



CaP Precipitation on Titanium under UV Lighting and Effect of Urea Concentration

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Abstract

This paper reports the precipitation of calcium phosphate (CaP) on alkaline hydrothermally treated titanium alloy under ultraviolet (UV) lighting. Urea (NH_2CONH_2) with different concentrations were added into a simulated body fluid buffered by sodium lactate. CO_2 and NH_3 gas release during immersion tests were detected. The pH values were also examined during the tests. The results show that the detected NH_3 and CO_2 concentrations near the solution are larger than those in the air. The release of NH_3 and CO_2 due to the hydrolysis of urea was confirmed both under UV lighting and under dark. It is shown by SEM photographs that the precipitated CaP particles are surrounded by organic which is supposed to be lactate deposition after 14 days immersion. A structure of embedded CaP particles in organic deposition matrix is considered as a primary stage of the formation of CaP-CO-NaCl layer. UV lighting causes the contents of Na and Cl increased, but C and O decreased.

Keywords: *CaP biomineralization, photocatalysis, NH_3 release, CO_2 release, urea, titanium alloy*

1. Introduction

Titanium and titanium alloy are used as biomaterials in the fields of hard tissue substitute, dental implant et al due to their higher mechanical properties and biocompatibility [1]. To improve their surface bioactivity other authors have proposed a method of manufacturing hydroxyapatite (HA) layer on titanium [2,3,4]. HA is proposed to promote osteoblast cell proliferation and growth [5,6]. Although the presentative of HA is a crystalline in the form of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ the existence in actual bone at different developing periods and different tissues do not have this format [7]. Amorphous phase or a combination of crystalline and non-crystalline and the presence of Cl, F, CO_3 were found [7]. Ca sometime is substituted by Mg, Zn or Na. Other authors [8,9,10] call the series compounds calcium phosphate (CaP).

The effect of TiO_2 photocatalysis on CaP formation has been investigated in the papers [11-13]. It is known that TiO_2 existing in the states of anatase and rutile has excellent photocatalysis [14]. The photocatalysis is supposed to be produced by photo-induced electrons and holes separation at the surface of TiO_2 , and causing redox reaction [15]. Photoactivated water molecular splitting is also considered as a source [16]. An acceleration effect of CaP formation photoactivated by ultraviolet (UV) lighting was reported [11,13]. The shape and the crystallinity of CaP, which influence the bioactivity and dissolution rate of CaP [17] may be altered by photocatalysis.

Urea is an existence in blood plasma and a metabolic result of protein in human body. There are several exploratory studies [18-20] synthesizing HA powder from urea added physiological solutions. In this work, in vitro CaP formation under photocatalysis by using a urea added simulated body solution (SBF) is investigated.

2. Experimentals

The experimental procedures include TiO_2 layer preparation on titanium alloy by alkaline hydrothermal method, immersion tests in a SBF under UV lighting and the CaP precipitation observation and composition analysis.

2.1. TiO_2 Layer Preparation on Titanium Alloy

Titanium alloy (Ti-6Al-4V) bars were purchased commercially (BaoTi, Co., Baoji, China). A piece with the size of $\phi 16\text{mm} \times 3\text{mm}$ was obtained by wire-cutting. The surfaces of pieces were ground with 400-1500# abrasive papers gradually. Then they were polished with

alumina powder (1.5 μm). They were cleaned in ethanol and deionized water 15 min under ultrasonic vibration progressively.

Alkaline hydrothermal treatment of the titanium surface was according to the method proposed by Peng [21]. The titanium alloy pieces were put into a stainless-steel autoclave with 2.5 M NaOH (GR >98.0%, Nanjing Chemical reagent Co., China) solution. The autoclave has a Teflon inner container which is corrosion-resistant. Then the autoclave was heated to 150°C and kept for 15 hours. After the pieces were cooled to the room temperature with the oven, the titanium pieces were then immersed in deionized water for 3 days. They were then immersed in 1 M HNO₃ solution (diluted from 60wt% HNO₃, Shanghai Pilot Chemical Industry, China) for 3 days, cleaned and dried, and finally annealed in an electric furnace at 600°C for 5 hours in air.

2.2. Immersion Tests

The solution used in the immersion tests was an SBF with the compositions of Na⁺ 142, Cl⁻ 103, Ca²⁺ 2.5, Mg²⁺ 1.5, HPO₄²⁻ 1, HCO₃⁻ 27, SO₄²⁻ 0.5, K⁺ 5mM, which are near those of human blood. The SBF were made by adding NaCl 5.26, CaCl₂·2H₂O 0.368, Na₂HPO₄·2H₂O 0.178, KCl 0.373, NaHCO₃ 2.268, MgCl₂·6H₂O 0.305, Na₂SO₄ 0.071g/L (all GR level purity, Shanghai Aladdin Co., China) into deionized water. Sodium L-lactate (60% aqueous solution, Shanghai Aladdin Co., China) was used as buffer reagent. Use L-lactic acid (90%, Shanghai Aladdin Co., China) for pH adjustment to 7.4. Lactate acid is one of the metabolic results of human body [22]. The solution was filtered by a 0.2 μm filter head. Then 2, 10 and 30mM urea (99.5%, Shanghai Aladdin Co., China) was added into the SBF separately.

Immersion tests of the hydrothermally treated pieces under UV lighting were carried out in a 37°C water bath. An Hg ultraviolet lamp (18W GPH303T5L, Kadind, USA) was hung over the liquid level 150mm. A quartz glass piece was covered on the dish for preventing dust and bacteria from entering into the solution while allowing UV light transmission. For UV lighting details refer to our previous work [23]. A UV radiation flux meter (SDR2040, 250~410nm wavelength UV probe, Shenzhen Speedre Co., China) was used to probe the radiation flux of UV behind the quartz glass piece and 150mm far away from the lamp. A maximum radiation flux of 4.2mW/cm² was obtained.

An irradiation pattern of 4 hours lighting and 20 hours dark was applied per day and lasted 14 days. Immersion tests under a total dark environment were also carried out for comparison. The releases of CO₂ and NH₃ gases during immersion were detected by a NDIR CO₂

transmitter and a NH₃ sensor (VMS 3002-CO₂, NH₃, Shangdong Vemsee Tech. Co., Jinan, China), respectively. The values of pH of the solution during immersion were examined by a pH meter (PHS-3C, INESA Instrument, Shanghai, China).

2.3. SEM and EDS

Immersed surfaces of titanium pieces were observed by a scanning electronic microscopy (SEM, JSM-6360LV JEOL, Tokyo Japan). The compositions of the interesting areas were probed by an energy dispersive spectroscopy (EDS, 2000XMS60, GENESIS) attached to the SEM.

3. Results and Discussion

3.1. NH₃ and CO₂ emission

During the immersion of the pieces in the urea added SBF the occurrence of NH₃ and CO₂ gas releases is because of the reaction of urea with water. The hydrolysis of urea is expressed by the follow [18],

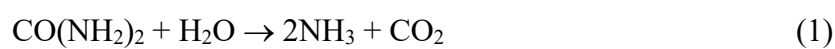


Table 1. NH₃ release during immersion of titanium pieces in urea added SBF.

Soaking time (h)	NH ₃ (ppm)						
	U2	U10	U30	D2	D10	D30	In air
4	12	13	12	19	23	24	7
28	16	18	17	21	18	22	14
52	33	35	35	39	37	42	22
76	21	19	20	19	20	20	11
100	23	24	26	27	30	31	18
124	13	14	16	15	16	17	11
148	12	12	13	14	15	13	8
172	10	12	14	11	12	11	8

196	15	18	18	19	17	19	12
220	12	14	15	20	19	19	9
244	9	11	11	13	12	15	7
268	17	20	21	20	22	22	11
292	25	29	28	29	28	31	11
316	31	29	30	32	31	32	17

Equation (1) can be accelerated with the help of catalysis [18] although it also occurs at room temperature without catalysis. Table 1 and Table 2 list the detected NH_3 and CO_2 gas concentrations in ppm near the solution surfaces, respectively. U2, U10 and U30 denote the samples immersed in the SBF with urea concentration of 2, 10, 30mM under UV lighting, respectively. D2, D10 and D30 denote the samples immersed in the SBF with urea concentration of 2, 10, 30mM under dark, respectively. The gas concentrations in the air at room temperature within the lab room were also probed during the soaking time and listed in Table 1 and 2, too. Corresponded figures are shown in Figure 1 and 2.

Table 2. CO_2 release during immersion of titanium pieces in urea added SBF.

Soaking time (h)	CO_2 (ppm)					
	U10	U30	D2	D10	D30	In air
4	629	618	622	631	638	518
28	1260	1264	1225	1215	1214	957
52	1222	1288	927	945	1003	974
76	717	705	739	722	699	620
100	1080	1028	1138	1123	1149	877
124	856	817	792	765	758	580
148	881	814	908	866	870	587
172	817	862	784	864	625	528
196	604	586	544	560	543	521

220	747	687	567	574	558	461
244	741	686	651	644	631	563
268	958	785	774	745	773	493
292	733	677	697	729	708	497
316	873	715	713	693	676	604

It is noted that the NH₃ and CO₂ gas concentrations change with the soaking time for all the samples. They are all more than those of the concentrations in air, indicating continuous release of these two kinds of gases from the solution. NH₃ released from urea is water soluble. The ionization of NH₃ · H₂O is expressed by,



Because Equation (2) is reversible, it is possible NH₃ releases under the condition that the reverse process is predominated, for an example, the increase in OH⁻.

The solubility of CO₂ in water is limited (0.1g/100g=CO₂/water at 37°C [24]). The dissolution of CO₂ into water is expressed in the follows,



From Equation (4) more CO₃²⁻ production can be obtained with the increase of OH⁻, i.e. the decrease of H⁺ and pH increment. The CO₃²⁻ is a component of carbonated CaP, for example, Ca₁₀(PO₄)₆CO₃ [24]. The necessary amount of CO₃²⁻ for forming carbonated CaP deposition is low because of the lower solubility of carbonated CaP [24].

It can be seen from Figure 1 and 2 that there is sharp increase of NH₃ and CO₂ release in the initial immersion stage. The release of CO₂ is faster than that of NH₃ which may be due to much lower solubility of CO₂ compared to NH₃. Slightly larger NH₃ release under dark than under UV lighting but less CO₂ under dark than under UV lighting can be confirmed as shown in Figure 1 and 2. It indicates the UV lighting inhibits NH₃ release but promotes CO₂ release. More CO₂ release under UV lighting is agreeable to the decreased carbon content in the carbonated CaP formed under UV lighting as showing in the results of EDS (Table 3)

It can be observed in Figure 1 that slightly increased release of NH₃ as the urea concentration is increased. This is because the source of NH₃ is only from urea. However, the release of

CO₂ (Figure 2) seems to be suppressed due to the urea addition particularly after 200h immersion under UV lighting. The reason may be due to that more urea causes the increase of free NH₄⁺ and OH⁻ according to Eq (2) and less CO₂ according to Equation (3) and (4).

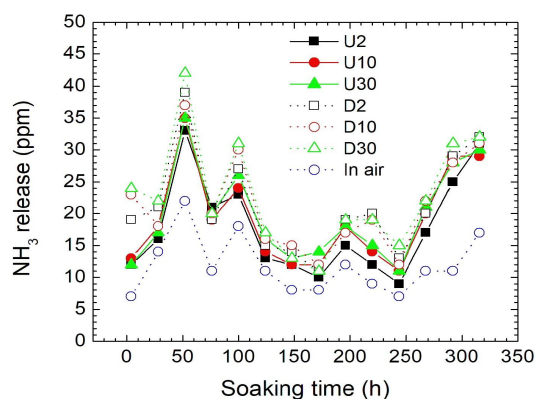


Figure 1. NH₃ release amounts probed at the places near the solution surfaces during immersion.

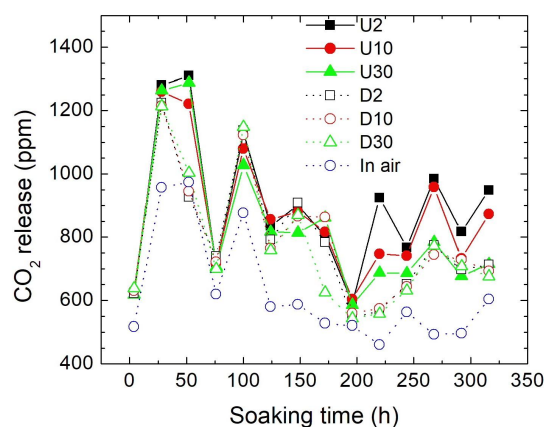


Figure 2. CO₂ release amounts probed at the places near the solution surfaces during immersion.

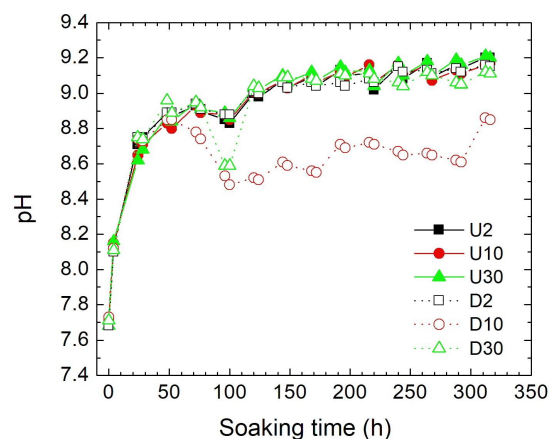
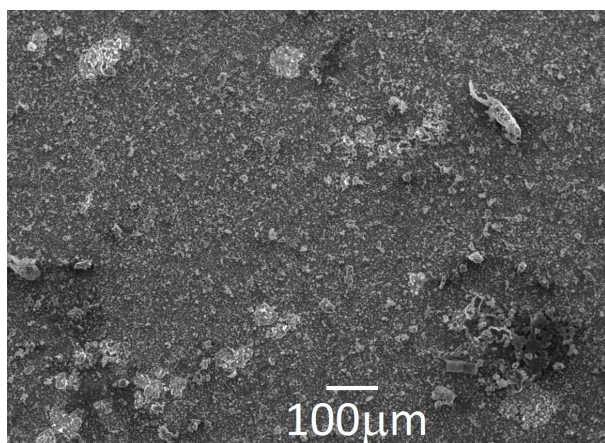


Figure 3. Values of pH of the solution during immersion.

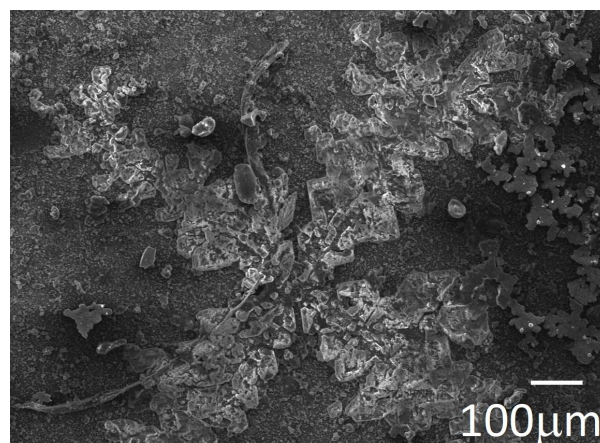
Figure 3 shows the pH plots against the soaking time. The increase of pH is fast at the initial stage (1-2 days), a slow increase stage is followed until the end of test. A pH decrease trend is seen for the D10 samples after 50 hours, the reason of which is unknown. Indifferent pH values evolution can be observed due to the urea concentration effect.

3.2. Surface Morphology and Composition

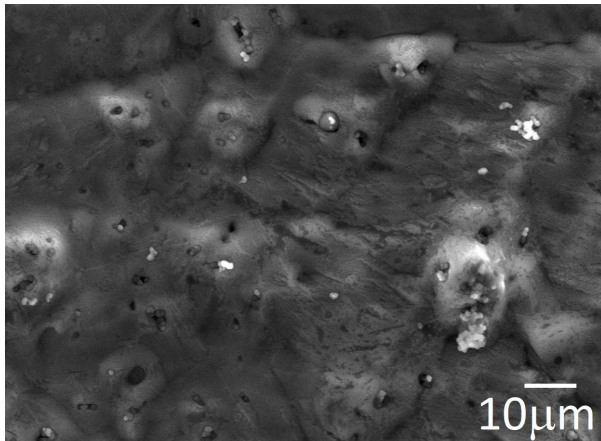
The results of the SEM observation of the surfaces after the immersion tests are shown in Figure 4. Different distributions of white particles areas between the U10 and D30 samples: isolated islands on U10 and dendritic shape on D30 are shown (Figure 4a and 4b, 4c and 4d). Such different morphologies were also observed on U2, U30, D2 samples. The dendritic shaped area detected by an EDS analysis (Figure 4f) is mainly composed of Na, Cl, C, O elements and small quantities of K, Ca, Ti and P elements. But the compositions of isolated islands mainly are Na, Cl, C, and small amounts of Ca, Ti and P (Figure 4e). It can be seen the white particles areas are composed of CaP particles and crystallized NaCl surrounding including C-O organic deposition. The existence of C in the crystalline under UV lighting and C-O under dark is interesting. The white CaP particles dotted crystallized NaCl-C surrounding can be seen on Figure 4c, but the CaP particles formed under dark are aggregated, NaCl crystal and organic (rectangular area) are formed separately (Figure 4d).



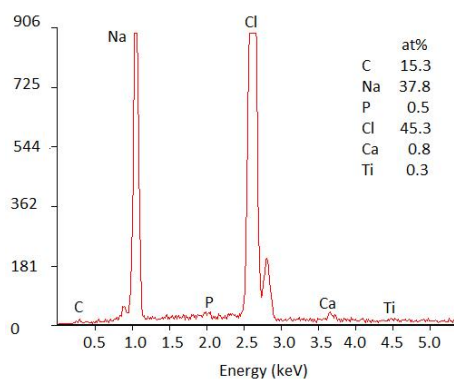
(a) U10



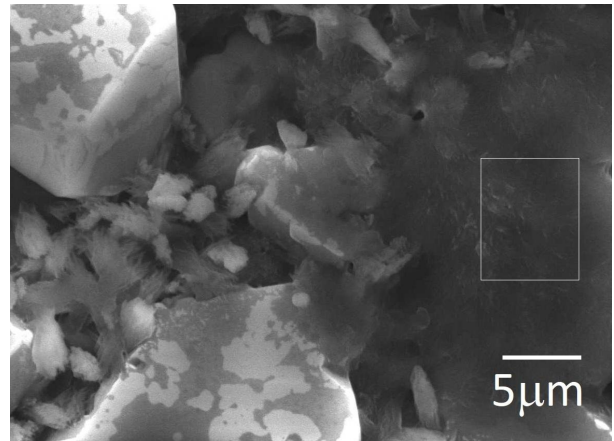
(b) D30



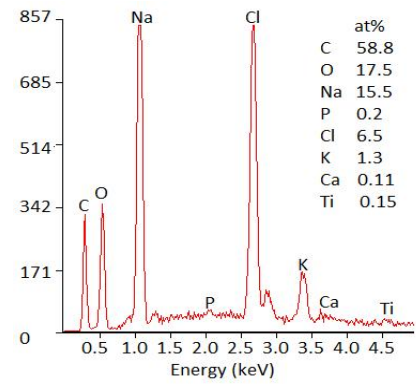
(c) U10



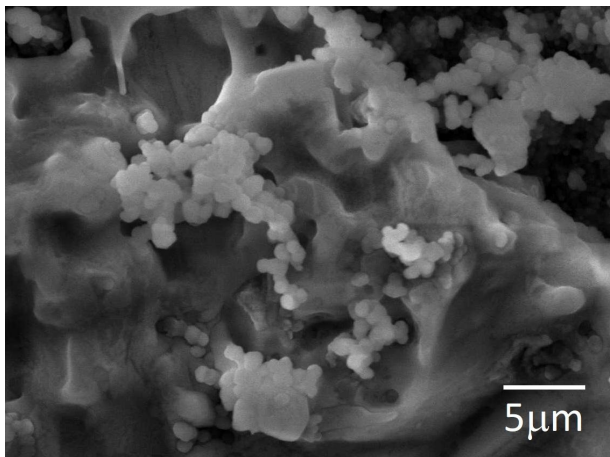
(e) U10



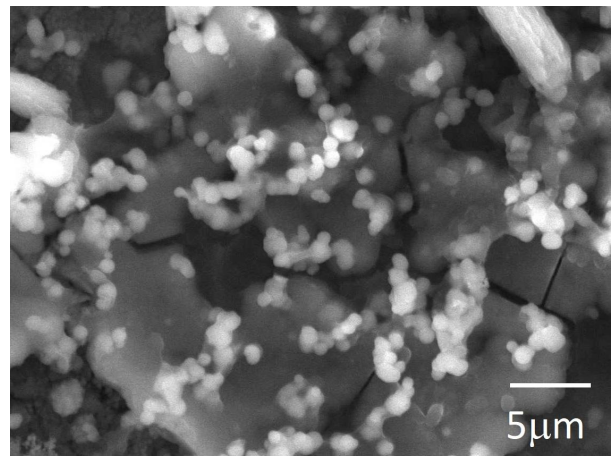
(d) D30



(f) D30



(g) U10



(h) D10

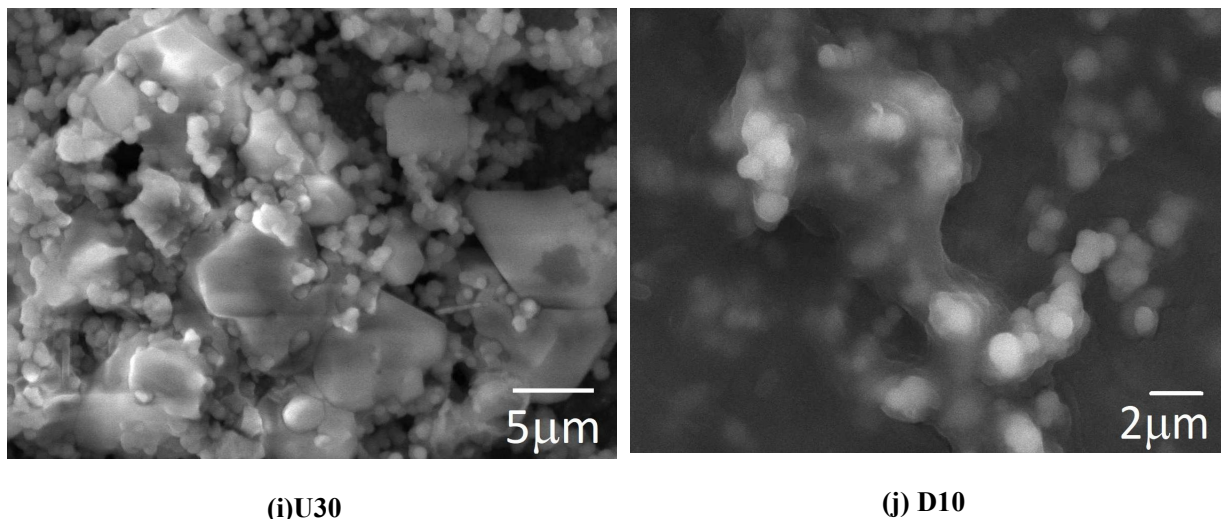


Figure 4. SEM observation of the surface morphologies of hydrothermally treated titanium alloy pieces after immersion tests in simulated body fluid containing urea. (a, c, g) U10, (i) U30, (b, d) D30, (e, f) corresponded EDS of (c) and the rectangular in (d), (h, j) D10.

More SEM photographs (Figure 4g and 4i) show CaP particles formed under UV lighting are surrounded by NaCl-C-O crystal. How the bond of C-O being connected with NaCl crystal needs more studies. We can see CaP particles embedded in C-O organic (Figure 4j). The C-O organic is supposed to be produced from lactate deposition. The organic includes Na and Cl elements obtained by EDS results (Table 3), but the contents of Na and Cl formed under UV lighting is increased largely compared to those under dark. Compared to the organic matter formed under dark, more crystals are formed under UV irradiation.

Table 3 lists the compositions of the elements in the deposition, it can be seen that less C and O, but more Na and Cl in the CaP particles on U series samples than D series samples. The contents of Ca and P are almost similar for U and D series samples, Mg, Na, K, Cl, Ti elements are included in the CaP particles. No nitrogen is found on the surfaces. It is supposed that nitrogen exists in the form of NH_4^+ and is released with the emission of NH_3 . The ratios of Ca/P are ranged in 1.61~1.64 under UV lighting, 1.31~1.53 under dark. The Ca/P of U series samples are bigger than those of D series. $\text{Ca}_6(\text{PO}_4)_{10}\text{CO}_3$ formation is supposed due to the Ca/P ratio of near 1.6 and NH_3 gas release. OH^- ion in the solution is increased with the soaking time according to Equation (2) until the NH_3 gas releases. Consequently no OH^- can be involved into the formation of $\text{Ca}_6(\text{PO}_4)_{10}(\text{OH})_2$.

Table 3. Compositions of the precipitated CaP (white particles in SEM) detected by EDS (at%)

	C	O	Na	Mg	K	Cl	Ti	P	Ca	Ca/P
U2	43.9	13.5	11.5	1.4	0.9	15.2	0.2	4.9	7.9	1.61
U10	35.9	15.6	14.6	1.4	3.3	14.0	0.2	4.8	7.9	1.64
U30	34.0	11.4	16.9	1.0	0.3	25.0	0.6	4.1	6.7	1.63
D2	50.0	17.9	7.5	1.9	0.5	6.6	2.6	5.1	7.8	1.53
D10	60.0	21.1	1.6	1.4	0.2	0.6	0.7	6.2	8.1	1.31
D30	43.1	29.2	4.0	2.2	0.5	0.4	4.8	6.8	9.1	1.34

4. Summary Remarks

An investigation of CaP precipitation on the hydrothermal treated Ti-6Al-4V by immersed in a simulated body fluid with urea addition under UV irradiation was carried out. The SBF used in this work is a sodium lactate and lactic acid buffered solution system. The release of NH₃ and CO₂ during the immersion tests was confirmed in consideration that detected NH₃ and CO₂ concentrations near the surface of the solutions are larger than those in the air. NH₃: 9-40 ppm, and CO₂: 543-1264 ppm. The release of two kinds of gases is related to the hydrolysis of NH₂CONH₂ which occurs both under UV lighting and under dark. NH₃ and CO₂ were first dissolved in the SBF solution, and then released. NH₃ release under UV lighting is slightly less than under dark, but CO₂ release under UV lighting is larger than under dark. Particularly, a larger CO₂ release was observed in the later immersion stage under UV lighting compared to those under dark. It seems UV lighting promotes CO₂ release, but limits NH₃ release.

No significant effect of the urea concentration within the range of 2 ~ 30 mM on CaP precipitation and NH₃ and CO₂ gas release have been observed. However, the photocatalysis caused by UV lighting had large influence on CaP precipitation and the ratio of Ca/P. The ratio of Ca/P near 1.6 and the CaP particles dotted in the crystallized NaCl-C-O matrix were observed on the photocatalytic titanium surfaces. A structure of embedded CaP particles into organic deposition matrix is considered as a primary stage of the formation of CaP-CO-NaCl layer. UV lighting causes the contents of Na and Cl increased, but C and O decreased.

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