of bovine-origin cylinders first $(NH_4)_2HPO_4$-treated and then heated at the temperatures shown

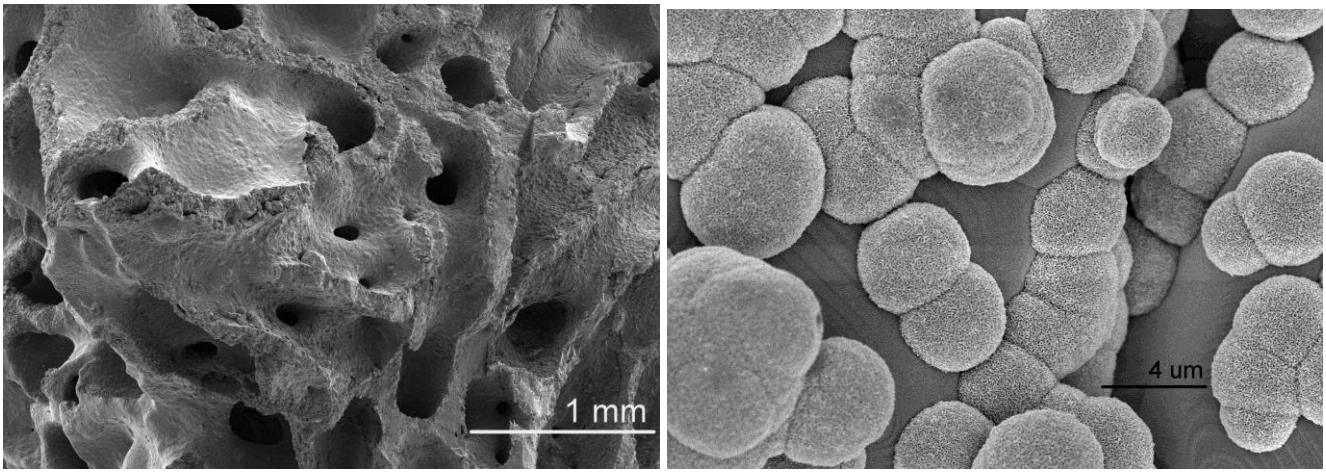

Figure 5 Surfaces of bovine bioceramics (a) after $(NH_4)_2HPO_4$ treatment, (b) 10xSBF-coated in 6h

ICP-AES analysis performed on six of the TCP-transformed cylinders, after 1225°C-heating, revealed that their Ca/P molar ratio was 1.52±0.015. The same ratio for the starting Endobon® cylinders was found to be in the vicinity of 1.70. The nanoporous CaP spherulites shown in Fig. 5 would also serve to increase the BET surface area of such macroporous β-TCP samples. In other words, having a β-TCP implant with an increased surface area will always be better, in terms of its osteointegrative properties, than having a β-TCP implant with low surface area. The follow-up studies will experimentally verify this assertion.

The characteristic SEM morphology of the as-received collagen membranes used in this study was shown in Fig. 6a. The typical microstructure after coating the membrane with apatitic calcium phosphate, by soaking in 10xSBF for 6 h, was given in Fig. 6b.

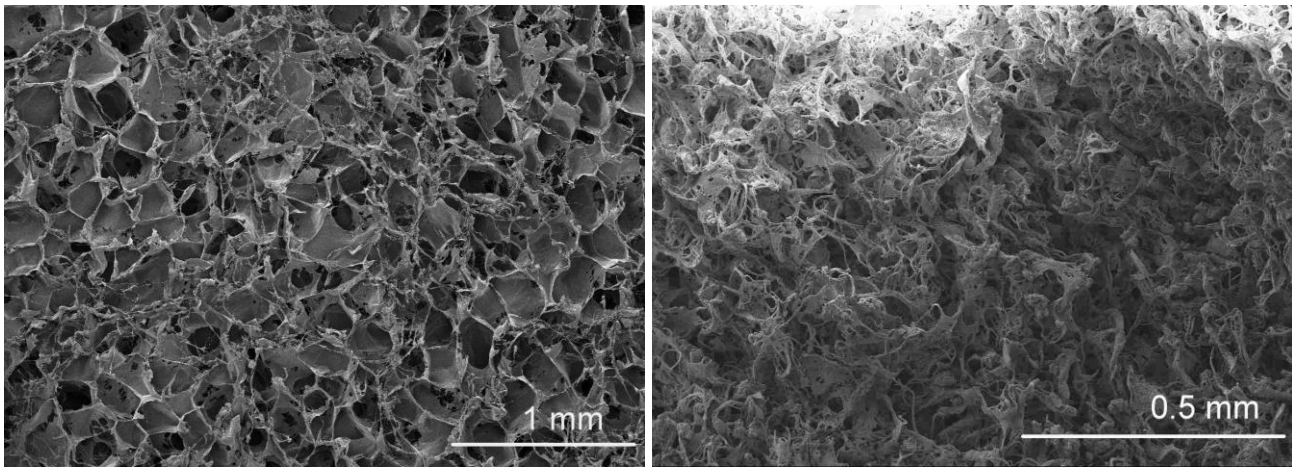

Figure 6 Collagen membrane microstructure (a) as-received, (b) 10xSBF soaked, 6 h at RT

The SEM photomicrographs of Figure 7 below depicted the microstructure of the 10xSBF-soaked collagen membranes at magnifications higher than those given in Figure 6. The procedure described here is one of the easiest ways of producing collagen-apatitic calcium phosphate composites. The membranes preserved their original flexibility after the 10xSBF coating. The rapid coating process also eliminated the problem of collagen swelling.

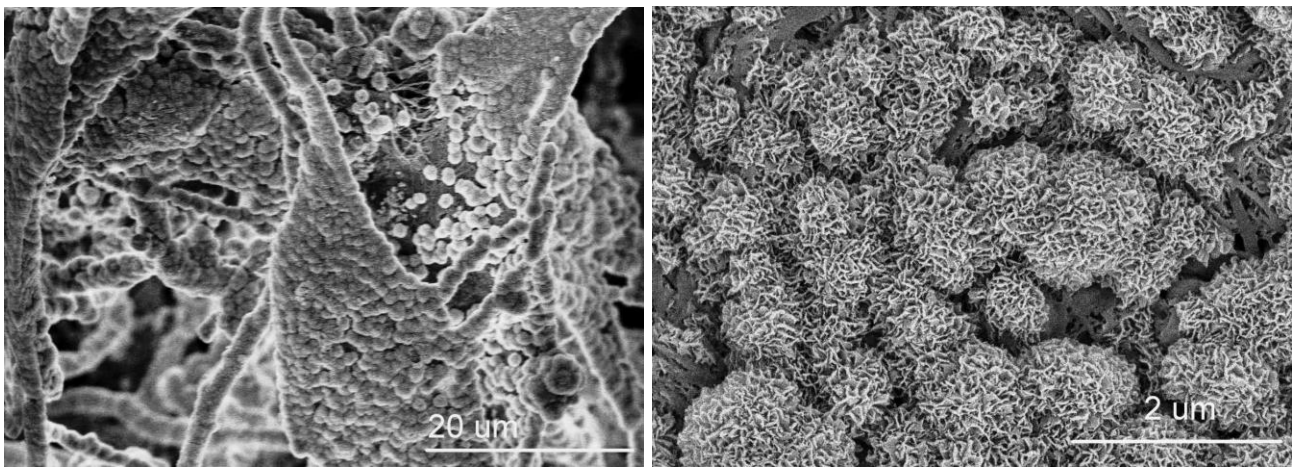

Figure 7 Collagen membranes soaked in 10xSBF solutions for 6 h at RT (*higher magnifications*)

Figure 8 showed the XRD data collected directly from the collagen membranes soaked in 10xSBF solution for 6 h at RT. This XRD chart was characteristic for the nanocrystalline (or poorly crystallized) nature of apatitic CaP formed in aqueous solutions of neutral pH. The data of Fig. 8 was collected from a 10xSBF-soaked membrane after placing it directly on the sample holder of the diffractometer.

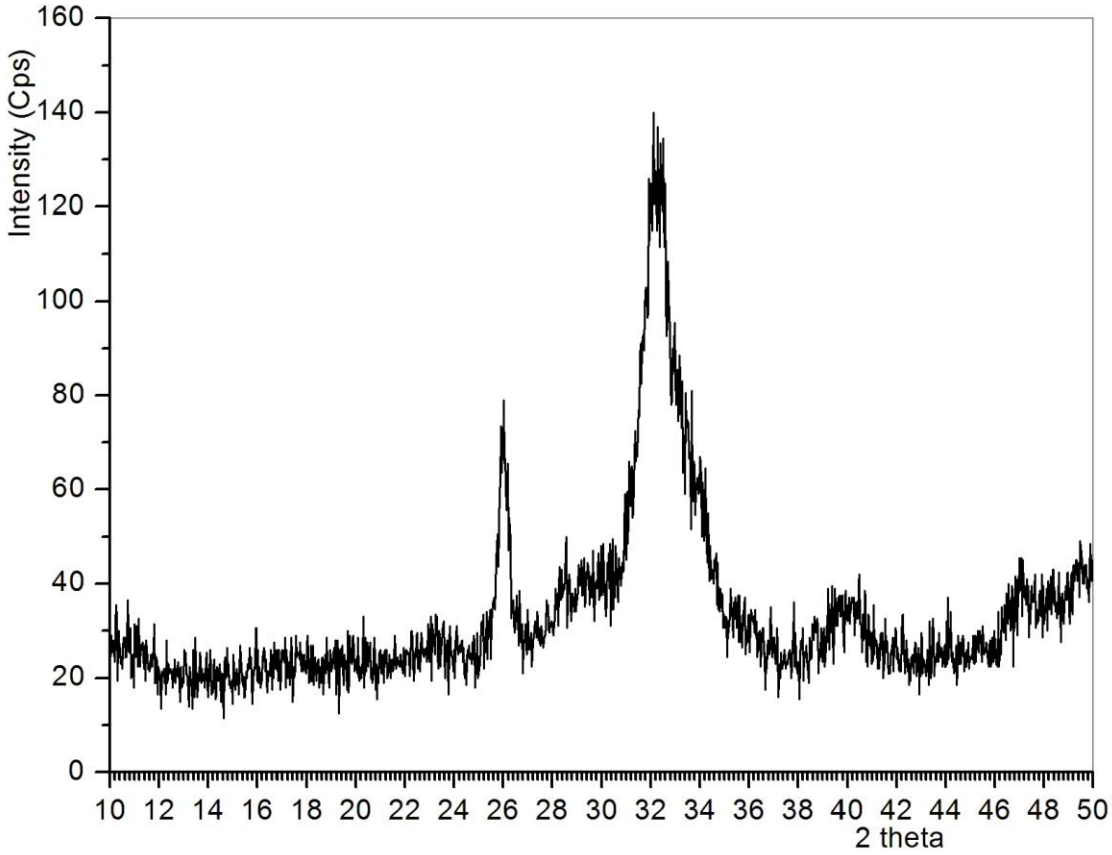

Figure 8 The typical XRD chart of 10xSBF-treated collagen membranes (for 6 h at RT)

Collagen is the *de facto* biopolymer in mammalians, whereas cellulose is that of plants and trees, and chitin is usually seen in the exoskeleton of crustaceans (sea-shells, oysters, crabs, etc.). Human bones are meticulous composites of collagen and carbonated, apatitic CaP mineralites. Collagen is tough and inextensible, with great tensile strength, and is the main component of cartilage, ligaments, tendons, and bones [38]. Multiple tropocollagen (with triple helix structures) molecules (300 nm long and 1.5 nm in diameter) form collagen fibrils, and multiple collagen fibrils form collagen fibers [39]. Soaking of collagen membranes or sponges in Tris–SBF-4.2 mM solution was first studied and reported by Rhee and Tanaka [40]. Lickorish *et al*. [41] later confirmed the findings of Rhee and Tanaka [40]. Girija *et al*. [42] tested the viability of a Hepes–NaOH-27 mM SBF solution [43] in synthesizing collagen–CaP composites by soaking fibrous collagen in that solution.

However, the process of 10xSBF-soaking of collagen membranes described here was unprecedented according to the best of our knowledge.

CONCLUSIONS

The use of $NaHCO_3$ with a concentrated (10 times of Ca^{2+} and HPO_4^{2-} ion concentrations) synthetic/simulated body fluid-like solution of ionic strength of 1137.5 mM allowed the formation of a bonelike apatitic calcium phosphate layer on Ti6Al4V at room temperature, within 2 to 6 hours. The coating solutions of pH 6.5 did not necessitate the use of buffering agents. The pH adjustment was achieved by a single-step addition of $NaHCO_3$. The coating process did not require the continuous bubbling of CO_2 during the process. This robust process had a linear and fast coating kinetics. The surfaces of the Ti6Al4V strips were chemically etched in 5 M KOH solution and thermally treated afterwards at 600°C, prior to soaking in 10xSBF solutions. KOH soaking and thermal treatment following it ensured the formation of potassium titanates on the strip surfaces. The coatings had a Ca/P molar ratio of 1.57 and contained 8 wt% CO_3^{2-}. Formation of colloidal nuclei, within the solution, was observed during the first hour of soaking at RT, but apparently the presence of those fine nuclei did not adversely affect the coating process.

Macroporous and bovine-origin HA cylinders were first converted into β-TCP, and then soaked in 10xSBF at RT for 6 h under conditions very similar to those employed in coating the Ti6Al4V strips. 10xSBF solutions were able to coat most of the available β-TCP surfaces.

Porous collagen (Type I) membranes were soaked at RT (for 6 h) in 10xSBF solutions and the collagene membranes were readily coated with apatitic and nanotextured calcium phosphate globules or spherulites. This process is a simple, rapid and robust way of producing porous collagen-apatitic calcium phosphate composites at room temperature within few hours.

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