Rapid coating of Ti6Al4V at room temperature with a calcium phosphate solution similar to 10× simulated body fluid

A. Cuneyt Tas and Sarit B. Bhaduri
School of Materials Science and Engineering, Clemson University, Clemson, South Carolina 29634

In this paper, we report the utilization of high ionic strength (>1100 mM) calcium phosphate solutions in depositing 20–65-µm-thick, bonelike apatitic calcium phosphate on Ti6Al4V within 2–6 h, at room temperature. The super-strength solution used here multiplied the concentrations of calcium and phosphate ions in human plasma or simulated body fluid (SBF) by a factor of ten. The interesting features of the technique are given in the following. First, the solutions did not contain any buffering agents, such as Tris or Hepes. Second, during the process, homogeneous formation of calcium phosphate nano-clusters took place. However, their presence did not adversely affect the coating process. Third, other than simple surface treatments to begin with, no other additional intermediate steps were necessary. The only step needed after the preparation of the solution from reagents is the addition of proper amounts of $NaHCO_3$ to raise the pH to 6.5 prior to the coating procedure. Fourth, there is no CO_2 bubbling required, and hence, this is a robust process. Fifth, such a procedure led to a significant enhancement of coating rate enabling the formation in as little as 2–6 h. Coating proceeded with a linear rate. Sixth, the adhesion strength (12 ± 2 MPa) of the present coatings was comparable to coatings produced by soaking in 1.5× SBF solutions over a prolonged period of time, typically two to three weeks. Finally, the carbonate content (8 wt%) and Ca/P molar ratio (1.57) qualify the coating as bonelike.

I. INTRODUCTION

Simulated body fluid (SBF) 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 and 1.0 mM, respectively.[6] Furthermore, to mimic human plasma, the 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]

With 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 obtain total surface coverage of a 10 × 10 × 1 mm titanium or titanium alloy substrate immersed into a 1.5 or 2× SBF solution, one typically needs to wait for 2 to 3 weeks, with frequent (at every 36 to 48 h) replenishment of the solution.[12] The broad motivation in this work is to enhance the kinetics of coating deposition.

To achieve the above objective, Barrere et al.[13–17] have recently developed unique 5× SBF-like solution recipes (with pH values close to 5.8), which did not use 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 h 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 5× SBF solution (to seed the surface with calcium phosphate nuclei) for 24 h at 37 °C, followed by another soaking in their second 5× SBF solution (to form the actual coat layers by a so-called growth process) for 6–48 h at 50 °C.[13] Dorozhkin et al.[18] recently modified this CO_2-bubbling technique, by using two different 4 SBF 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 recently provided by Dorozhkina et al.[11] They reported CO_3^{2-} weight percentages of 19, 26, and 33 (in the resultant calcium phosphates) for 2, 4, and 8 SBF 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 for the first time [at room temperature (RT), 22 ± 1 °C] rapidly, in as little as 2 h. It is shown that it is not necessary to use biomimetic conditions (i.e., 37 °C and pH 7.4) for coating purposes.

II. EXPERIMENTAL

A. Preparation of Ti6Al4V strips

Sheets of Ti6Al4V (Grade 5 ASTM B265) were cut into rectangular strips with typical dimensions of 10 × 10 × 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 de-ionized 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 (with water) strips were finally heat-treated at 600 °C for 1 h in Al_2O_3 boats, with heating and cooling rates of 3 °C/min.

B. Coating solutions

Solution preparation recipe (for a total aqueous volume of 2 l) is given in Table I. The chemicals given in Table I are added, in the order written, to 1900 ml of de-ionized water in a glass beaker of 3.5-l capacity. Before the addition of the next chemical, the previous one was completely dissolved in water. After all the reagents were dissolved at RT, the solution was made up to 2 l by adding the proper amount of water. This stable stock solution of pH value of 4.35–4.40 can be stored at RT in a capped glass bottle for several months without precipitation.

Just prior to coating a Ti6Al4V strip, a 200-ml portion of this 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 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 2–6 h during coating.

C. Characterization

After the experiments were over, the strips were taken out of the solutions and rinsed with an ample supply of de-ionized water and ethanol, followed by drying in air. Samples were characterized by x-ray diffraction (XRD; XDS 2000, Scintag Corp., Sunnyvale, CA; operated with Cu K_α radiation at 40 kV and 30 mA), Fourier transform infrared (FTIR; Bruker, ATR-FTIR and Nicolet 550, Thermo-Nicolet, Woburn, MA), scanning electron microscopy–energy dispersive x-ray spectroscopy (SEM-EDXS; Hitachi, Tokyo, Japan S-4700 in the secondary electron mode, acceleration voltage 5–15 kV), and inductively coupled plasma–Auger electron spectroscopy

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

(ICP-AES; Thermo Jarrell Ash, Model 61E, Woburn, MA). Platinum sputtering was used to make the coating surfaces conductive for the SEM investigations. To measure the thickness of the coat layers, the strips were tilted by 45° and studied by SEM.

Bonding strength of coatings to the substrates was measured by using the method described in ASTM C-633 standard.[21] Both sides of the substrates were attached with a drop of glue (with a tensile strength of 15 MPa) to cylindrical stainless steel jigs 10 mm in diameter and 15 mm in length. Tensile load was applied normally to the substrates with an Instron testing machine (Measurements Technology, Inc., Roswell, GA) at a crosshead speed of 1 mm/min until fracture occurred. The above sample preparation technique was previously utilized by Kokubo et al.[22] in testing the adhesion strength of bonelike apatite layers (about a few microns thick) obtained from SBFs of conventional recipes.

III. 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 in lieu of 5 M NaOH. Figures 1(a) and 1(b) show the surface of 5 M KOH + 600 °C treated metal surface. The aggregated rosettes seen on the surface [Fig. 1(b)] 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. 1(b). A phase of similar stoichiometry (i.e., $Na_2Ti_5O_{11}$) was also observed in the 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. 1(c). The peak positions (labeled with "1") for the potassium titanate phase of the XRD chart in Fig. 1(c) 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.

K^+ ions originating from the potassium titanates formed on the surfaces of KOH^- and heat-treated Ti6Al4V strips, when exposed to the coating solution, are released into the solution in exchange of H_3O^+ ions and eventually result in the formation of a Ti–OH layer.
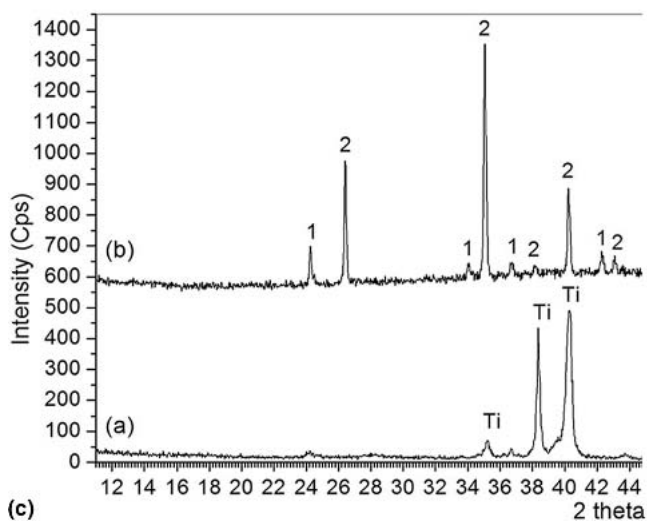

(a)

(b)

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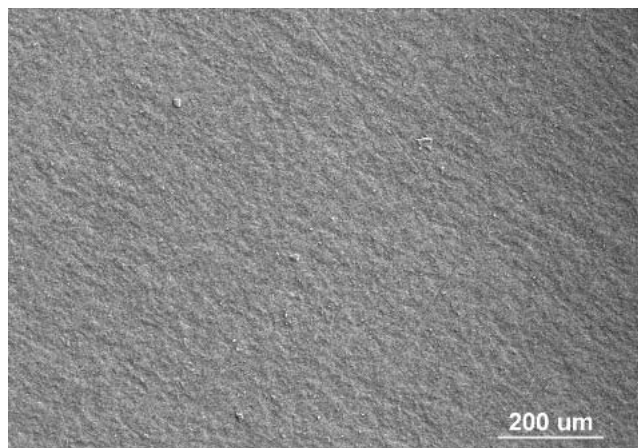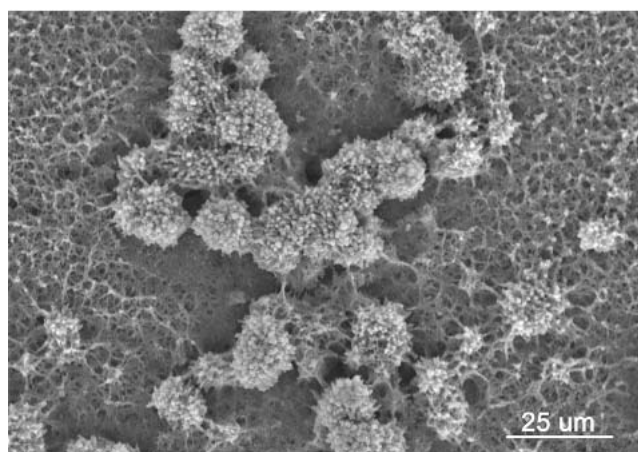
FIG. 1. (a) Surface of 5 M KOH + 600 °C treated Ti6Al4V strips prior to coating (low mag). (b) Surface of 5 M KOH + 600 °C treated Ti6Al4V strips prior to coating (high mag). (c) XRD data of 5 M KOH^- (trace-a) and 5 M KOH + 600 °C treated Ti6Al4V strips; phase 1: potassium titanate, phase 2: rutile,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

Ca^{2+} ions from the coating solution are then incorporated in this basic layer and act as embryonic sites for the nucleation of carbonated apatitic calcium phosphates.[23] The dimensions of crevices or pits created on Ti6Al4V surface in the etching step of 5 M KOH-soaking was found [Fig. 1(b)] to be much larger than those created in using 5 M NaOH.[24] Bigger crevices (as compared to submicron pits obtained with NaOH) are more suitable for the attachment of calcium phosphate globules a few microns in size.

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. 2). The pH versus time curve depicted in Fig. 2 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 21 °C) given in Table I. The stability against homogeneous precipitation only lasted from 5 to 10 min at RT, following the addition of $NaHCO_3$. After that period, solutions containing the metal strips slowly started displaying turbidity (from 10 min to the end of the first hour), and by the end of 2 h the solution turned opaque. The colloidal precipitates 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 h of soaking period stayed the same or slightly increased to around 6.57 or 6.58. That 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 h 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 two-hour segment. The start of
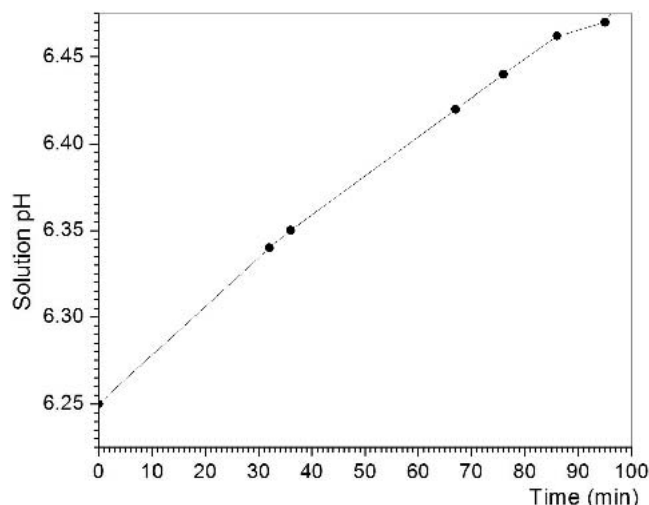
precipitation indicated the stage where the solution reached supersaturation.

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 the 5 SBF + 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 deposition time, attention must be paid 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 metal piece to be coated are placed in a closed, undisturbed glass container, and after keeping it at RT for 2 to 6 h, the strip 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 time.

Such a linear and enhanced coating rate has never been achieved before by using either 1.5 SBF or 5 SBF solutions. With the use of 5 SBF solutions under constant CO_2 bubbling, for instance, the maximum coating thickness attained was around 35 μm after 3 days (i.e., 72 h) 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. 3(a)–3(e)] at RT. By using 10 SBF solutions described here, the surface of the alkali- and heat-treated surface of Ti6Al4V strips are rapidly covered within the first hour of immersion [Fig. 3(a)] with a smooth, nano-textured calcium phosphate layer 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. 3(b)]. 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

FIG. 2. pH versus time curve of solution given in Table I immediately after the addition of 1.68 g $NaHCO_3$ (at 21 °C).

TABLE II. Coating thickness as a function of soaking time at RT in 10 SBF.

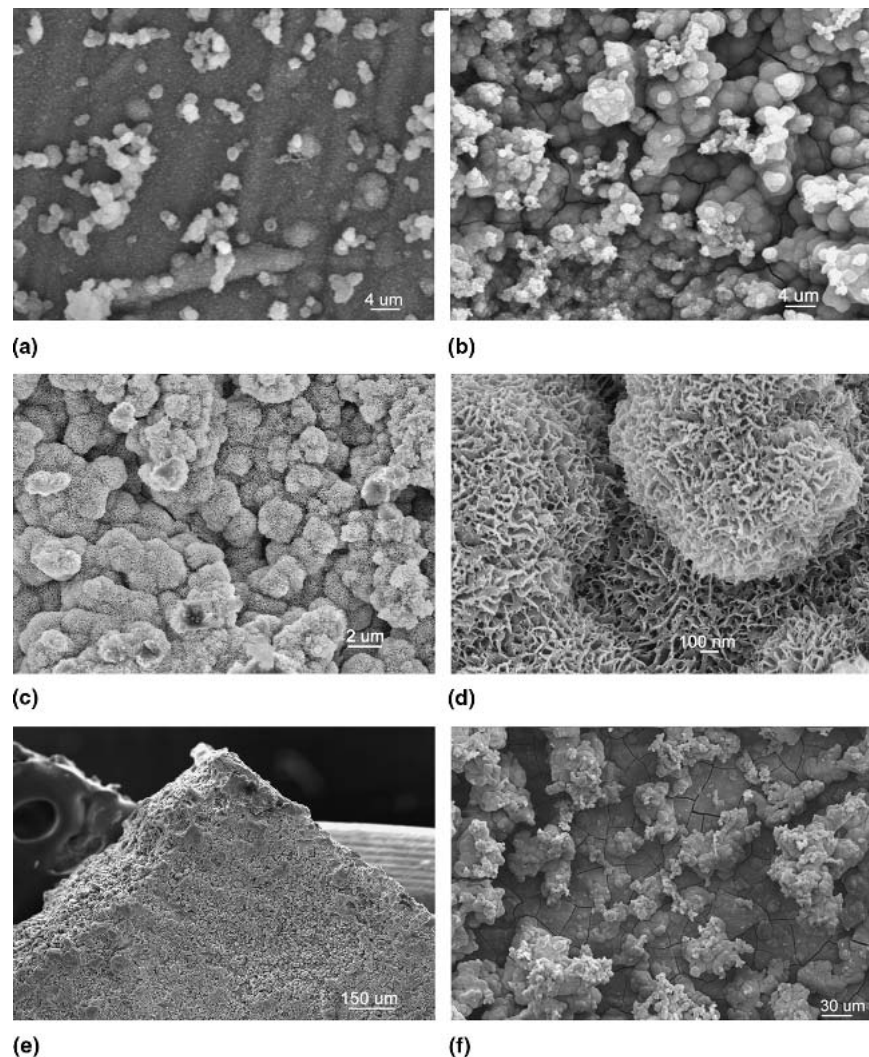| Soak time (h)	Coating thickness (μm)
1	13 ± 2
2	22 ± 2
4	46 ± 4
6	68 ± 5

FIG. 3. (a) Surface of Ti6Al4V strip after soaking at RT for 1 h. (b) Soaking time at RT: 2 h. (c) Soaking time at RT: 6 h. (d) Soaking time at RT: 6 h. (e) Soaking time at RT: 6 h. (f) Surface of Ti6Al4V strip coated in 1.5 SBF for 2 weeks at 37 °C.

not met in our study. The SEM micrograph given in Fig. 3(f) 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., 1.5 × *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 h at RT. High-magnification photomicrographs of Figs. 3(c) and 3(d) showed that the globules actually consisted of petal-like nanoclusters. The significant extent of surface coverage of the Ti6Al4V strip, in only 6 h of coating, was exemplified in the macro-scale SEM picture depicted in Fig. 3(e).

The adhesion strength of the coat layers was found to be 12 ± 2 MPa, and there was virtually no difference in adhesion strength as compared to 1.5 × SBF-coated Ti6Al4V strips. Kokubo et al.[22] previously reported the adhesive strengths of "bonelike apatite layers formed (by immersion in conventional SBFs) on chemically treated titanium metals to range from 9.8 to 11.5 MPa."

XRD data of the coated strips also confirmed the apatitic nature of these globules, as shown in Fig. 4. The intermingling morphology of the colloidal precipitates obtained from a coating solution at the end of 2 h is shown in Fig. 5. These precipitates were filtered from their mother liquor by centrifugation, washed three times with water, and once with ethanol, followed by drying at RT overnight. The SEM micrograph of Fig. 5, and the XRD data of these precipitates given in Fig. 4, as well as the FTIR data supplied in Fig. 6(a), also indicated that the nanocrystalline apatitic calcium phosphates formed in the solution aggregated during filtration and drying. Bands of the O–H stretching and bending of H_2O were seen at 3440 and 1649 cm^{-1}, respectively. 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 needs only 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 a SBF

formulation simply retards the coating process to the level that requires one to wait 1 or 3 weeks to obtain a decent surface coverage.[6–8,24]

Fast coating solutions, sometimes called 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 after 16 h of immersion. More recently, Choi et al.[32] reported the room-temperature coating (about 10 μm thick in 24 h) 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.

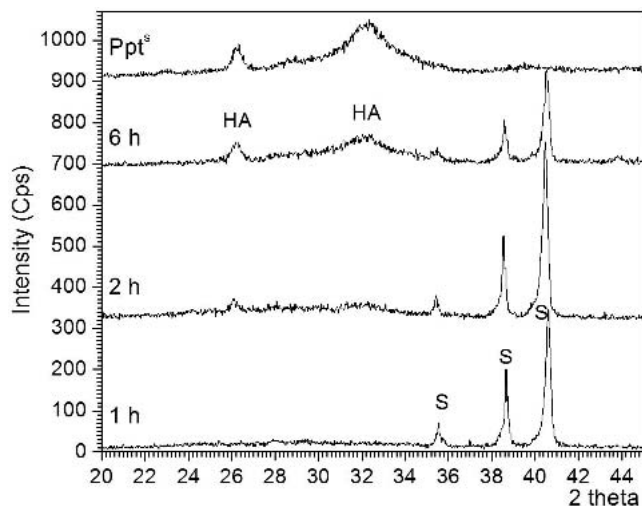It is known that an amorphous calcium phosphate

FIG. 4. XRD data of the coated Ti6Al4V strips as a function of immersion time (S: peaks of substrate) and those of colloidal precipitates
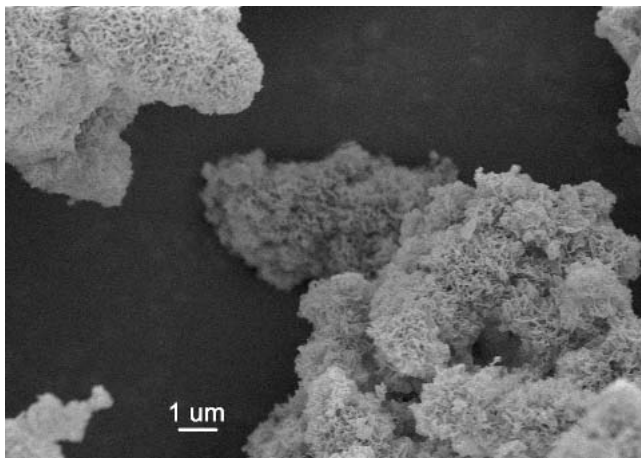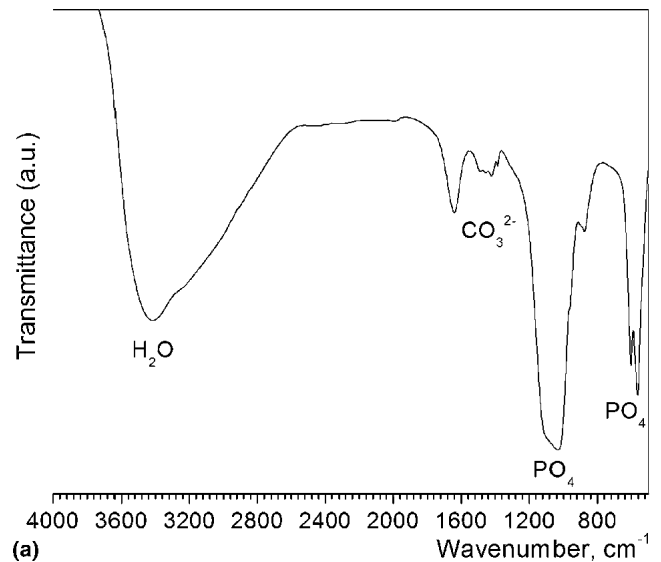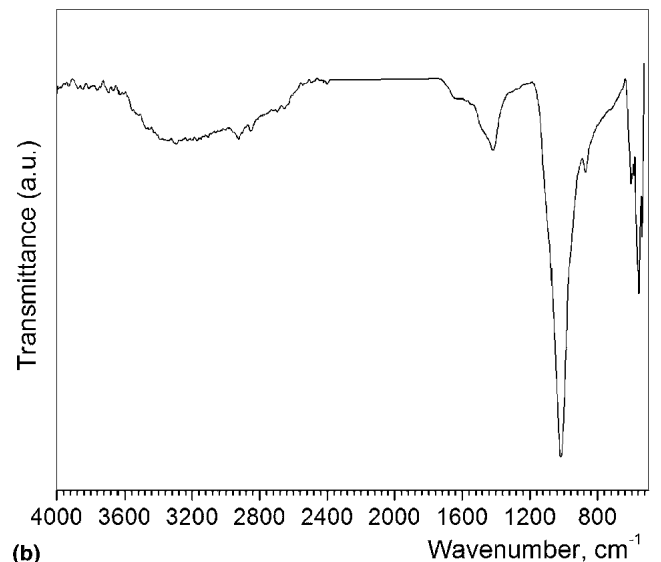

FIG. 5. Apatitic precipitates recovered from the coating solutions.

FIG. 6. (a) FTIR data of the colloidal precipitates formed in coating solutions. (b) FTIR data of the coated (6 h) Ti6Al4V strip

(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. 2(d) and 4], with the intercluster space filled with water. Such clusters (with a diameter of about 9.5 Å[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]

The 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 ± 0.3%). This means that the deposited material consists of "carbonated, calcium-deficient, poorly crystallized hydroxyapatite." This is how de Groot 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 5–10 min, 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 undercoat layer is formed [Figs. 3(a), 3(b), and 3(f)] 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 the 2nd or 3rd day of soaking at 37 °C. Since the Ca/P molar ratio of all SBF solutions (including the one presented here) is 2.50, they are not stable against hydroxyapatite precipitation when the solution pH is higher than 6.4.[10,11]

IV. CONCLUSIONS

The use of $NaHCO_3$ with a concentrated (10 times that of Ca^{2+} and HPO_4^{2-} ion concentrations) 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 2–6 h. The coating solutions of pH 6.5 did not necessitate the use of buffering agents. The pH adjustment was achieved by a single addition of $NaHCO_3$. The coating process did not necessitate 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 10 × SBF. KOH soaking and thermal treatment following it ensured the formation of potassium titanates on the strip surfaces. Bonding strength of the present bonelike coatings (with a Ca/P molar ratio of 1.57 and 8 wt% CO_3^{2-}) was found to be 12 ± 2 MPa, which was comparable to that obtained in 1.5 SBF, i.e., so-called biomimetic coatings. Formation of colloidal precipitates within the solution was observed during the first hour of soaking at RT, but apparently the presence of those fine precipitates did not hinder the coating process.

REFERENCES

1. T. Kokubo: Surface chemistry of bioactive glass-ceramics. *J. Non-Cryst. Solids* **120,** 138 (1990).
2. T. Kokubo: Apatite formation on surfaces of ceramics, metals and polymers in body environment. *Acta Mater.* **46,** 2519 (1998).
3. T. Kokubo, H.M. Kim, M. Kawashita, and T. Nakamura: Bioactive metals: Preparation and properties. *J. Mater. Sci. Mater. Med.* **15,** 99 (2004).
4. J.H. Hanks and R.E. Wallace: Relation of oxygen and temperature in the preservation of tissues by refrigeration. *Proc. Soc. Exp. Biol. Med.* **71,** 196 (1949).
5. L. Frauchiger, M. Taborelli, B.O. Aronsson, and P. Descouts: Ion

adsorption on titanium surfaces exposed to a physiological solution. *Appl. Surf. Sci.* **143,** 67 (1999).

6. H.M. Kim, H. Takadama, F. Miyaji, T. Kokubo, S. Nishiguchi, and T. Nakamura: Formation of bioactive functionally graded structure on Ti-6Al-4V alloy by chemical surface treatment. *J. Mater. Sci. Mater. Med.* **11,** 555 (2000).

7. A. Oyane, K. Onuma, A. Ito, H.M. Kim, T. Kokubo, and T. Nakamura: Formation and growth of clusters in conventional and new kinds of simulated body fluids. *J. Biomed. Mater. Res.* **64A,** 339 (2003).

8. A.P. Serro and B. Saramago: Influence of sterilization on the mineralization of titanium implants induced by incubation in various biological model fluids. *Biomaterials* **24,** 4749 (2003).

9. E.I. Dorozhkina and S.V. Dorozhkin: Surface mineralisation of hydroxyapatite in modified simulated body fluid (mSBF) with higher amounts of hydrogencarbonate ions. *Colloids Surf. A* **210,** 41 (2002).

10. A.C. Tas: Synthesis of biomimetic Ca-hydroxyapatite powders at 37°C in synthetic body fluids. *Biomaterials* **21,** 1429 (2000).

11. E.I. Dorozhkina and S.V. Dorozhkin: Structure and properties of the precipitates formed from condensed solutions of the revised simulated body fluid. *J. Biomed. Mater. Res.* **67A,** 578 (2003).

12. H. Takadama, H.M. Kim, T. Kokubo, and T. Nakamura: TEM-EDX study of mechanism of bonelike apatite formation on bioactive titanium metal in simulated body fluid. *J. Biomed. Mater. Res.* **57,** 441 (2001).

13. P. Habibovic, F. Barrere, C.A. van Blitterswijk, K. de Groot, and P. Layrolle: Biomimetic hydroxyapatite coating on metal implants. *J. Am. Ceram. Soc.* **85,** 517 (2002).

14. F. Barrere, C.A. van Blitterswijk, K. de Groot, and P. Layrolle: Influence of ionic strength and carbonate on the Ca-P coating formation from SBF x 5 solution. *Biomaterials* **23,** 1921 (2002).

15. F. Barrere, C.A. van Blitterswijk, K. de Groot, and P. Layrolle: Nucleation of biomimetic Ca-P coatings on Ti6Al4V from SBF x 5 solution: Influence of magnesium. *Biomaterials* **23,** 2211 (2002).

16. F. Barrere, C.M. van der Valk, R.A.J. Dalmeijer, C.A. van Blitterswijk, K. de Groot, and P. Layrolle: In vitro and in vivo degradation of biomimetic octacalcium phosphate and carbonate apatite coatings on titanium implants. *J. Biomed. Mater. Res.* **64A,** 378 (2003).

17. F. Barrere, C.M. van der Valk, G. Meijer, R.A.J. Dalmeijer, K. de Groot, and P. Layrolle: Osteointegration of biomimetic apatite coating applied onto dense and porous metal implants in femurs of goats. *J. Biomed. Mater. Res.* **67B,** 655 (2003).

18. S.V. Dorozhkin, E.I. Dorozhkina, and M. Epple: A model system to provide good in vitro simulation of biological mineralization. *Cryst. Growth Design* **4,** 389 (2004).

19. M.C. Dalconi, C. Meneghini, S. Nuzzo, R. Wenk, and S. Mobilio: Structure of bioapatite in human foetal bones: An x-ray diffraction study. *Nucl. Instrum. Meth. B* **200,** 406 (2003).

20. R.Z. LeGeros: *Calcium Phosphates in Oral Biology and Medicine* (Karger Publications, Basel, Switzerland, 1991), pp. 4–44; 108–114.

21. Designation C-633. Standard test method for adhesion strength of flame-sprayed coatings, *Annual Book of ASTM Standards,* Vol. 3.01 (American Society for Testing and Materials, Philadelphia, PA, 1993), pp. 665–669.

22. T. Kokubo, F. Miyaji, H.M. Kim, and T. Nakamura: Spontaneous formation of bonelike apatite layer on chemically treated titanium metals. *J. Am. Ceram. Soc.* **79,** 1127 (1996).

23. L. Jonasova, F.A. Mueller, A. Helebrant, J. Strnad, and P. Greil: Biomimetic apatite formation on chemically treated titanium. *Biomaterials* **25,** 1187 (2004).

24. H.M. Kim, F. Miyaji, T. Kokubo, and T. Nakamura: Effect of heat treatment on apatite-forming ability of Ti metal induced by alkali treatment. *J. Mater. Sci. Mater. Med.* **8,** 341 (1997).

25. N. Masaki, S. Uchida, H. Yamane, and T. Sato: Characterization of a new potassium titanate, $KTiO_2(OH)$ synthesized via hydrothermal method. *Chem. Mater.* **14,** 419 (2002).

26. Z.Y. Yuan, X.B. Zhang, and B.L. Su: Moderate hydrothermal synthesis of potassium titanate nanowires. *Appl. Phys. A* **78,** 1063 (2004).

27. P.A.A.P. Marques, M.C.F. Magalhaes, and R.N. Correia: Inorganic plasma with physiological CO_2/HCO_3^- buffer. *Biomaterials* **24,** 1541 (2003).

28. H. Takadama, M. Hashimoto, M. Mizuno, K. Ishikawa, and T. Kokubo: Newly improved simulated body fluid. *Key Eng. Mater.* **254-256,** 115 (2004).

29. P. Layrolle, K. de Groot, J.D. de Bruijn, C.A. van Blitterswijk, and Y. Huipin: Method for coating medical implants. U.S. Patent No: 6 207 218 (March 27, 2001).

30. G. Vereecke and J. Lemaitre: Calculation of the solubility diagrams in the system $Ca(OH)_2$-H_3PO_4-KOH-HNO_3-CO_2-H_2O. *J. Cryst. Growth* **104,** 820 (1990).

31. H.B. Wen, J.G.C. Wolke, J.R. de Wijn, Q. Liu, F.Z. Cui, and K. de Groot: Fast precipitation of calcium phosphate layers on titanium induced by simple chemical treatments. *Biomaterials* **18,** 1471 (1997).

32. J. Choi, D. Bogdanski, M. Koeller, S.A. Esenwein, D. Mueller, G. Muhr, and M. Epple: Calcium phosphate coating of nickel-titanium shape memory alloys. Coating procedure and adherence of leukocytes and platelets. *Biomaterials* **24,** 3689 (2003).

33. X. Yin and M.J. Stott: Biological calcium phosphates and Posner's cluster. *J. Chem. Phys.* **118,** 3717 (2003).

34. A.S. Posner and F. Betts: Synthetic amorphous calcium phosphate and its relation to bone mineral structure. *Acc. Chem. Res.* **8,** 273 (1975).

35. P.J. Li, I. Kangasniemi, K. de Groot, and T. Kokubo: Bonelike hydroxyapatite induction by a gel-derived titania on a titanium substrate. *J. Am. Ceram. Soc.* **77,** 1307 (1994).

36. K. Onuma and A. Ito: Cluster growth model for hydroxyapatite. *Chem. Mater.* **10,** 3346 (1998).