

A new approach in biomimetic synthesis of calcium phosphate coatings using lactic acid–Na lactate buffered body fluid solution

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ABSTRACT

The main objective of this study was to investigate calcium phosphate (CaP) coatings on Ti6Al4V substrates by using the biomimetic technique. To this purpose, a new solution was developed to coat CaP on Ti6Al4V alloy substrates. The newly formulated body fluid (*Lac*-SBF) contained appropriate amounts of sodium lactate (NaL) and lactic acid (HL), as well as all the other ionic constituents of the human blood plasma. The inorganic ion concentrations of the *Lac*-SBF solutions were identical with those of human blood plasma. The new *Lac*-SBF solution of this study eliminated the need for using Tris/HCl or Hepes/NaOH buffers. Prior to coating, Ti6Al4V substrates were chemically treated in NaOH and/or NaOH + H₂O₂ solutions as an alternative route and then heated at 600 °C for 1 h in air. In the previous applications, the Cl[−] ion concentration was found to be higher than blood plasma 103 mM, which exists in human blood plasma as a result of Tris/HCl which are used to prevent precipitation and to keep the pH level at certain values. In this study, instead of using Tris/HCl, HL/NaL which are generated by human body and do not show any toxic behavior, are used and Cl[−] concentration was kept at 103 mM value for the first time. The prepared *Lac*-SBF was shown to have similar concentration to human blood plasma in terms of all inorganic ions for the first time. Solution properties were evaluated by using turbidimeter, pH meter and rheometer. The coatings were characterized using Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and a scratch tester. The obtained results are presented and discussed.

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1. Introduction

Titanium alloy (Ti6Al4V) is a material which is commonly used in the manufacturing of orthopedic implants such as hip prostheses, bone plates, and bone screws. The excellent mechanical properties and inert nature of this material made it desirable for restoring the structural integrity of hard tissues that have undergone physical or pathogen-related trauma. The non-corrosive nature of titanium has many biological benefits. Commercially pure titanium and Ti6Al4V do not induce a negative immune response from the body and exhibit very low incidences of toxicity and rejection. Nevertheless, this same lack of interaction with the biological environment partially prevents the implant from integrating with the surrounding hard tissues [1].

Many methods, such as physical machining and controlled oxidation, have been used to improve the in vivo osseointegration of titanium-based implants [2,3]. More recently, calcium phosphate

(CaP) coatings, such as hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂), have been used on orthopedic implants [4–6]. The addition of this bio-ceramic to the surface of oxidized metallic implants has been shown to dramatically enhance the hard tissue integration, thereby increasing the in situ mechanical stabilization [7]. This hard tissue integration and stabilization would lead to quicker patient recovery times and extended life for orthopedic implants [2].

In the so-called biomimetic technique, the apatite-like CaP coating is achieved by placing the substrates to be coated into a simple biocompatible aqueous medium kept at the body temperature of 37 °C and at the blood pH of 7.4. The calcification solutions (usually called as SBF) allow the in situ chemical precipitation from a metastable supersaturated solution similar in ion concentrations similar to those of human blood plasma [5,6]. These solutions typically have a Ca/P molar ratio of 2.50, and, therefore, are supersaturated with respect to the apatite (whose Ca/P ratio is only 1.667) nucleation. The chemical precipitation is triggered by the OH[−] ions present on the surfaces of the immersed substrates. The incorporation of OH[−] ions onto the metallic substrates are made possible by aging those in a concentrated NaOH [5] or KOH [8] solution at

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temperatures between 60 and 90 °C, just prior to the SBF soaking. The biomimetic coating method was shown to elicit film thicknesses of over 15 μm in about 5 days, with apatite-to-substrate adhesion strengths varying over the range of 10–30 MPa [4].

The solutions commonly used for this type of coating are named as simulated or synthetic body fluids, which mimic the composition of blood plasma with respect to inorganic ions. Kokubo-SBF having a HCO₃⁻ concentration of 4.2 mM [5] and Tas-SBF of 27 mM HCO₃⁻ [9] are the two commonly known Tris-buffered SBFs. The pH values of these solutions were adjusted to 7.4 by using the Tris/HCl pair. The description of such calcification or SBF solutions as “metastable supersaturated solutions” was explained by Combes et al. [10].

Tas and Bhaduri [8] had previously shown that it was possible to coat Ti6Al4V substrates at room temperature with an apatite-like CaP layer much more rapidly (in few hours) by using a solution having ten times the concentrations of Ca²⁺ and HPO₄²⁻ ions than present in a typical SBF solution, also without a need for the presence of a buffering agent, such as Tris or Hepes. Later, Bigi et al. [6] also demonstrated a supersaturated calcium phosphate solution, but buffered with Hepes, to maintain the pH in the vicinity of physiological levels.

In this study, we exemplified a new approach in preparing solutions similar to body fluids, and sodium lactate (NaL) and lactic acid (HL) were used to adjust the pH at the physiological level, without using Tris or Hepes.

Lactic acid was used instead of Tris. It is important to note that while lactic acid is present in the human body, Tris or Hepes are not. We used high-purity starting chemicals of CaCl₂·2H₂O, MgCl₂·6H₂O, KCl, NaCl, Na₂HPO₄·2H₂O, Na₂SO₄, NaHCO₃, sodium lactate, and lactic acid in preparing these solutions in distilled water. The resultant *Lac*-SBF solutions precisely matched all of the ion concentrations of the human blood plasma. CaP coatings were formed on Ti6Al4V substrates by using these novel *Lac*-SBF solutions.

2. Materials and methods

Chemical composition of the Ti implant alloy (i.e., Ti6Al4V) used in this study is given in Table 1. Ti6Al4V specimens with the dimensions of 10 × 10 × 1.2 mm were first abraded with a #320 SiC paper and then cleaned with acetone and distilled water. Both NaOH and NaOH + H₂O₂ solutions were used for the chemical etching process to compare their merits after the biomimetic coating. One half of the total substrates were chemically etched in 5 M NaOH aqueous solution at 60 °C for 24 h. After adding an appropriate amount of H₂O₂ solution to a 5 M NaOH solution, the other half of the substrates was chemically treated at 60 °C for 24 h in the NaOH + H₂O₂ solution. All the substrates were thoroughly washed with an ample supply of distilled water, followed by drying at 40 °C for 24 h in air. The etched substrates were finally heat-treated at 600 °C in a microprocessor-controlled furnace with heating rates

of 5 °C min⁻¹, soaked at 600 °C for 1 h and were then cooled slowly in the furnace down to room temperature.

Lac-SBF was prepared by using the salt amounts given in Table 2. It must be noted that the recipe of Table 2 was for preparing 2.5 × *Lac*-SBF solutions in a total volume of 2500 ml. In preparing the 2.5 × *Lac*-SBF solutions, only the Ca²⁺ and HPO₄²⁻ ion concentrations were multiplied by a factor of 2.5, with respect to those of human blood plasma. Following the addition of CaCl₂·2H₂O and MgCl₂·6H₂O into the solution, a total volume of 40 ml of 1 M HL was periodically added into the solution to prevent precipitation. The ion concentrations of the *Lac*-SBF solutions were compared with those of Tris-buffered SBFs in Table 3. The ion concentrations of 1 × *Lac*-SBF solutions, on the other hand, were perfectly identical with those of human blood plasma.

Before biomimetic synthesis, the sterilization of the substrates was performed in ethylene oxide. The substrates to be coated were placed in glass containers into a constant-temperature water bath (maintained at 37 °C) and the coating time was 48 h at 37 °C. 2.5 × *Lac*-SBF solution was fed to the sample containers by a peristaltic pump at the feed rate of 4 ml h⁻¹, meaning that the solution was replenished at the stated rate during the entire biomimetic coating period of 48 h at 37 °C. At the end of 48 h, the coated samples were taken out of the solution and were washed with distilled water. Finally, the samples were dried at 30 °C for 4 h in air.

Turbidity, pH value, and viscosity of the prepared solutions were measured. Turbidity properties of the fresh solutions were also measured, to use as standard solutions for the coating process, by using a TB-1 Velp Scientifica Model turbidimeter by observing the standard ISO 7027-nephelometric method. The sample was placed in a vessel with a diameter of 25 mm and a height of 50 mm. Formazin was the primary standard, and formazin solution was used to calibrate the turbidity measurement results. Measurement window was taken over the range of 0 and 1000 ntu (nephelometric turbidity unit).

After the preparation of transparent solutions, pH values were measured by using a standard pH meter (WTW Inolab, Germany). The viscosities of the solutions were measured by using a CVO 100 Digital Rheometer (Bohlin Instrument). The ionic strength of the solutions was calculated by using the equation:

$$I = \frac{1}{2} \sum_{i=1}^n c_i z_i^2 \quad (1)$$

where c_i is the molarity of ion i , z_i is the charge of that ion, and the sum was taken over all the ions present in the solution. The ionic strength was thus calculated to be 173.25 mM for the 2.5 × *Lac*-SBF solution.

FTIR (Perkin–Elmer) absorption spectra of the CaP samples were measured over the range of 4000–400 cm⁻¹. XRD patterns of the CaP coatings were gathered to identify the present phases with a Rigaku (D/MAX-2200/PC) diffractometer by using Cu K α radiation. The surface topographies of the CaP coatings were examined by using SEM (JEOL-JSM 6060). Surface roughness and the thickness

Table 1
Chemical composition of Ti alloy substrate.

Element	(wt.%)
N	0.0030
C	0.0050
H	<0.0005
Fe	0.1000
O	0.0900
Al	6.2100
V	3.8700
Y	<0.0010
Others	<0.3000
Ti	Balance

Table 2
Preparation of 2.5 × *Lac*-SBF (total volume = 2.5 l).

Reagents	Amount (g)
CaCl ₂ ·2H ₂ O	2.2973
MgCl ₂ ·6H ₂ O	0.7625
KCl	0.9325
NaCl	12.0533
Na ₂ HPO ₄ ·2H ₂ O	1.1125
Na ₂ SO ₄	0.1775
NaHCO ₃	5.6708
Na-lactate (70–72%, d: 1.375–1.385)	10.4573
Lactic acid (1 M)	40.0 (ml)

Table 3
Comparative ion concentrations of Tris-SBFs and the *Lac*-SBF of this study.

Ion	Kokubo-SBF ⁵ (mM)	Tas-SBF ⁶ (mM)	<i>Lac</i> -SBF _x 1 (mM)	<i>Lac</i> -SBF ×2.5 (mM)	Blood plasma	
					(mM)	meq l ⁻¹
Na ⁺	142.0	142.0	142.0	142.0	142.0	142.0
Cl ⁻	147.8	125.0	103.0	103.0	103.0	103.0
HCO ₃ ⁻	4.2	27.0	27.0	27.0	27.0	27.0
K ⁺	5.0	5.0	5.0	5.0	5.0	5.0
Mg ²⁺	1.5	1.5	1.5	1.5	1.5	3.0
Ca ²⁺	2.5	2.5	2.5	6.25	2.5	5.0
HPO ₄ ²⁻	1.0	1.0	1.0	2.5	1.0	2.0
SO ₄ ²⁻	0.5	0.5	0.5	0.5	0.5	1.0
Na-lactate	–	–	22	26.5	(+) ion: (155 meq l ⁻¹ or 162.5 meq l ⁻¹) (–) ion:	
Lactic acid (1 M)	–	–	36 mL	40 mL	(133 meq l ⁻¹ + 22 meq l ⁻¹ organic anions) or	
Tris	50	50	–	–	(136 meq l ⁻¹ + 26.5 meq l ⁻¹ organic anions)	
Ionic str. (mM)	–	–	160.5	173.25	–	–

of the coatings were evaluated by a standard surface roughness machine (TIME, TimeSurf For TR200 V1.0) and the SEM.

Adhesion strength of CaP coatings was determined by using a SHIMADZU scanning scratch tester (Model SST-W101). Scratch test was carried out to determine and analyze the adhesion strength of the CaP coatings to the substrates. In the test, a diamond stylus was scratched over the coated surface with a constant speed under progressing normal load until a critical force is reached, at which adhesion failure was detected. This critical load was used as a measure for the adhesion between coating and the substrate. In this study, adhesion strength of coatings was evaluated by using a scanning scratch tester with a 15 μm tip radius diamond stylus. The applied load was 98 mN for all the samples. During the test, a stylus was drawn on the coating surface with a sliding speed of 5 μm s⁻¹ keeping scanning amplitude of 10 μm which was perpendicular to the scratching direction. Load was progressively exerted on the stylus with a loading speed of 2 μm s⁻¹. Friction on the stylus increased with increasing load, which caused a delay in movement between the cartridge body and stylus. This delay was defined as the cartridge output, and reported as such.

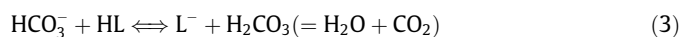
3. Results and discussion

Turbidimeter is an instrument for measuring the loss in intensity of a light beam shined through a solution that contains suspended particulate matter. Turbidimetric or nephelometric measurements were used to confirm the complete dissolution of powder-based salts in the perfectly transparent solutions. The turbidity values of the *Lac*-SBF solutions were given in Table 4. Their turbidity values before and after the coating process were, respectively, found to be 3.315 and 0.928 ntu, indicating that the salts of the starting chemicals were completely dissolved in the solutions. However, the difference between those two solutions (i.e., before and after coating) was mainly due to the consumption of aqueous Ca²⁺ and HPO₄²⁻ ions during the coating process. On the other hand, in more general terms, it can be expressed that no significant change in turbidity values were observed.

Oyane et al. [11] have demonstrated, by using dynamic light scattering, the presence of CaP clusters from 0.7 to 1.0 nm in size

even in transparent SBF solutions. They reported that CaP clusters were present in SBF even when there was no precipitation. Posner et al. [12] proposed that the process of CaP formation in solution involved the formation first of Ca₉(PO₄)₆ clusters, which then aggregated randomly to produce the larger spherical particles or globules as observed in the biomimetic SBF coatings, with the inter-cluster space filled with water. Such clusters (with a diameter of about 9.5 Å [13]) were the transient solution precursors to the formation of carbonated globules with the stoichiometry of a calcium-deficient hydroxyapatite, namely, Ca_{10-x}(HPO₄)_x(PO₄)_{6-x}(OH)_{2-x}, where *x* might be converging to one [3]. Turbidity measurements were indicating to the presence of Posner's clusters in the *Lac*-SBF solutions prior to their use in biomimetic coating.

The pH value of such supersaturated solutions is an important factor influencing the solubility, stability, and the nucleation-growth of apatite-like CaP phases, and this should be taken into account in preparing solutions. The pH value of the *Lac*-SBF solution should be adjusted to approximately 7 because precipitation easily forms in the solution when pH value increases [14]. During the coating of CaP on Ti6Al4V substrates by the biomimetic technique at 37 °C, the pH value of the transparent solution increased from 7 to around 8.9. Generally speaking, the cause of this pH increase could be explained by the reactions of the HPO₄²⁻ and HCO₃⁻ ions in the solution with lactic acid, as seen in the following equation:



Initially, HPO₄²⁻ and HCO₃⁻ were converted to about 39% H₂PO₄⁻ and 76.5% CO₂, respectively. Since HCO₃⁻ is soluble in water, CO₂ easily formed and escaped off of the solution during the process. Due to the fact that the pH value increases slightly at the end of the process, some Ca ions in the solution approach to Ti–O–Na, Al–O–Na groups which are formed on substrate surface during chemical cleaning of Ti and Al, are replaced with Na ions, and enhance the deposition of Ca–P on the surface of substrate by bonding on activated metal such as (Al–O–CaCa₄(PO₄)₃), (Ti–O–CaCa₄(PO₄)₃). Additionally, replacement of H⁺ ions of the water molecule with Na⁺ ions increases alkalinity of the solution because Na ions have weak bonds to Ti–O–Na and Al–O–Na groups. This situation is explained by the increase of pH values of the solution. More specifically, these chemical reactions are shown as:

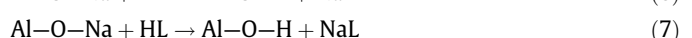
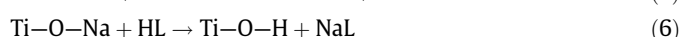
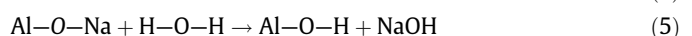
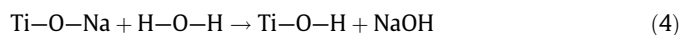


Table 4
Turbidity of solutions before and after coating process.

Solutions	Turbidity
Before	3.315
After coating	0.928

Increase of pH value after this reaction provides faster Ca–P coating on the surface of the substrate. Because of this reason, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ starts to form at pH 7 and then $\text{Ca}_3(\text{PO}_4)_2$ and $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ phases form when pH increases.

Fig. 1 denotes the viscosities and shear stresses of solutions before and after the coating process. The data of Fig. 1 illustrate the viscosities and shear stresses measured as a function of increasing test time. Viscosities of the solutions, at 400 Hz, before and after the coating process were found to be 25 Pa s and 35 Pa s, respectively (Fig. 1a). The shear stresses of the solutions, at 400 Hz, before and after the coating process, were measured to be 10,000 Pa and 14,000 Pa, respectively. It should be noted that the viscosity and shear stress values of the solutions were different from one another since the Ca^{2+} and HPO_4^{2-} ions were depleted from the solutions. On account of the fact that the thin films were formed by the Ca^{2+} and HPO_4^{2-} ions-based solutions having high concentrations, these results were reasonable for the biomimetic technique. In addition to these, some of CaP-based phases leaving from thin films on the substrate's surface in the solutions during biomimetic synthesis. When the process finishes, solution mixture contains CaP particles. Because of this reason, viscosity and shear stress of the solutions increase after the biomimetic process.

The FTIR spectra of the biomimetic coatings formed after 2 days in SBF2.5 are summarized in Fig. 2. A broad absorption band at 3450 cm^{-1} and the bending mode at 1650 cm^{-1} originating from H_2O were observed in the spectra of precipitated coatings. The small peaks between 3600 and 3000 cm^{-1} indicate stretching frequencies of OH group. Of these, the peaks at

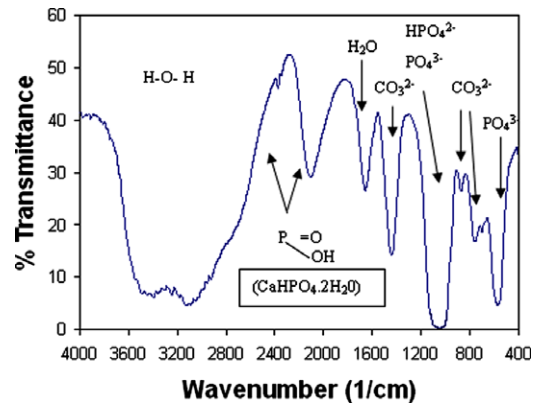


Fig. 2. FTIR analysis of biomimetic coatings formed on chemically pretreated Ti6Al4V after soaking in $2.5 \times \text{Lac-SBF}$ for 2 days.

$3600\text{--}3400\text{ cm}^{-1}$ band show intermolecular bands and the peaks at $3200\text{--}3000\text{ cm}^{-1}$ denote the intramolecular bands. The 2700 and 2080 cm^{-1} bands belong to $\text{PO}(\text{OH})$ and POH bands in $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ structure. Here Ca is bonded with P in different configurations such as $\text{PO}(\text{OH})$ and POH groups. $\text{PO}(\text{OH})$ bonds are determined at $2700\text{--}2560\text{ cm}^{-1}$ and $2350\text{--}2080\text{ cm}^{-1}$. POH bond is found at 2400 cm^{-1} . The bands detected at $1490\text{--}1410\text{ cm}^{-1}$, $900\text{--}860\text{ cm}^{-1}$ and $710\text{--}690\text{ cm}^{-1}$ were assigned to the CO_3^{2-} group of carbonated apatite and brushite where PO_4^{3-} groups are substituted by CO_3^{2-} . Broad phosphate stretching modes from the P–O asymmetric stretching mode of the PO_4^{3-} and HPO_4^{3-} groups were found in the region from 1100 to 1000 cm^{-1} indicating a deviation of the phosphate ions from their ideal tetrahedral structure. Also PO_4^{3-} is determined at $550\text{--}500\text{ cm}^{-1}$ band. The triply and doubly degenerated bending modes of phosphates O–P–O bonds were found at 604 , 567 , and 474 cm^{-1} . Additionally, the absence of the usually sharp vibration band derived from hydroxyl ions at $3500\text{--}3700\text{ cm}^{-1}$ probably indicates the substitution of hydroxyl ions by carbonate ions in the apatite and brushite structures.

Fig. 3 depicts the XRD traces of CaP coatings obtained on the Ti6Al4V alloy substrates. $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (apatite, PDF#01-084-1998), $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (brushite, PDF#01-072-0713), titanium (Ti,

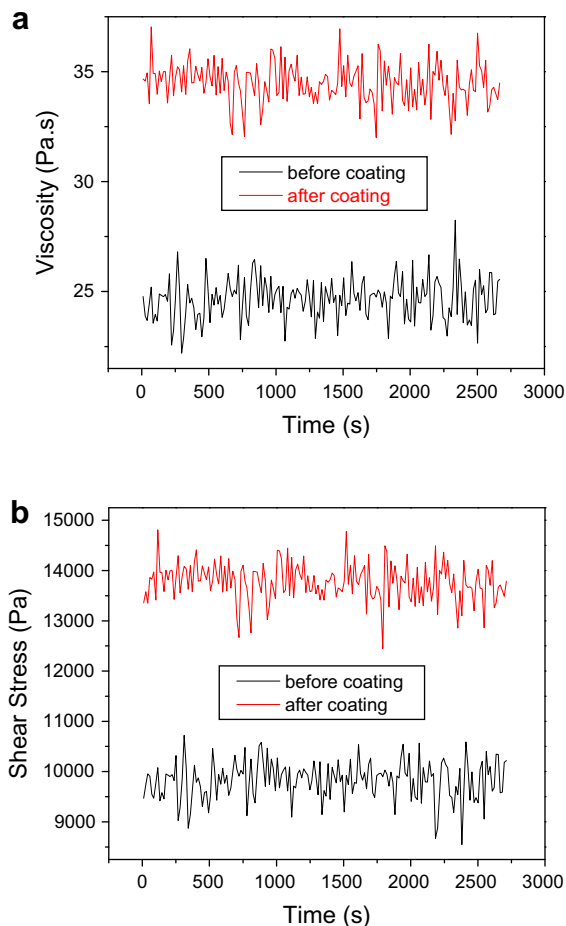


Fig. 1. (a) Viscosities and (b) shear stresses of Lac-SBF solutions before and after coating process.

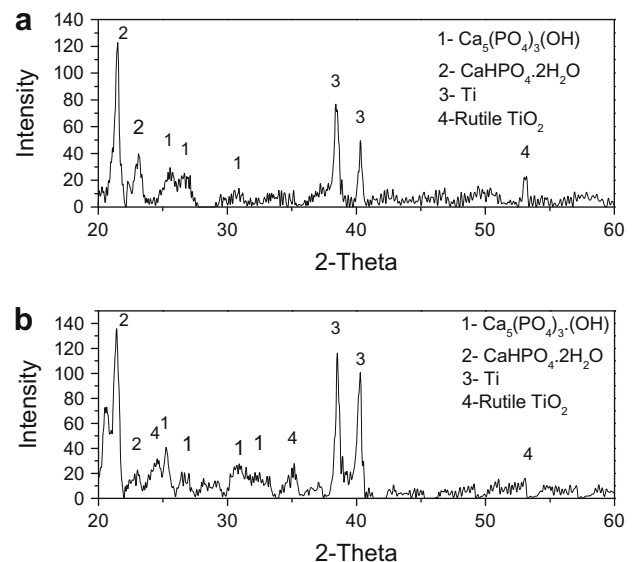
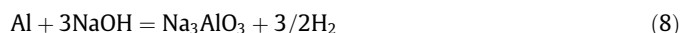


Fig. 3. XRD patterns of CaP coatings on Ti6Al4V alloy substrates pretreated in (a) NaOH and (b) $\text{NaOH} + \text{H}_2\text{O}_2$ solutions.

PDF#00-044-1294), and rutile (TiO_2 , PDF#00-021-1276) phases were detected on the coatings obtained on Ti6Al4V substrates which were chemically treated in NaOH and NaOH + H_2O_2 . It is clearly seen from Fig. 3 that apatite peaks generally were found at 2θ of 25.90° , 28.15° , 31.79° , and 32.90° corresponding to (0 0 2), (1 0 2), (1 2 1), and (3 0 0) orientations for the both samples. Brushite was the major crystalline phase. Small and broad brushite peaks were obtained at 2θ of 20.95° and 23.40° corresponding to (1 2 -1) and (0 4 0) orientations for the both samples. It was also obvious from Fig. 3 that the Ti peaks obtained at the 2θ value of 38.47° and 40.26° corresponded to the (0 0 2) and (0 1 1) planes. The rutile TiO_2 phase was formed on the Ti6Al4V substrates during their heat treatment at 600°C . It is believed that the TiO_2 phases were formed between CaP coating and the substrate after heat treatment process.

The SEM photomicrographs given in Figs. 4 and 5 show the CaP coating morphology obtained by using the $2.5\times$ Lac-SBF solutions of this study on Ti6Al4V substrates treated in NaOH + H_2O_2 and NaOH, respectively. The coating morphology depicted in Figs. 4 and 5 were almost identical to those obtained by using the more common Tris-SBF solutions of the previous literature [5,9]. The cracks visible in the SEM photomicrographs (Figs. 4 and 5) were formed during the drying process, owing to the thermal expansion difference between the Ti6Al4V substrates and the coat layer on those. Jalota et al. [15] have suggested a remedy for the elimination of such cracks from the SBF-based coatings on titanium alloy substrates. Fig. 6 demonstrates EDS analysis of the CaP coatings

on Ti6Al4V substrates pretreated in NaOH and NaOH + H_2O_2 solutions. Ca/P ratios of the CaP coatings pretreated in NaOH and NaOH + H_2O_2 solutions were calculated to be 1.26. This value is reasonable because Ca/P ratios of $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ and $\text{CaHPO}_4\cdot 2\text{H}_2\text{O}$ were 1.67 and 1, respectively. Total ratio is approximately 1.5. A relationship between NaOH and NaOH + H_2O_2 etching solutions can be clarified using EDS analysis. To illustrate this, an interaction between Al and etching solutions can be explained. According to Reaction (8), while Al metal in the TiAl6V4 substrate dissolves during the etching process with NaOH, H_2 gas covers surface of the substrate:



This gas prevents the effect of NaOH, in depth, on the substrate. In this case, dissolution of Al content is prohibited on the surface of the substrate.

In Reaction (9), the effect of NaOH + H_2O_2 can be easily explained:



According to this reaction, Al dissolves from the substrate because of H_2O_2 . NaOH effect can be seen and NaOH descends to pits of the substrate. Due to this reason, size of pits and surface roughness increases on the substrate. As a result of NaOH + H_2O_2 effect, Al ratio decreases in the substrate. These effects can be seen in EDS results.

The coating thickness and surface roughness values were reported in Table 5. $2.5\times$ Lac-SBF solutions were able to coat a

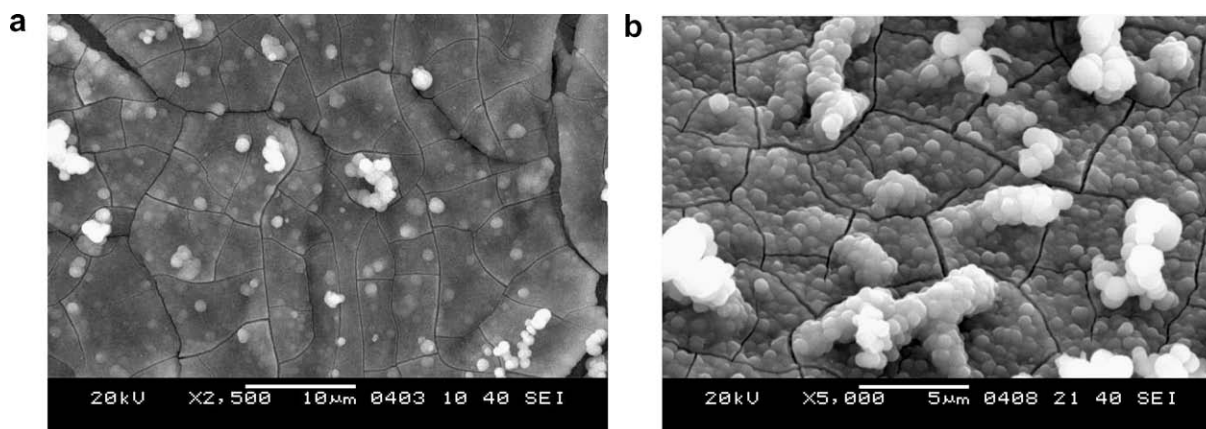


Fig. 4. SEM micrographs of the CaP coatings on Ti6Al4V substrates produced using the $2.5\times$ Lac-SBF solutions. The substrates were pretreated in NaOH + H_2O_2 solutions prior to coating process. The scale bars are (a) $10\ \mu\text{m}$ and (b) $5\ \mu\text{m}$.

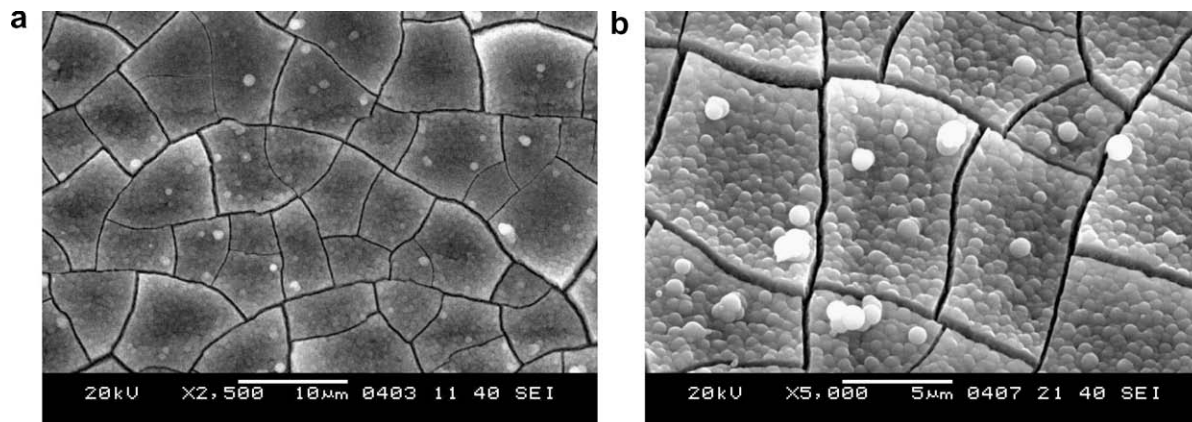


Fig. 5. Surface morphologies of the CaP coatings on Ti6Al4V substrates produced using the $2.5\times$ Lac-SBF solutions. The substrates were pretreated in NaOH solutions prior to coating process. The scale bars are (a) $10\ \mu\text{m}$ and (b) $5\ \mu\text{m}$.

4 μm -thick layer in 48 h on the Ti6Al4V substrates of this study. The surface roughness values of CaP coatings on the substrate pretreated with NaOH and NaOH + H₂O₂ are 0.140 μm and 0.246 μm , respectively. When Ti6Al4V substrates which were polished to remove their surface oxide layer were soaked in 2.5 \times Lac-SBF solution, a dense layer of CaP was formed on the available surfaces. The CaP nuclei grew spontaneously by consuming the calcium and phosphate ions from the Lac-SBF solution. The resultant CaP layer was found to be tightly bonding to the Ti6Al4V substrates. This integration of the coating to the Ti6Al4V substrate was facilitated by the hydrated TiO₂ and Na-titanate layers placed there by the hydrothermal etching and thermal treatments [5,8]. Furthermore, the surface roughness values of the coatings treated in NaOH and NaOH + H₂O₂ were found to be 0.140 μm and 0.246 μm , respectively (see Table 5).

The CaP coating of titanium implants following chemical and heat treatments would significantly increase the bone-implant interface bonding strength, and thus, the biomimetic method was found to be quite advantageous especially for the load-bearing metallic implants [16,17]. Fig. 7 shows the adhesion characteristics of the CaP coatings on Ti6Al4V substrates pretreated in NaOH and NaOH + H₂O₂ solutions. Scratch testing is normally performed under controlled conditions (increasing load and constant speed or vice versa, or both constant) and by scratching of a coated surface with a specified indenter. A diamond-shaped indenter is usually used and from an exact knowledge of all test parameters, and by measuring the critical load at failure of the coating, the adhesion strength can be estimated [18]. In this study, the critical loads of the CaP coatings treated in NaOH and NaOH + H₂O₂ approximately were found to be 9 mN and 3 mN, respectively. That was to say that the adhesion strength of coatings applied on Ti6Al4V surfaces

Table 5

Coating thickness and surface roughness of the CaP coatings.

Materials	Coating thickness (μm)	Surface roughness (μm)
Ti6Al4V	–	0.192 \pm 0.005
CaP coating on NaOH + H ₂ O ₂ treated substrates	4 \pm 0.134	0.246 \pm 0.006
CaP coating on NaOH treated substrates	4 \pm 0.107	0.140 \pm 0.007

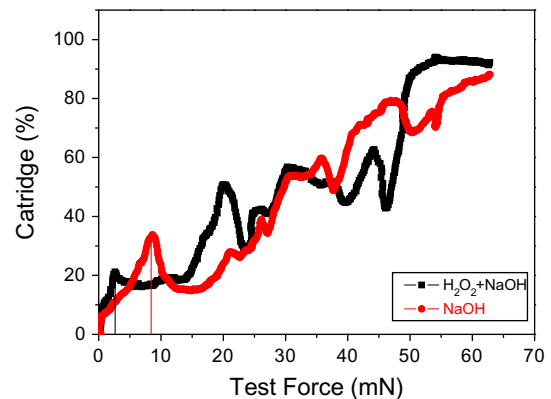


Fig. 7. Adhesion characteristics of the CaP coatings on Ti6Al4V substrates pretreated in NaOH and NaOH + H₂O₂ solutions.

pretreated in NaOH was better than those treated in NaOH + H₂O₂ solutions.

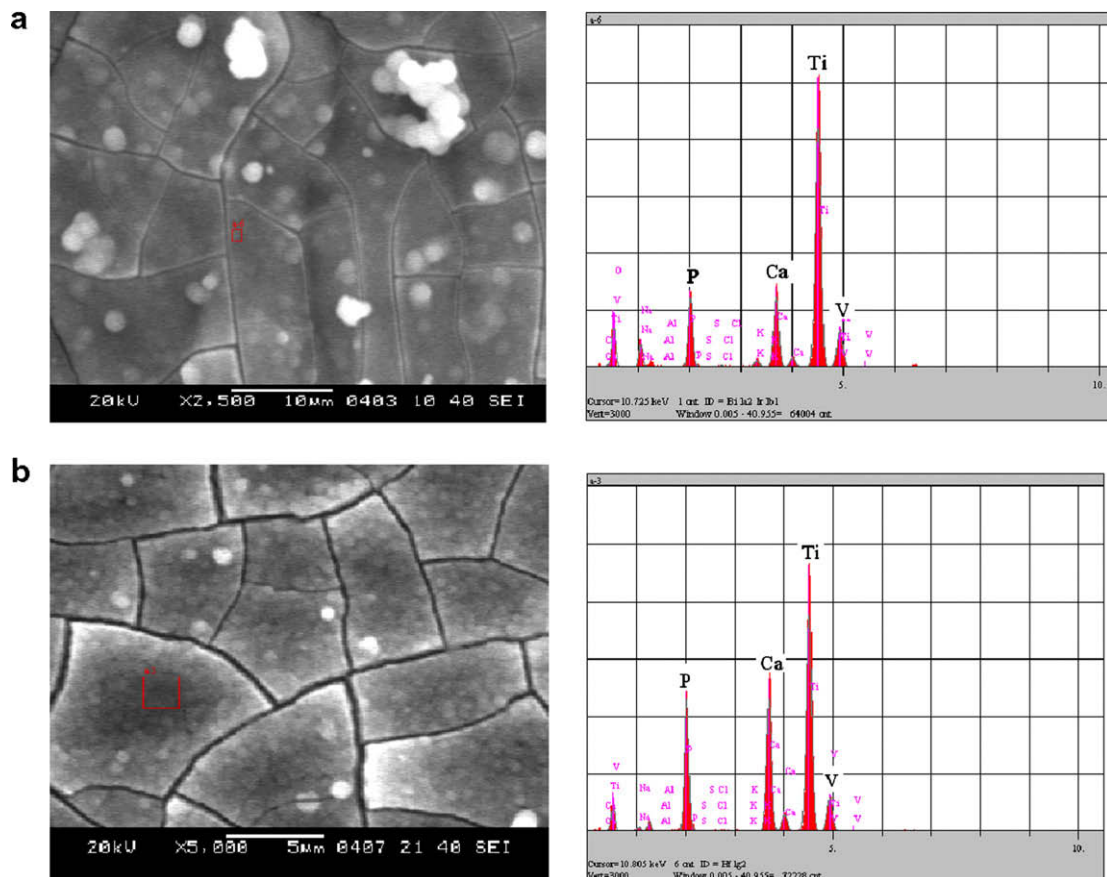


Fig. 6. SEM micrographs and EDS analysis of the CaP coatings on Ti6Al4V substrates pretreated in (a) NaOH and (b) NaOH + H₂O₂ solutions. EDS analysis was performed from the pointed area in SEM micrographs. Ca, P, Ti, and V elements were found in EDS analysis. The scale bars are (a) 5 μm and (b) 10 μm .

4. Conclusion

In summary, a new SBF solution was suggested to deposit CaP coatings on Ti6Al4V substrates by using the biomimetic technique. The new SBF solution contained the same (not similar) Ca^{2+} , Na^+ , Mg^{2+} , K^+ , HPO_4^{2-} , HCO_3^- , Cl^- , and SO_4^{2-} ion concentrations with those of human blood plasma. The pH adjustment of the SBF solution was achieved by using Na-lactate and lactic acid. The solution does not contain any Tris or Hepes, and thus called as “*Lac*-SBF solution”. In the previous applications, the Cl^- ion concentration was found to be higher than blood plasma 103 mM which exists in human blood plasma as a result of Tris/HCl which are used to prevent precipitation and to keep the pH level at certain values. In this study, instead of using Tris/HCl, HCl/NaL which are generated by human body and do not show any toxic behavior, are used and Cl^- concentration was kept at 103 mM value for the first time. The prepared *Lac*-SBF was shown similar concentration to human blood plasma in terms of all inorganic ions for the first time. When the Ca^{2+} and HPO_4^{2-} ion concentrations (in mM units) of the *Lac*-SBF solution were multiplied by the factor of 2.50, the new solution can be prepared, namely, $2.5 \times$ *Lac*-SBF. This new solution was able to coat CaP on Ti6Al4V substrates much quicker than the *Lac*-SBF solution at 37 °C.

Ti6Al4V substrates were etched in NaOH or NaOH + H₂O₂ at 60 °C. The surface roughness values of the CaP coatings on substrates pretreated in NaOH and NaOH + H₂O₂ were found to be 0.140 μm and 0.246 μm, respectively. NaOH + H₂O₂ solutions assist to increase the surface roughness of the Ti6Al4V substrates. It was found that Ca₅(PO₄)₃(OH), CaHPO₄·2H₂O, titanium and rutile TiO₂ phases were present in CaP coatings on Ti6Al4V substrates. The coating morphology is almost identical to those obtained by using the more common Tris-SBF solutions. The coating thickness values were determined as 4 μm-thick layer. The adhesion strengths of the CaP coatings obtained on Ti6Al4V samples pretreated in NaOH and NaOH + H₂O₂ solutions were found to be 9 mN and 3 mN, respectively.

CaP coating on metal implant is an effective method to enhance bioactive properties of the metal surface. It improves the bonding strength on bone tissue without inducing the growth of fiber tissue. CaP coating can act as a barrier between the body and the metallic implant, and provide a surface on which bone can easily grow, generating mechanical interlocking and chemical bonding at the bone-implant interface. This study will present a great service to mankind with using the metal implants which are coated with CaP in the *Lac*-SBF solution in living creatures, that the implant material is strongly bonded to bone surfaces, and that it shortens a patient recovery period by quickly knitting to bones.

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figures 1, 2, 6 and 7, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi: [10.1016/j.actbio.2009.12.013](https://doi.org/10.1016/j.actbio.2009.12.013).

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