

CRYSTALLIZATION OF CALCIUM PHOSPHATES ON ALKALI-TREATED Ti-SUBSTRATE

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KRYŠTALIZÁCIA Ca-P ZLÚČENÍN NA POVRCHU Ti –SUBSTRÁTU UPRAVENÉHO V ALKALICKOM PROSTREDÍ

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Abstrakt

Sledoval sa účinok koncentrácie iónov Na⁺ v koróznej vrstve Ti6Al4V substrátu na proces precipitácie Ca–fosforečnanov v prekalciфикаčných roztokoch SCS), t.j. roztokoch nasýtených vápenatými a fosforečnanovými iónmi, ktoré nie sú pufrované a ktorých pH bolo 6,2. Povrch vzoriek Ti6Al4V-zliatiny bol najprv predupravený lúhovaním v koncentrovanom roztoku hydroxidu sodného ($c_{(\text{NaOH})} = 10 \text{ mol.l}^{-1}$, pri teplote 60 °C, po dobu 24 hodín). V nakorodovanej vrstve ostáva vysoký podiel sodíka, ktorý sa následným uložením vzoriek na 7 dní do vodných roztokov s rozdielnou koncentráciou NaOH (0; 0.1; 10 mol.l⁻¹) výrazne, v závislosti na koncentrácii roztoku zmenil. Ukázalo sa, že rozdiely v koncentrácii Na⁺ iónov v povrchovej vrstve ovplyvňujú proces vylučovania Ca-fosforečnanov na povrchu Ti-substrátu. Výmena Na⁺ z povrchu za Ca²⁺ z roztoku po ponorení vzoriek do vodného roztoku SCS je rýchla. Povrch vzoriek s vysokou koncentráciou Na⁺ iónov sa rýchlo pokryl produktmi precipitácie, t.j. kryštálmi Ca-fosforečnanov, pričom povrch vzoriek prostý Na⁺ iónov nepokryli kryštály Ca-fosforečnanov súvislou vrstvou. Kryštály sa objavovali skôr sporadicky, ale boli väčšie.

Stabilita fáz Ca- fosforečnanov (HA–hydroxyapatit / Ca₁₀(PO₄)₆(OH)₂; OCP–octakalcium fosfát / Ca₈(HPO₄)₂(PO₄)₄.(5H₂O); TCP–tricalcium fosfate / Ca₃(PO₄)₂ a ďalšie, o ktorých sa predpokladá, že precipitujú z roztoku SCS a SBF /simulovaná telová tekutina) vo vodnom roztoku v závislosti na pH bola overená výpočtom. V roztokoch SCS ($c_{(\text{Ca}^{2+})} = 5 \text{ mmol.l}^{-1}$ a $c_{(\text{PO}_4^{3-})} = 2,5 \text{ mmol.l}^{-1}$) o pH 6,2 sú fazy HA, TCP a OCP termodynamicky stabilné.

Abstract

Effect of the Na⁺ ion concentration in the surface layer of alkali - treated Ti6Al4V alloy on the heterogeneous nucleation of calcium phosphate was verified in non-buffered

precalcificated solution (SCS) at pH 6.2. The alkali-treated Ti6Al4V samples ($c_{(\text{NaOH})} = 10 \text{ mol.l}^{-1}$, 60 °C and 24 hrs.) were stored in aqueous solutions with different concentrations of Na^+ ions (0; 0.1; 10 mol.l^{-1}) resulting in dramatic changes in Na^+ concentration in the surface layers. Substitution of Na^+ by Ca^{2+} ions in SCS solution is rapid. Capacity of samples to be covered by crystals when immersed in SCS solutions (super - saturated by Ca^{2+} , PO_4^{3-}) decreases with diminishing Na^+ concentration in the hydrated layers of Ti6Al4V.

Stability / capability of calcium phosphates nucleation (HA–hydroxyapatite / $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$; OCP–octacalcium phosphate / $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$; TCP–tricalcium phosphate / $\text{Ca}_3(\text{PO}_4)_2$; a.o.) in SCS solution and in SBF solution (simulated body fluid) was assessed by thermodynamic driving forces. Phases HA, TCP a OCP are thermodynamical stable in the solution SCS ($c_{\text{Ca}^{2+}} = 5 \text{ mmol.l}^{-1}$ and $c_{\text{PO}_4^{3-}} = 2,5 \text{ mmol.l}^{-1}$) by pH 6,2 .

Keywords: Precalcification, SCS an SBF Fluids, Activation of Titanium

1. Introduction

Titanium and its alloys are materials used for surgical implants due to their excellent mechanical properties and chemical stability. They are applied as implants of hard bone tissue. Titanium is bio-inert material with good biocompatibility. The bioactivity of titanium is attributed to the formation of titanium oxide (TiO_2) film on its surface. Formation of satisfactory bonding between the titanium materials and the living tissue is of great importance. Therefore various techniques have been developed for depositing bioactive calcium phosphate coating on top of titanium materials, such as plasma spraying, electrochemical deposition, sol-gel deposition etc. The implant with hydroxyapatite layer [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ – HA] becomes bioactive and grows faster together with the bone tissue.

Great attention is currently been paid to investigating the chemical activation of Ti-surface in the alkaline or acidic solutions and to effects of precalcification in supersaturated calcium solutions (SCS) [1-8]. The chemical treatment of Ti-materials in NaOH solutions with elevated concentration ($c_{\text{NaOH}} = 5\text{--}10 \text{ mol.l}^{-1}$, $T = 273\text{--}337 \text{ K}$, $\tau = 1\text{--}24 \text{ hr}$) significantly increases the surface reactivity and accelerates process of the heterogeneous nucleation of calcium phosphate in the simulated body fluid (SBF) having chemical composition similar to blood plasma [2,5,9]. Exposure of Ti - material to NaOH solution results in slow formation of several micrometers thick-hydrated layer, consisting of $n\text{H}_2\text{O} - \text{TiO}_{2-x}$ and Na^+ ions. Formation of calcium phosphate coating at the chemically treated surface can be accelerated by precalcification, which is carried out by immersing the substrate into the supersaturated solution containing Ca^{2+} , PO_4^{3-} ions for a short period of time [1,3,7,10-12]. The Ca^{2+} , PO_4^{3-} concentrations in these solutions are usually 2-3 times higher than corresponding concentrations in blood plasma and in SBF (model solutions, Table 1). The increase of Ca^{2+} and PO_4^{3-} concentration accelerates precipitation of calcium phosphates from the solution, and promotes formation of Ca-P nuclei on the substrate. In the first stage the ability of the substrate to form a uniform hydroxyapatite layer on the surface of implant with living tissues was tested “in vitro” in SBF followed by in vivo tests [1,6,10,13,14].

This paper discusses the effect of Na^+ concentration in the hydrated layer on the rate of calcium phosphate deposition on the Ti6Al4V surface, which is takes place through formation of Ca-P crystal in SCS solution and compares thermodynamic stability of Ca-P precipitates in SCS and SBF solution.

2. Experimental

The samples of titanium alloy Ti6Al4V (90 : 6 : 4 wt%) with the dimension of 10 x 10 x 0.8 mm were prepared from rolled sheet alloy. The samples were washed for 5 min in acetone by an ultrasonic cleaner, then washed in distilled water and subsequently etched for 90 min in concentrated hydrochloric acid at 298 K. Finally the samples were 3-times washed in distilled water. The cleaned samples were chemically treated in NaOH solution with concentration 10 mol.l⁻¹ at 60 °C (330 K) for 24 hours and then washed 3-times in 50 ml distilled water.

The samples treated in alkaline solution were divided in four groups, each containing three test samples that were then stored under different conditions.

Group 1: washed samples were immersed in SCS solution where they remained for 3 days,

Group 2: washed samples were dried at room temperature and stored in desiccator for 7 days at laboratory temperature,

Group 3: samples were immersed in 0.1 mol.l⁻¹ NaOH solution (S/V = 0.08 cm⁻¹ (S - surface of sample [cm²]; V - volume of solution [cm³]) for 7 days at laboratory temperature,

Group 4: samples were immersed in distilled water (S/V = 0.08 cm⁻¹) for 7 days at laboratory temperature).

Group 5: reference samples were not alkali-treated.

All samples in groups 1 – 5 were then immersed in SCS (S/V = 0.044 cm⁻¹) under stationary conditions and stored in a biological thermostat at 310 K for 3 days. After exposure to SCS, the samples were 3-times washed in distilled water. Finally, the bioactivity of the samples was tested in vitro in SBF solution (S/V = 0.044 cm⁻¹) at temperature of 309.5 K for 7 days.

Table 1 Solution composition, calcification solution (SCS); simulated body fluid (SBF); blood plasma [3,15].

Solution	Ion concentration in solutions /mmol.l ⁻¹								
	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	Cl ⁻	H ₂ PO ₄ ⁻	SO ₄ ²⁻	HCO ₃ ⁻	pH
SCS	4	–	5	–	10	2.5	–	1.5	6.4
SBF*	142	5	2.5	1	131	1	1	5	7.4*
Blood plasma	142	3.6-5.5	2.1-2.6	1	95-107	0.65-1.45	1	27	7.4

*buffer TRIS/HCl

After 3 days of exposure to SCS as well as after 7 days exposure to SBF the sample surfaces were observed under optical microscope Neophot 32 (Carl Zeiss, Jena), by electron microscope SEM–EDS (JEOL JSM-7000 F), and by X-ray diffractometers (Philips X'Pert PRO and PANalytical X'Pert PRO). These analytical methods were used to evaluate morphology of Ti6Al4V surface layer developed in each stage, number of nucleation sites per unit area as well as size of crystalline calcium phosphate phase (Fig. 1, 2 and 4). Variations of Ca²⁺ concentration in SCS solutions were determined by complexometric analysis (KIII) using eriochrome black indicator, and PO₄³⁻ concentration was determined by spectrophotometric method by spectrophotometer Spekol 11 (Carl Zeiss, Jena) at 690 nm wavelength (Fig. 3).

3. Results and discussion

Structural changes of the Ti6Al4V resulting from chemical treatment in acidic and alkaline solutions are depicted in Fig. 1 and 2. Internal stress existing within the surface layer as a result of its preliminary hot and cold rolling was eliminated by chemical etching in hydrochloric acid solution, as a result of which the surface layer becomes uniform.

Previous studies [2-5,8,9] showed that alkali treatment in concentrated NaOH solution results in activation of Ti – substrate, which is realized by formation of a porous hydrated gel-layer containing Na^+ ions (Fig. 2 a-b). The hydrated layer consisting of $\text{Na}(\text{H})\text{-O-TiO}_x$ is several micrometers thick and can easily crack during drying. On rinsing the alkali-treated surface in water, Na^+ ions slowly dissolve depending on intensity of washing, but certain amount of Na^+ ions may still remain in the hydrated layer. In case that substrate covered by hydrated layer is stored in water for a longer time, Na^+ ions can be completely removed (Table 2). Elimination of Na^+ from the structure of hydrated layer results in higher porosity in comparison with the porosity of the layer subjected to alkali treatment (Fig. 2 b and 2 d). This change in structure may be attributed to Na^+ ions being “eaten out” from the network present in the matrix.

Chemical composition of the initial solution SCS is summarized in Table 1. Fig. 3 depicts Ca^{2+} (white bar) concentration ratio analysed in samples prior and after they have been soaked in SCS solution and PO_4^{3-} (black bar) is concentration ratio analysed in samples prior and after they have been soaked in SCS solution. The most extensive decrease in Ca^{2+} and PO_4^{3-} concentration in solution is evident in case of samples in groups 1 – 3, which had elevated Na^+ concentration in the surface layer (Fig. 3). The solution analyses are in agreement with the surface coverage by calcium phosphates crystals (Fig 4).

Table 2 Concentration of Na^+ ions in Ti6Al4V surface after chemical treatment in NaOH ($c_{\text{NaOH}} = 10 \text{ mol.l}^{-1}$, 60°C , 24 h); EDS – analysis at least of three samples.

Sample after alkali treatment ($c_{\text{NaOH}}=10 \text{ mol.l}^{-1}$)		at %					
		Ti	Al	V	O	Na	Ca
1.	3-times washed in distilled water	29.4-36.8	1.2- 2.3	0.6-0.9	54.3-61.4	6.2–8.5	Traces < 1 at%
3.	7 days storage in 0.1 mol.l^{-1} NaOH and then 3-times washed in distilled water	29.8-33.2	1.2- 1.5	0.6	60.5-62.1	5.3- 5.8	
4.	7 days storage in distilled water	29.9-35.5	1.3- 3.1	0.6-1.0	62.3-68.5	0*	

* below detection limit

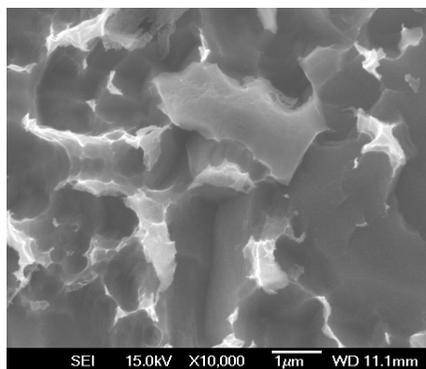


Fig.1 (SEM-EDS) Surface of Ti6Al4V after leaching in HCl solution (1:1).

Needle-shaped crystals grow in spherulites on the surface of the substrate. A great number of smaller-size crystals were observed in samples in group 1 and 2, i.e. within the hydrated layers containing the highest Na^+ concentration (about 6 - 7 at.%). On the other hand,

larger crystals ($\approx 100 \mu\text{m}$) were formed in the hydrated layer but their number was much lower and with lower Na^+ concentration and/or completely free of Na^+ . The samples in group 5 were inactive. Their surface was not covered by crystals, which could be observed under optical microscope.

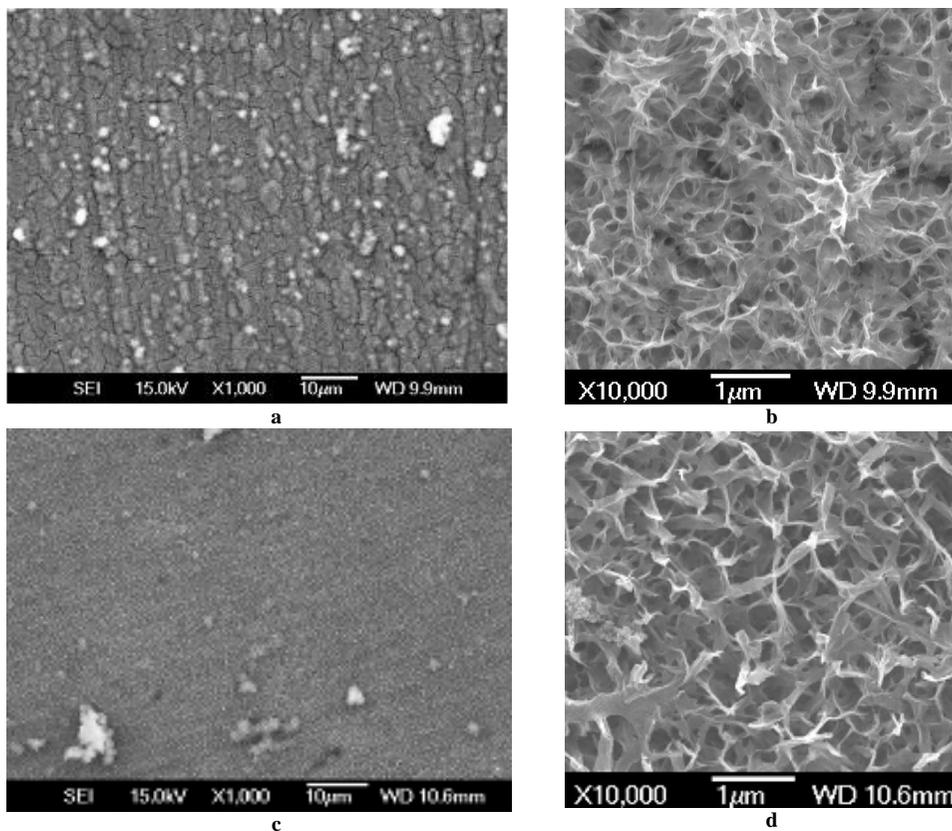


Fig.2 (SEM-EDS) Morphology of Ti6Al4V surface after alkali treatment in NaOH solution: a) b) sample from group -1 (3-times washed in distilled water); c), d) samples from group 4 (7- day storage in distilled water).

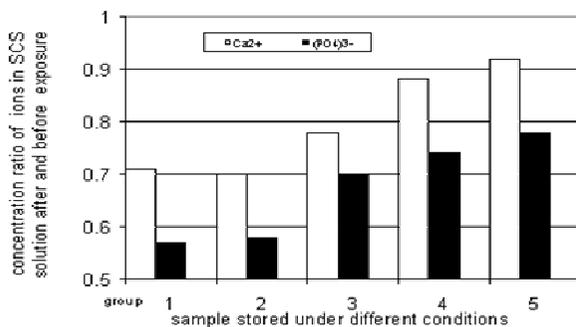


Fig.3 Decrease of Ca^{2+} and PO_4^{3-} concentration in SCS solution after 3 days of exposure. Exposed were the alkali-treated Ti6Al4V samples that were stored under different conditions: 1- 3-times washed in distilled water; 2- dried and stored for 7 days in desiccator; 3- storage for 7 days in 0.1 mol.l^{-1} NaOH solution; 4- storage for 7 days in distilled water; 5- reference samples, i.e. no-alkali treatment.

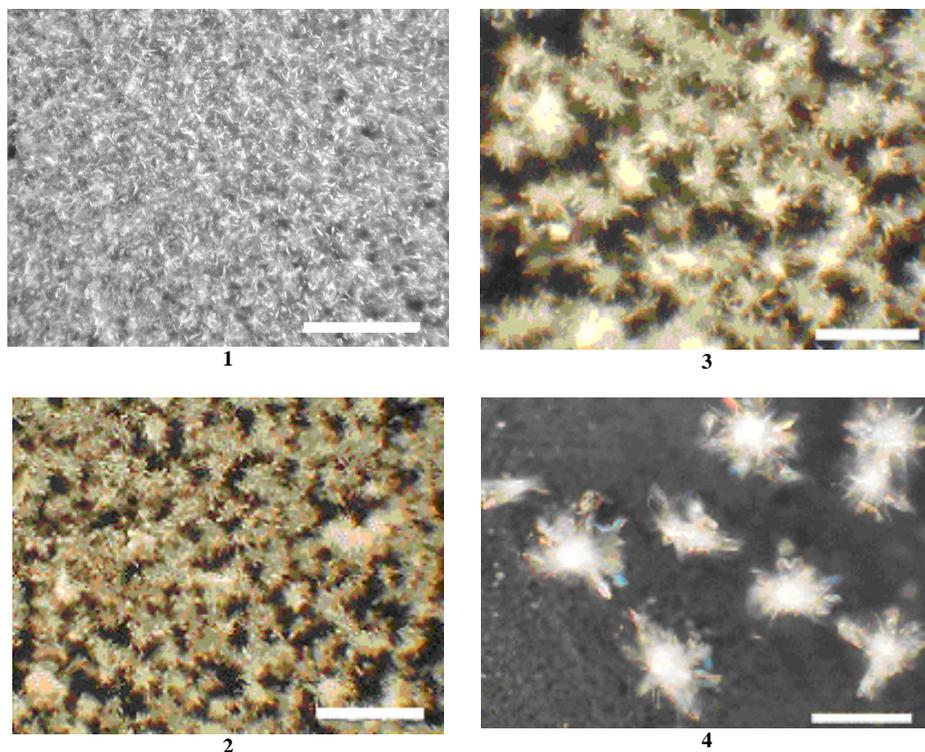


Fig.4 (OM) Ti6Al4V samples of groups 1 – 4 after 3 days of exposure to SCS. They are alkali-treated (10 mol.l^{-1} NaOH) samples of group: 1- 3-times washed in distilled water; 2- dried and stored for 7 days in desiccator; 3- stored for 7 days in 0.1 mol.l^{-1} NaOH solution; 4 – stored for 7 days in distilled water. Scale: $100 \mu\text{m}$

It seems that Na^+ concentration in the hydrated layer of Ti6Al4V plays an important role in nucleation of calcium phosphate mainly from supersaturated solutions (SCS). At the initial stage, immediately after immersing alkali-treated samples in SCS solution the concentration of Ca^{2+} in the surface layer rapidly increases whereas Na^+ decreases. In this initial stage phosphorus (within 0.5 h) was either not detectable by EDS or there were only traces. The growth of calcium phosphate coating in SCS was observed as depicted in Fig 5. It appears that a nucleus of calcium phosphate phase is created at the less energy-demanding site corresponding to the site with elevated Na^+ concentration. The EDS analysis of convex notches of alkali-treated samples confirmed elevated concentrations of Na^+ ions. The Ca/P atomic ratio measured by EDS at the surface of the needle-shaped crystals of spherulites is ≈ 1.2 after 3-days exposure to SCS.

Coherent crystalline coatings several micrometers thick were created on the alkali-treated surfaces after being exposed to SCS for 3-days and to SBF for 7 days. Preliminary calcification of the alkali-treated surface is not the prerequisite for growth of Ca-P coating in SBF solution whereas the alkali-treatment is inevitable. The growth of the globular-shaped HA-crystals in SBF was observed as depicted in Fig. 6. Shapes of crystals depicted in Fig. 5 suggest that it is possible that crystals, which precipitate from SCS solution correspond to a different phase than the one crystallizing from SBF (Fig. 6). Concentrations of Ca^{2+} , PO_4^{3-} and their respective ratios in SCS and SBF solutions are different (Table 1).

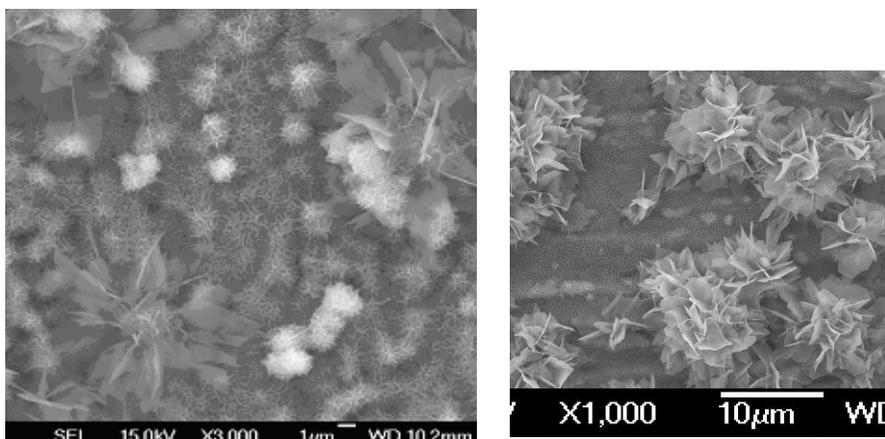


Fig.5 (SEM) The growth of spherulites on the alkali-treated surface (group 1) in the initial stage of exposure to SCS .

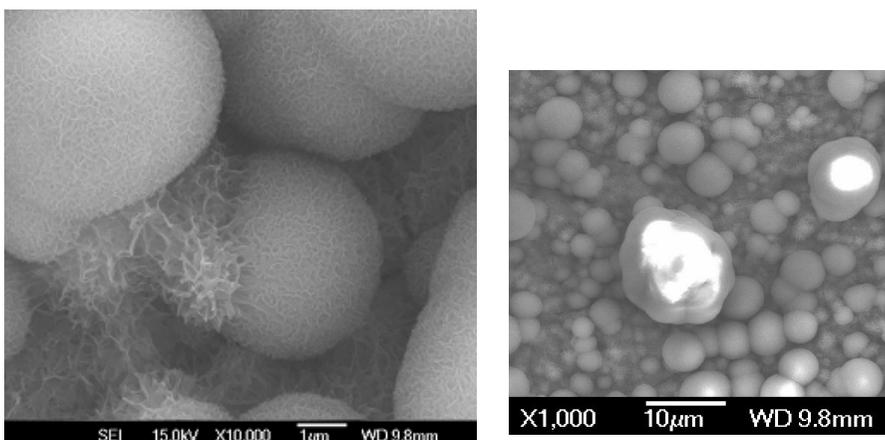


Fig.6 (SEM) The growth of HA-coating on the alkali-treated of Ti6Al4V (group 1, without exposure to SCS) during short time exposure to SBF

Diffraction analyses of calcium phosphate films (Fig. 7) show differences in crystalline phases that precipitated from the SCS and SBF solutions. High intensity of signal in the $5-20^\circ$ range of 2θ confirms the occurrence of amorphous phases that are created at the surface of samples during their exposure to super-saturated solutions. The stretched peaks situated below 30° of 2θ angel indicate that these are in fact mixed phases. Crystalline phases in films created at the surface of Ti6Al4V samples after 3 days of exposure to SCS solution were difficult to be analysed by X-ray diffraction method (see in Fig. 7a). Identified were the lines corresponding to metallic titanium and whitloctite ($\text{Ca}_3(\text{PO}_4)_2$); the lines corresponding to octacalcium phosphate $\text{Ca}_4\text{H}(\text{PO}_4)_3$ were less intensive.

Thick and uniform HA-layer was formed after 7 days of exposure to SBF on all samples, which had been preliminarily treated in NaOH solution. The Ca/P atomic ratio in HA – coating after 7 days of exposure to SBF is ≈ 1.45 . HA-coating ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) was identified

by X-ray diffraction on the surface of Ti6Al4V exposed to SBF (see in Fig. 7b). The lower diffraction line shown in the diffractogram corresponds to Ti6Al4V substrate.

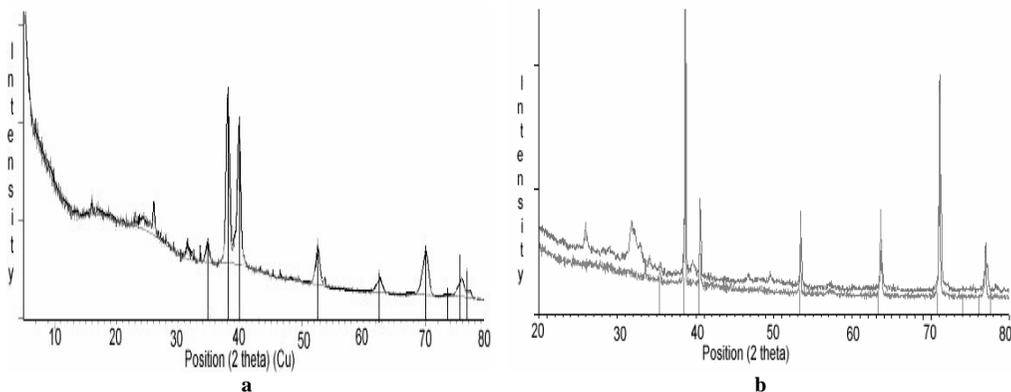


Fig.7 X-ray diffraction patterns of coating on Ti6Al4V a) after exposure to SCS (identified are the lines corresponding to Ti) (PANanalytical X'Pert PRO); b) after exposure to SBF (upper diffraction line) and on Ti6Al4V substrate (lower diffraction line)(Philips X'Pert PR.)

4. Thermodynamic equilibrium in SCS and SBF

Calcium phosphate coatings created in SCS and SBF solutions under stationary conditions at 310 K were different in nature (Fig. 5 and 6). This might be caused by different ion concentration in the solution. Ca^{2+} concentration in SCS solution was two-times higher than in SBF, and the $\text{Ca}^{2+} : \text{H}_2\text{PO}_4^-$ ratio in SCS and SBF is $5 : 2.5 = 2$ and $2.5 : 1 = 2.5$, respectively. The SBF solution is buffered (pH= 7.4) whereas SCS solution was not (pH = 6.2).

Previous studies [3,6,12,16] showed that calcium phosphates such as $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot (5\text{H}_2\text{O})$ (OCP–octacalcium phosphate), $\text{Ca}_4\text{O}(\text{PO}_4)_2$ (TTCP–tetracalcium phosphate), $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (DCPD–dicalcium phosphate dihydrate), $\text{Ca}_3(\text{PO}_4)_2$ (TCP–tricalcium phosphate), $\text{Ca}_9(\text{HPO}_4)(\text{PO}_4)_5(\text{OH})$ (DOHA–calcium-deficient apatite) and/or $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (HA–hydroxyapatite) and $\text{Ca}_{10}(\text{PO}_4)_6(\text{CO}_3)_{0.5}(\text{OH})$ (SCHA–carbonated apatite) may precipitate from SBF solution at temperature of 310 K and pH \approx 7.4. These calcium phosphates may recrystallize and recrystallization depends on conditions (pH, temperature, solution concentration).

Stability of solid substances precipitated from the solution increases with the decreasing solubility product (K_{sp}) and is characterized by Gibbs free energy changes of ionic species that are present in Ca-P solution (ΔG° [$\text{J} \cdot \text{mol}^{-1}$]). Concentrations of respective ionic species in SCS and SBF solutions were calculated in a similar way as in reference [6] (Table 1). The concentrations of ionic species in solutions at pH ranging from 4 to 10 were calculated by X(PLORE) software from the selected dissociation reactions and from four mass balance equations (Table 3).

Equilibrium ionic concentrations were calculated for the following cases:

Case A - all 17 dissociation equations in SBF and dissociation equations 1–11 in SCS in Table 3,
Case B - only equations with calcium and/or phosphoric ions, equations 1–7 in which Mg^{+2} and CO_3^{2-} ions are absent.

Table 3 Simultaneous dissociation reactions in the solutions and mass balance equations based on [6]

Dissociation reaction	K (T= 310 K)	
$\text{H}_3\text{PO}_4(\text{aq.}) \leftrightarrow \text{H}^+ + \text{H}_2\text{PO}_4^-$	$10^{-2.196} = 6.37 \cdot 10^{-3}$	(1)
$\text{H}_2\text{PO}_4^- \leftrightarrow \text{H}^+ + \text{HPO}_4^{2-}$	$10^{-7.185} = 6.53 \cdot 10^{-8}$	(2)
$\text{HPO}_4^{2-} \leftrightarrow \text{H}^+ + \text{PO}_4^{3-}$	$10^{-12.19} = 6.46 \cdot 10^{-13}$	(3)
$\text{Ca}^{2+} + \text{H}_2\text{PO}_4^- \leftrightarrow \text{CaH}_2\text{PO}_4^+$	31.9	(4)
$\text{Ca}^{2+} + \text{HPO}_4^{2-} \leftrightarrow \text{CaHPO}_4(\text{aq.})$	6.81×10^2	(5)
$\text{Ca}^{2+} + \text{PO}_4^{3-} \leftrightarrow \text{CaPO}_4^-$	3.46×10^6	(6)
$\text{Ca}^{2+} + \text{OH}^- \leftrightarrow \text{CaOH}^+$	$25.12 = 2.51 \cdot 10^1$	(7)
$\text{Ca}^{2+} + \text{HCO}_3^- \leftrightarrow \text{CaHCO}_3^+$	$10^{1.16} = 1.45 \cdot 10^1$	(8)
$\text{Ca}^{2+} + \text{CO}_3^{2-} \leftrightarrow \text{CaCO}_3(\text{aq.})$	$10^{3.38} = 2.40 \cdot 10^3$	(9)
$\text{H}_2\text{CO}_3(\text{aq.}) \leftrightarrow \text{H}^+ + \text{HCO}_3^-$	$10^{-6.31} = 4.9 \cdot 10^{-7}$	(10)
$\text{HCO}_3^- \leftrightarrow \text{H}^+ + \text{CO}_3^{2-}$	$10^{-10.25} = 5.62 \cdot 10^{-11}$	(11)
$\text{Mg}^{2+} + \text{HCO}_3^- \leftrightarrow \text{MgHCO}_3^+$	$10^{0.62}$	(12)
$\text{Mg}^{2+} + \text{CO}_3^{2-} \leftrightarrow \text{MgCO}_3(\text{aq.})$	$10^{1.87} = 7.4 \cdot 10^1$	(13)
$\text{Mg}^{2+} + \text{OH}^- \leftrightarrow \text{MgOH}^+$	$10^{2.19} = 1.54 \cdot 10^2$	(14)
$\text{Mg}^{2+} + \text{H}_2\text{PO}_4^- \leftrightarrow \text{MgH}_2\text{PO}_4^+$	$10^{0.4}$	(15)
$\text{Mg}^{2+} + \text{HPO}_4^{2-} \leftrightarrow \text{MgHPO}_4(\text{aq.})$	$10^{1.8}$	(16)
$\text{Mg}^{2+} + \text{PO}_4^{3-} \leftrightarrow \text{MgPO}_4^-$	$10^{3.3}$	(17)
Mass balance equations		
$c(\text{Ca}^{2+}) = [\text{Ca}^{2+}] + [\text{CaOH}^+] + [\text{CaHCO}_3^+] + [\text{CaCO}_3] + [\text{CaH}_2\text{PO}_4^+] + [\text{CaHPO}_4] + [\text{CaPO}_4^-]$		(18)
$c(\text{Mg}^{2+}) = [\text{Mg}^{2+}] + [\text{MgOH}^+] + [\text{MgHCO}_3^+] + [\text{MgCO}_3] + [\text{MgH}_2\text{PO}_4^+] + [\text{MgHPO}_4] + [\text{MgPO}_4^-]$		(19)
$c(\text{CO}_3^{2-}) = [\text{CaHCO}_3^+] + [\text{CaCO}_3] + [\text{MgHCO}_3^+] + [\text{MgCO}_3] + [\text{CO}_3^{2-}] + [\text{HCO}_3^-] + [\text{H}_2\text{CO}_3]$		(20)
$c(\text{PO}_4^{3-}) = [\text{CaH}_2\text{PO}_4^+] + [\text{CaHPO}_4] + [\text{CaPO}_4^-] + [\text{MgH}_2\text{PO}_4^+] + [\text{MgHPO}_4] + [\text{MgPO}_4^-] + [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}] + [\text{PO}_4^{3-}]$		(21)

Variations of ion concentrations in SBF and SCS solutions with pH are depicted in Fig. 8. It seems that concentration of magnesium ions in solution SBF remains at the approximately same level with increasing pH value, whereas above pH 8 association of Ca^{2+} to create CaCO_3 can be observed (Fig. 8. a). Concentration curves shown in Fig. 8 b/c and d/e corresponding to Ca^{2+} curve are similar suggesting a more dramatic decrease in Ca^{2+} concentration in case of calculation in Case A with increasing pH, i.e. in case that presence of carbonates in equations 8 - 13 is taken into account (Fig. 8 b/c). Better agreement between results of calculation obtained in Case A and B, (trends of curves corresponding to HCO_3^- , HPO_4^{2-} , CaHPO_4 , CaPO_4^- and Ca^{2+} concentrations, Fig. 8 d/e) has been achieved in case of SCS solution in which magnesium is absent and where concentration of CO_3^{2-} - containing species is much lower. The concentrations of ionic species (Ca^{2+} , PO_4^{3-}) in SCS are actually about too times higher than in SBF. Curves depicting variation of HCO_3^- , HPO_4^{2-} and CaHPO_4 reach maximum at pH 8. The dissociation reactions 8 -17 have negligible effect on resulting PO_4^{3-} , HPO_4^{2-} , H_2PO_4^- and Ca^{2+} concentrations at pH between 4 and 8.

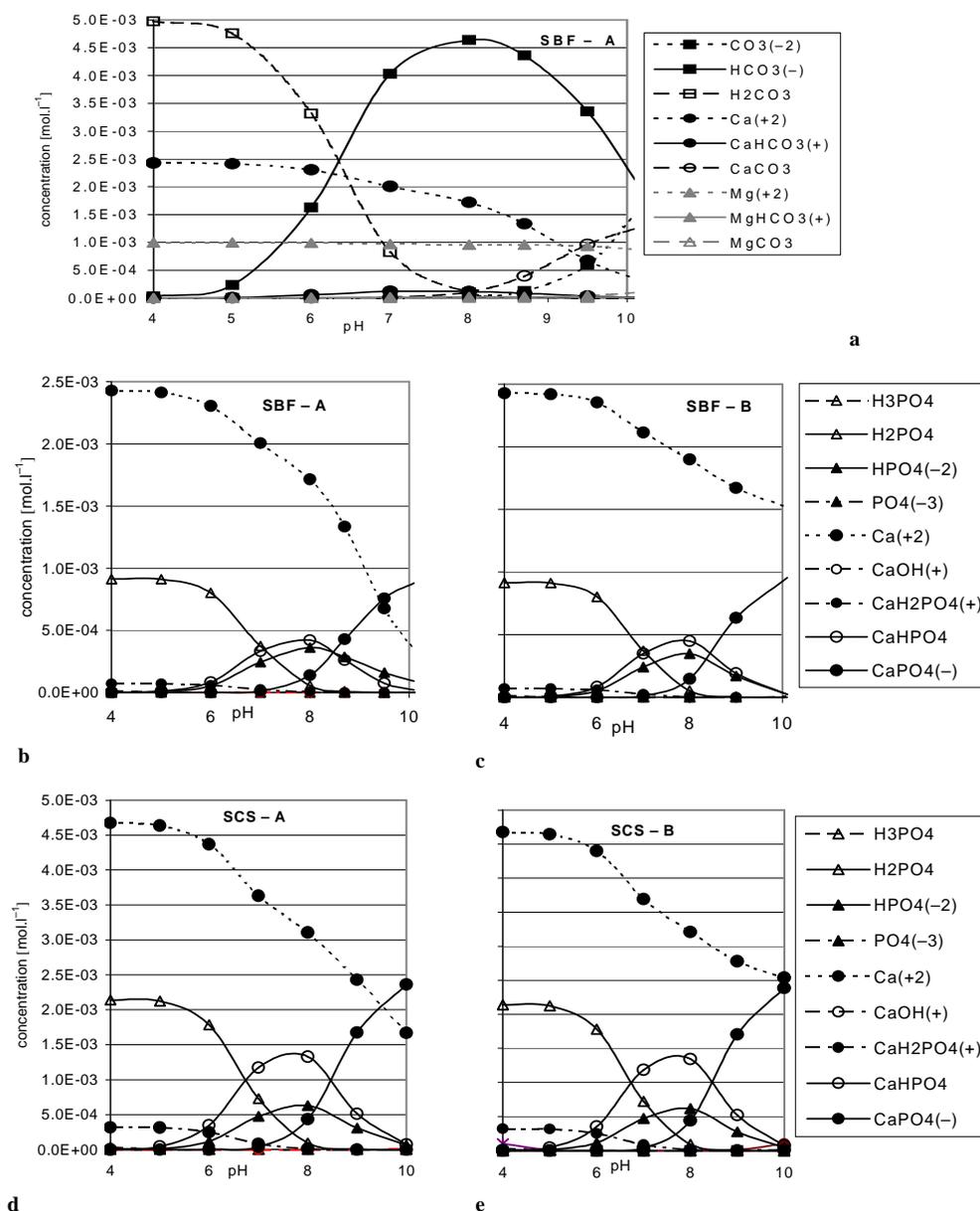
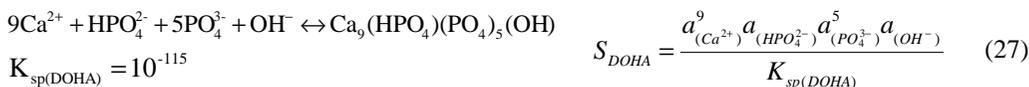
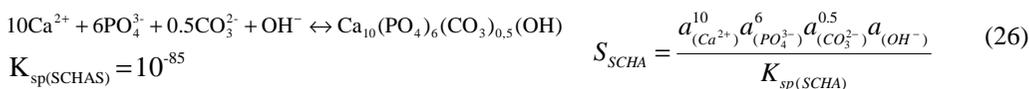
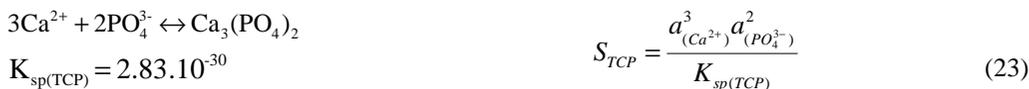


Fig.8 Variation of ion concentrations with pH in SBF and SCS solutions; **a), b), d)** case **A**: calculated from all 17 dissociation reactions in Table 3.; **c), e)** case **B**: calculated only from reactions 1 –7.

Mean ionic activity coefficients obtained from Debye-Hückel equation and the corresponding calculated ion concentrations in both SBF and SCS solutions are necessary for determining the solutions' super-saturation, S_p , by several calcium phosphates as well as for calculating reaction equilibria (22 – 27) [6]. Data of K_{sp} to equations 22 and 25 - 27 are taken over from work [6], to equations 22 - 25 from work [17] and to equations 24 - 25 from [13].

Super-saturation, S_p , is expressed in terms of ratio of product of activities of ionic species, which created precipitates ($a_{(i)}^n$), to the corresponding solubility product (K_{sp}).



The relative degree of super-saturation (σ) is defined by equation 29 [6,14].

$$\sigma = S_p - 1 = \left(\frac{IP}{K_{sp}} \right)^{\frac{1}{v}} - 1 \quad (28)$$

in which v is the number of ions that occur in the chemical formula corresponding to the precipitated phase, S_p is supersaturation, IP is ionic activity of ionic species and K_{sp} is the solubility product. The latter directly affects the rate of nucleation.

Relationships between extend of super-saturation of SCS and SBF solutions with regard to precipitation of and pH of the solutions are shown from results in Table 4. Super-saturation by calcium phosphate (HA, TCP, OCP, DCPD, DOHA and SCHAS) increases with increasing pH, Table 4. In this case the values of supersaturation at pH corresponding to SCS and SBF solutions are significant (6.2 and 7.4).

Table 4 Relative degrees of super-saturation $\sigma = (S_p - 1)$ in SCS and SBF solutions at corresponding pH values.

Solution	pH	$\sigma = S_p - 1$					
		HA	TCP	OCP	DCPD	DOHA	SCHAS
SCS	6.2	4.86	0.72	0.4	0.016	-0.19	-0.81
	7.4	26.5	6.70	3.1	0.74	2.32	1.04
SBF	6.2	0.44	-0.65	-0.65	-0.72	-0.62	1.06
	7.4	6.17	0.70	0.15	-0.48	3.11	9.16
	8	12	1.91	0.62	-0.478	17.1	8.54

As shown in Table 4, increasing pH results in several times higher degree of super-saturation of the solution and in accelerated formation of nuclei of calcium phosphate.

Compounds with negative degree of super-saturation would not precipitate from the solutions. The solution supersaturation is the highest in case of HA followed by TCP and OCP. Relative degrees of super-saturation of SCS at pH = 6.2 by HA, TCP and OCP are comparable with SBF at pH = 7.4. The Ca^{2+} and PO_4^{3-} concentrations in SBF are two times lower and carbonate concentration is three times higher as compared to SCS and therefore the degrees of super-saturation of DOHA and SCHA are significantly increased.

In case of calcium phosphate the change of pH in the range between 5 and 7 has great effect on solubility/stability of solid. The thermodynamic driving forces for precipitation and stability of Ca-P precipitates depend on Gibbs free energy changes in supersaturated solutions $\Delta G_{\text{Ca-P}}$ [$\text{J}\cdot\text{mol}^{-1}$] defined as

$$\Delta G_{(T)} = -RT \ln \left(\frac{IP}{K_{SP}} \right)^{\frac{1}{v}} \quad (29)$$

where $\Delta G_{(T)}$ - the Gibbs free energy per one mole of ionic species of Ca-P precipitate in the solution, R- the gas constant [$8.314 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$], T - the absolute temperature [K]. The remaining parameters v, IP, K_{sp} are defined in equation 28.

Variations of $\Delta G_{(\text{Ca-P})}$ with pH of SCS and SBF solutions for precipitation of HA, OCP, TCP, DCPD, SCHA, DOHA are illustrated in Fig. 9. a-b and summarized in Table 5. The relative stabilities all of precipitates were calculated from concentrations of ionic species determined in Case A (equations 1-17) and Case B (equations 1 - 7) in the pH range 4 – 10. As differences between concentrations calculated according to methods applied in Case A and B were negligible, results of $\Delta G_{(T)}$ versus pH for the same kind of precipitate were identical, the exception to this rule being SCHA (carbonated apatite). In the case of SCHA show in the Fig. 9 two curves.

Growth of nuclei and formation of new phase depend on the driving forces behind Ca-P precipitation, i.e. Gibbs free energy changes - ΔG . The negative value of $\Delta G_{(\text{Ca-P})}$ indicates super-saturation, whereas positive value signifies under-saturation. Stability of precipitate increases with the decreasing value of $\Delta G_{(\text{Ca-P})}$. Increasing concentration of Ca^{2+} and PO_4^{3-} in the solution shifts precipitation equilibria/stability of solid phases towards lower pH values.

Super-saturation of SCS by HA at pH 6.2 is the highest among all precipitates taken into consideration (Table 4). Thermodynamically less stable types of calcium phosphate forms, such as HA are: OCP, TCP and DOHA; their stability rapidly increases with increasing pH. In terms of thermodynamics the feasibility of DCPD precipitation is minimal. As can be seen in the Fig. 9a), the OCP and TCP precipitation from SCS (pH = 6.2) is possible only at higher pH.

SCHA, DOHA and HA phases precipitate preferably from SBF solution at pH above 7. Results of X-ray diffraction analyses (Fig. 7) indicated occurrence of HA-coating on the Ti6Al4V substrate, which was exposed to SBF for 7 days.

As the characteristics (appearance, form) of crystalline phases present in the substrate after exposure to SBF and SCS were very different (Fig. 5 and 6), a new type of Ca-P phase had to be formed instead of HA in SCS solution, presumably corresponding to TCP and/or OCP phase. This assumption is also referred to in the literature [3,11] suggesting that the OCP phase preferentially grows at the surface of Ti- substrate in SCS solution at temperature of 310 K. This conclusion does not disagree with Ostwald rule [18] according to which the initial nucleation of

precipitate from the solution would correspond to substance or to the compound having lower stability. These nuclei will subsequently re-crystallize to a stable phase. Unlike SBF, the SCS solution is not buffered, therefore Na^+ ions present at the surface layer of alkali-treated Ti-substrate, which immediately after its immersing are being released to the solution, can increase the pH at an interface. The Na^+ concentration has higher effect on pH variation, especially in the sensitive range of pH between 6 and 8. The thermodynamic driving forces for HA, OCP, TCP and DOHA precipitation increase, which means that the stability of precipitates also increases.

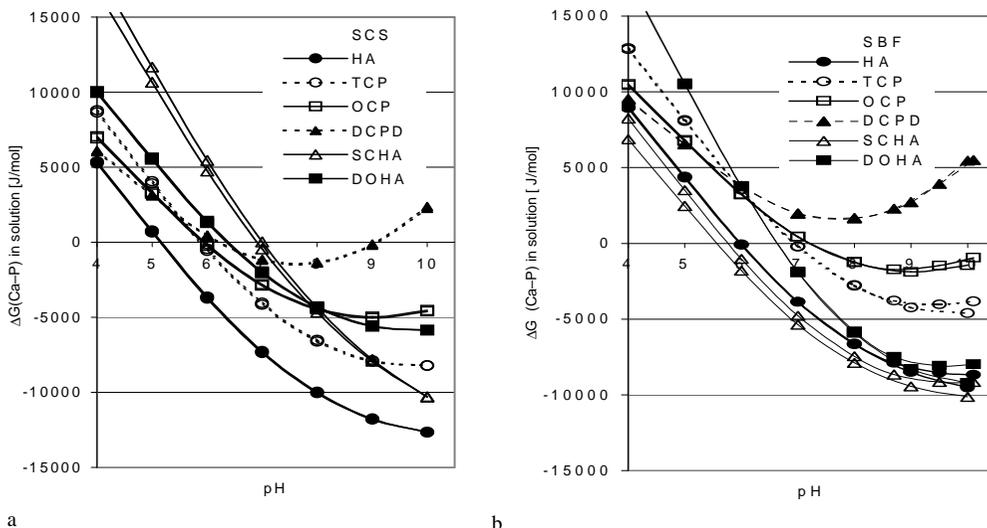


Fig.9 Effect of pH on Ca-P precipitation in a) SCS and b) SBF solutions

Table 5 ΔG of Ca-P precipitate from SCS and from SBF solutions at pH 6.2 and 7.4.

Solution	pH	ΔG [J. mol ⁻¹]					
		HA	TCP	OCP	DCPD	SCHA	DOHA
SCS	6.2	-4 550	-1 390	-1 270	-42	4 270	531
	7.4	-8 540	-5 260	-4 430	-1 430	-1 840	-3 090
SBF	6.2	-940	2 680	2 600	3 310	-1 870	2 480
	7.4	-5 080	-1 360	-750	1 690	-5 970	-3 640

The results of experiments confirmed our assumption that presence of a weakly bonded Na^+ in the hydrated layer is a prerequisite for rapid and immediate nucleation of calcium phosphates at the surface contacted with SCS solution. Its importance is equivalent or even higher than the extent of solution super-saturation. The extent to which the surface is covered by Ca-P crystals depends on Na^+ concentration in the hydrated layer. Observed rapid covering of substrate by the needle-shaped crystals during the initial period (several minutes), i.e. is immediately after the solid was immersed in the non-buffered SCS solution (Fig. 4., group 1 and 2), is probably induced by an increase in pH value at the surface of the chemically pretreated (NaOH) Ti6Al4V – substrates. Hydrated surface after chemical treatment consists of the porous hydrated gel-layer $\equiv\text{Ti}-\text{O}-(\text{H}/\text{Na})$ (Fig. 1, Table 2), which provides for its good wettability; Na^+ ions can be easily substituted by H_3O^+ or Ca^{2+} . The CaOxTiO_2 compounds are more stable than $\text{Na}_2\text{OxTiO}_2$, H_3O^+ or Ca^{2+} rapidly substitute Na^+ ions because calcification process is rapid and

may be completed within several hours. Ion substitution and Ca-P precipitation are consecutive processes. The porous structure and chemically active layers create good conditions for direct bonding and rapid growth of Ca-P crystals. Fast covering of substrate with coherent calcium phosphate-layer can prevent porous space in hydrated layer of surface substrate from being filled in.

5. Conclusions

Experiments confirmed that the presence of Na⁺ ions at the surface of Ti-substrate has an extensive effect on heterogeneous nucleation of Ca-P compounds from non-buffered SCS solutions. In case that Na⁺ ions have been removed from the layer, the nucleation process slows down dramatically. If concentration of Na⁺ in the hydrated layer remains to be high, number of nuclei are created on Ti6Al4V surface immediately after it had been immersed in SCS solution at pH = 6.2. High Ca²⁺ and PO₄³⁻ concentrations in solution therefore result in nuclei growth to create large crystals. Different types of crystalline phases, which may precipitate from SCS and SBF solutions, are presumably due to i) two times higher saturation of SCS by Ca²⁺ and PO₄³⁻ ions as compared to SBF, ii) buffering of SBF at pH = 7.4 and non-buffering of SCS at pH= 6.2.

Precipitation of calcium phosphates such as HA, TCP, OCP and DOHA is enhanced by this increase in pH. There is no significant change in pH which remains approximately constant at 7.4 at the solution/substrate interface in buffered SBF, therefore the predominant products of precipitation are HA, (DOHA, SCHA) compounds. Unlike SBF, SCS is the non-buffered solution. It is very likely that a sudden release of large number of Na⁺ ions from alkali-treated Ti6Al4V from the surface results in an increase of pH of the solution above 6.2 and probable precipitation of OCP.

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