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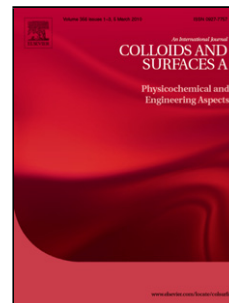
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Surface structure and in vitro apatite-forming ability of titanium doped with various metals

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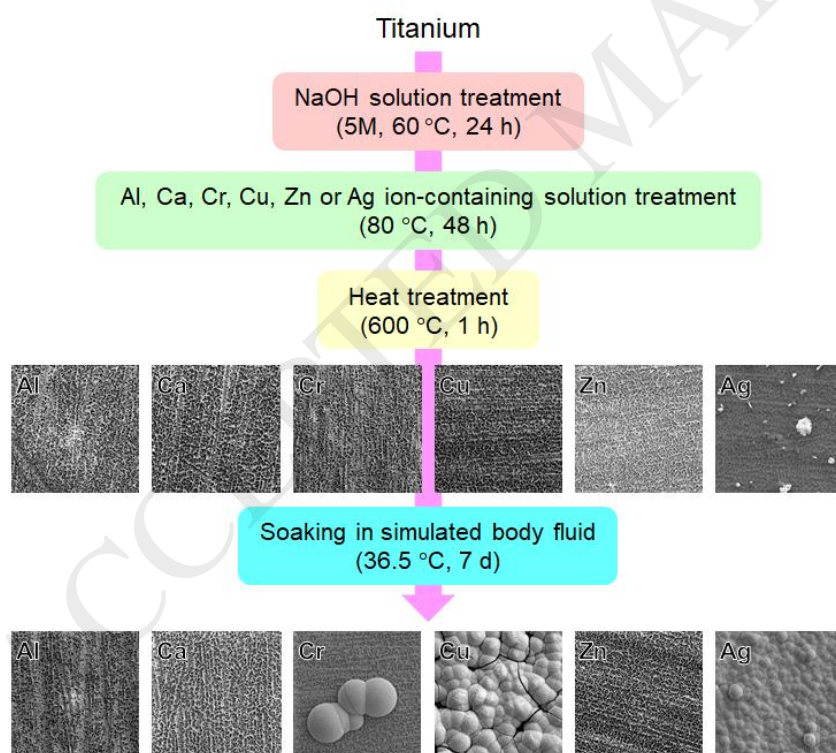
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Graphical abstract



Abstract

Surface modification by sodium hydroxide (NaOH) and subsequent heat treatment can provide titanium and its alloys an apatite-forming ability in simulated body fluid (SBF), and is used clinically to treat titanium alloy hip joints in Japan. However, there is no systematic study on the relationship between the amount and chemical state of metals incorporated into titanium and the apatite-forming ability in SBF. We have studied this relationship herein by treating NaOH-modified titanium with aqueous solutions containing different types (aluminum, calcium, chromium, copper, zinc, and silver) and concentrations (0.01, 0.1, and 1 M) of metal ions. As a result, we found that formation of anatase and the chemical states of the metals incorporated into titanium control the apatite-forming ability. Further, the chemical states of the metals can be interpreted in terms of the ionization tendency. These findings would aid future design of metal-doped bioactive titanium for orthopedic or dental implants.

Keywords: surface modification; bioactive titanium; simulated body fluid; apatite-forming ability

1. Introduction

Titanium and its alloys are frequently used as orthopedic and dental implants due to their good biocompatibility. However, the osteoconductivity of untreated titanium has scope for improvement. Various surface modifications have been applied to titanium and its alloys to induce bone-bonding ability (i.e., bioactivity). Of these, hydroxyapatite is the most representative; however, sodium hydroxide (NaOH) and subsequent heat treatment can also induce bioactivity to titanium and its alloys [1], and this technique has been used to produce bioactive titanium alloy hip joints that have been clinically used in more than 20,000 patients in Japan since 2007 [2]. Titanium and its alloys treated with NaOH and heat can form an apatite surface layer, through which they can bond to living bone when they are implanted into bone defects [3]. Apatite formation on titanium and its alloys can be reproduced in vitro in simulated body fluid (SBF) [4] with ion concentrations nearly equal to those of human plasma [5]. Although in vitro apatite-forming ability in SBF does not always lead to in vivo bone-bonding ability of artificial materials [6], it is often a good indicator.

Since the development of the NaOH and subsequent heat treatment, additional surface modifications using metal ion-containing aqueous solutions have been proposed to fabricate bioactive titanium with new functions such as antibacterial activity and enhanced osteoconductivity [7-13]. Antimicrobial activity can be induced in titanium by the incorporation of silver [7-9,13], and osteoconductivity can be enhanced by the incorporation of calcium or strontium [10,11]. Further, introducing zinc to the NaOH-modified titanium surface, by treatment with a solution containing $[\text{Zn}(\text{OH})_4]^{2-}$ complex, has been shown to

result in modified titanium implants with significantly stronger bone fixation [12].

The apatite-forming ability of surface-modified titanium is attributed to the formation of Ti-OH groups and surface charge [2], but there have been no systematic studies on the relationship between the amount and chemical state of doped metals and their apatite-forming ability in SBF. In this study, aqueous solutions containing different of metal ions (aluminum, calcium, chromium, copper, zinc, or silver) were prepared in concentrations of 0.01, 0.1, or 1 M, to investigate the relationship between the amount and chemical state of doped metals and apatite-forming ability in SBF as fundamental research. Like silver, copper can show antibacterial activity [14], and an antibacterial Ti-Cu alloy has been proposed [15,16]. Aluminum is added to titanium to produce alloys with high mechanical strength, such as Ti-6Al-4V [17,18]. Chromium is also an important alloy ingredient of Co-Cr-Mo alloys and offers excellent corrosion resistance [18,19]. Considering this, despite the fact that some of the resultant samples are unlikely to be used in clinical applications—aluminum oxide (Al_2O_3), a major chemical compound of aluminum, cannot show osteoconductivity [20], and hexavalent chromium ion (Cr^{6+}) is highly toxic [21]—the present findings are expected to aid future design of metal-doped bioactive titanium for orthopedic or dental implants.

2. Materials and methods

2.1. Sample preparation

Commercially available pure titanium plates (10 mm × 10 mm × 1 mm; purity, 99.9%; Kojundo Chemical Laboratory, Saitama, Japan) were abraded using No. 400 diamond abrasive paper and washed with pure acetone and ultrapure water in an ultrasonic cleaner. The Ti plates were then soaked in 5 mL of 5 M NaOH solution at 60 °C for 24 h followed by 7 mL of metal ion-containing aqueous solution at 80 °C for 48 h, and then gently washed with ultrapure water and dried. Special grade NaOH, aluminum nitrate nonahydrate ($\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$), calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), chromium(III) nitrate nonahydrate ($\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$), copper(II) nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$), zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), and silver nitrate (AgNO_3) (Wako Pure Chemical Industries, Osaka, Japan) were used in this study. The concentration of metal ions in the aqueous solutions was fixed at 0.1 M for samples treated with $\text{Al}(\text{NO}_3)_3$, $\text{Ca}(\text{NO}_3)_2$, and $\text{Cr}(\text{NO}_3)_3$ aqueous solutions, but concentrations of 0.01, 0.1, and 1 M were used for treatment with $\text{Cu}(\text{NO}_3)_2$, AgNO_3 , and $\text{Zn}(\text{NO}_3)_2$ aqueous solutions. Heat treatment of all samples was carried out at 600 °C. The samples were heated at a rate of 5 °C·min⁻¹ in a desktop high-temperature muffle furnace (MSFS-1218, Yamada Denki Co., Ltd., Tokyo, Japan), held at 600 °C for 1 h, and then naturally cooled to room temperature in the furnace. The sample names are listed in Table 1 along with the metal ions and the concentrations of solutions used in this study.

2.2. Characterization of sample surfaces

The surface structures of the treated titanium samples were investigated using a RINT-2200VL thin-film X-ray diffractometer (TF-XRD; Rigaku, Tokyo, Japan), a VE-8800 scanning electron microscope (SEM; Keyence, Osaka, Japan), and an AXIS Ultra DLD X-ray photoelectron spectrometer (XPS; Kratos Analytical, Manchester, U.K.). The following settings were used for the TF-XRD measurements: X-ray source, Ni-filtered Cu K α radiation; X-ray power, 40 kV, 40 mA; scanning rate, 4 $^\circ$ ·min $^{-1}$; sampling angle, 0.01 $^\circ$. The following settings were used for the XPS measurements: X-ray source, monochromatic Al K α radiation (1486.7 eV); X-ray power, 15 kV, 10 mA. The binding energy was calibrated using the C_{1s} photoelectron peak at 284.8 eV as a reference. XPS peak analysis was performed using CasaXPS Version 2.3.15 software, and the Shirley background was subtracted from all spectra prior to fitting. The atomic concentration was calculated from the XPS spectra using the specific relative sensitivity factors for the Kratos Axis Ultra (O_{1s}, 0.780; Ti_{2p}, 2.001; N_{1s}, 0.477; C_{1s}, 0.278; Al_{2p}, 0.193; Ca_{2p}, 1.833; Cr_{2p}, 2.427; Cu_{2p}, 3.547; Zn_{2p}, 3.726; Ag_{3d}, 5.987).

2.3. Evaluation of the apatite-forming ability of samples in simulated body fluid

Treated titanium samples were soaked at 36.5 $^\circ$ C in 30 mL of simulated body fluid (SBF) containing ions at concentrations of: Na⁺, 142.0 mM; K⁺, 5.0 mM; Ca²⁺, 2.5 mM; Mg²⁺, 1.5 mM; Cl⁻, 147.8 mM; HCO₃⁻, 4.2 mM; HPO₄²⁻, 1.0 mM; SO₄²⁻, 0.5 mM. These values are nearly identical to those found in human blood plasma, according to the ISO 23317:2014 standard. After being immersed in SBF for 7 days, the samples were removed and gently washed with ultrapure water.

3. Results and discussion

3.1. Effect of metal type on surface structure and apatite-forming ability of samples

To discuss the effect of metal type on surface structure and apatite-forming ability of doped Ti samples, we will first consider only samples treated by solutions with a 0.1 M concentration of metal ions (samples names beginning with 01). As shown in the SEM micrographs in Fig. 1, fine porous network structures formed on the surface of all these samples, while small particles were observed on the porous surface structure of sample 01-Ag. The atomic concentrations of the metals doped on the surfaces of the samples—calculated from the XPS spectra—differed depending on the type of metal, but with no apparent relationship (Table 2).

Figure 2 shows the TF-XRD patterns of the samples. All of the samples displayed TF-XRD peaks ascribed to metallic titanium (PDF #01-089-3725), anatase (PDF #01-072-7058), and rutile (PDF #01-077-0445), whereas samples 01-Cu, 01-Zn, and 01-Ag had additional TF-XRD peaks ascribed to copper oxide (CuO) (PDF #01-080-1916), zinc titanate (ZnTiO₃) (PDF #01-089-0190), and metallic silver (PDF #03-065-2871), respectively. These results

indicate that aluminum, calcium, and chromium ions were mainly incorporated on the sample surface in an amorphous state, while copper, zinc, and silver were mainly incorporated on the sample surface in a crystalline state. It is believed that the small particles that formed on the porous surface structure of sample 01-Ag (Fig. 1) are metallic colloids as reported in previous studies [7-9].

Figure 3 shows the Al_{2p} , Ca_{2p} , Cr_{2p} , Cu_{2p} , Zn_{2p} , and Ag_{3d} XPS spectra. In samples 01-Al, 01-Ca, 01-Cr, 01-Zn, and 01-Ag, the doped aluminum, calcium, chromium, zinc, and silver formed Al–O (around 74 eV) [22], Ca–O (around 350 and 347 eV) [23], Cr–O (around 1045 and 1021 eV) [24], Zn–O (around 1045 and 1021 eV) [25] and Ag–Ag (around 374 and 368 eV) [26,27] bonds, respectively. In sample 01-Cu, the Cu_{2p} XPS peaks were ascribed not only to Cu–O (around 962 and 934 eV) [28] but also Cu–Cu (metallic copper, around 952 and 933 eV) [28]. Figure 4 shows the Na_{KLL} XPS spectra of the samples, in comparison with those subjected to NaOH treatment or NaOH and heat treatments. In samples subjected to NaOH treatment or NaOH and heat treatments, the sodium formed Na–O bonds (around 496 eV) [29], but no remarkable XPS peak was observed around 496 eV for the other samples, with the exception of 001-Zn, 01-Zn, and 1-Zn, which produced a Zn_{LMM} Auger line around 498 eV that can be ascribed to Zn–O bonds [30]. This suggests that the majority of Na^+ ions introduced during NaOH modification were replaced by metal ions from the aqueous solutions.

According to the results in Figs. 1-3, it can be concluded that aluminum, calcium, chromium, and zinc were incorporated into the surface of the samples to form chemical compounds, binding to titanium and/or oxygen, whereas silver and copper were incorporated into the surface of samples as metallic colloids with partially oxidized surfaces. These results suggest that the chemical states of doped metals differ depending on the type of metal, which might be related to the standard electrochemical potential. The standard electrochemical potentials for Cu^{2+}/Cu and Ag^+/Ag are positive (+0.3419 V and +0.7996 V, respectively) [31], which means that these metals are more likely to be in a metallic state such as metallic colloids. In contrast, the values for Al^{3+}/Al , Ca^{2+}/Ca , Cr^{3+}/Cr , and Zn^{2+}/Zn are all negative (–1.662 V, –2.868 V, –0.744 V, and –0.7618 V, respectively) [31], which means that these metals are more likely to remain in the ionic state and bond to titanium and oxygen to form titanate compounds. Notably, the copper colloids cannot be seen in the micrographs (Fig. 1), likely because they are too small to be observed by SEM, although the reason why silver and copper colloids are of different sizes is currently unclear.

Figures 4 and 5 shows SEM micrographs and TF-XRD patterns, respectively, of the samples soaked in SBF for 7 days. Samples 01-Cu and 01-Ag formed apatite on their surfaces, whereas sample 01-Cr partially formed apatite and samples 01-Al, 01-Ca, and 01-Zn did not. Similarly, it has been reported previously that titanium treated with NaOH, calcium chloride ($CaCl_2$), and heat does not form apatite on its surface in SBF [9].

Apatite deposition in SBF on titanium subjected to NaOH and heat treatments is triggered by ion exchange between the treated titanium and the SBF [1,2]. Furthermore, the apatite-forming ability is lost in a humid environment [10]. These findings suggest that the release of metal ions from titanium to SBF could lead to apatite formation. In this study, the Na^+ ions introduced by NaOH treatment were replaced with other metal ions, hence the release of these metal ions to SBF might be important for apatite deposition. Although we have not measured the concentration of metals released from samples into SBF, the released amounts of copper and silver from samples 01-Cu and 01-Ag might be higher than those of aluminum, calcium, chromium, and zinc from samples 01-Al, 01-Ca, 01-Cr, and 01-Zn, because copper and silver form metallic colloids that are not chemically bonded to the titanium substrate, whereas aluminum, calcium, chromium, and zinc are bound by stable chemical compounds to titanium and/or oxygen (Figs. 2 and 3); furthermore, silver incorporated on the surface of titanium is known to release into phosphate buffered saline more easily than calcium or zinc [7,32]. Therefore, the difference in the apatite-forming abilities of the samples (Fig. 1) can be interpreted in terms of the difference in chemical state of the doped metals.

On the other hand, sample 01-Cr partially formed apatite on its surface (Fig. 1) even though chromium was not incorporated on the sample surface as metallic colloids (Figs. 2 and 3). In this sample, anatase is thought to be responsible for the apatite deposition [33] because, as shown in TF-XRD patterns in Fig. 2, the intensity ratio of the anatase peak to the rutile peak was greater in sample 01-Cr than that in the samples with no apatite-forming ability (01-Al, 01-Ca, and 01-Zn). Furthermore, we have previously reported that anatase might play an important role in apatite formation for NaOH- and heat-treated titanium [34]. The reasoning behind the variation in the ratio of anatase to rutile vary for samples with different metal ions should be examined in the future.

In summary, it is believed that control of both the chemical states of metals and the crystalline structure of TiO_2 are essential to obtain titanium with in vitro apatite-forming ability by chemical and heat treatments.

3.2. Effect of ion concentration on surface structure and apatite-forming ability of samples

Next, we will discuss the effect of the concentration of the metal ion-containing solution on surface structure and apatite-forming ability. Samples [001/01/1]-[Cu/Zn/Ag] were treated in 0.01, 0.1, and 1 M aqueous solutions of copper, zinc, and silver ions. According to the SEM micrographs in Fig. 1, only sample 1-Cu had a flat surface. This might be because the fine network structure which was formed by NaOH treatment was dissolved by the acidic 1 M $\text{Cu}(\text{NO}_3)_2$ aqueous solution. In fact, the pH values of 0.01, 0.1 and 1 M $\text{Cu}(\text{NO}_3)_2$ aqueous solutions were 4.8, 4.2, and 2.7, respectively. Small particles were formed on samples 001-Ag, 01-Ag, and 1-Ag, i.e., irrespective of the silver concentration. In samples 001-Cu, 01-Cu,

001-Zn, 01-Zn, and 1-Zn, the fine network structure formed by NaOH modification remained after treatment with the metal ion-containing aqueous solution. On the other hand, according to the TF-XRD patterns in Fig. 2, the concentration of the solutions hardly affected the crystalline structure of sample surface.

According to the XPS spectra in Fig. 3, the concentrations of the zinc and silver ion-containing solutions did not affect the chemical state of the zinc and silver at the sample surfaces. The atomic concentration of zinc in samples 001-Zn and 01-Zn was around 13 at.%, and was slightly lower in sample 1-Zn (Table 2), indicating that the amount of zinc doped on the sample surface saturates at around 13 at.%, even when in solutions with low concentrations (0.01 M). The atomic concentration of silver on the surface increased from 5.1 to 6.8 at.% with increasing concentration of solution, but this increase is extremely small compared to the difference in the silver concentrations in the solution, suggesting that the atomic concentrations of silver saturated at around 6 at.%. Interestingly, the chemical state of deposited copper had a different relationship with the concentration in the solution. That is, the atomic concentrations of metallic copper in samples 001-Cu (Cu–Cu, 3.6 at.%) and 01-Cu (Cu–Cu, 5.3 at.%) were higher than that in sample 1-Cu (Cu–Cu, 1.0 at.%). Furthermore, the total atomic concentration of copper decreased from 12.1 to 7.8 at.% as the concentration in solution increased. The low concentration of copper in sample 1-Cu might be attributed to the dissolution of the fine network structure by the acidic 1 M $\text{Cu}(\text{NO}_3)_2$ aqueous solution (Fig. 1).

Samples 001-Cu, 01-Cu, and 1-Cu formed apatite in SBF, but the apatite-forming ability of sample 01-Cu was higher than that of samples 001-Cu and 1-Cu (Figs. 5 and 6). As discussed in the section 3.1, the difference in the apatite-forming ability of these samples might be related to the difference in the chemical states of copper. According to Fig. 3 and Table 2, the copper exists mainly as metallic colloids (Cu–Cu) in sample 01-Cu, whereas samples 001-Cu and 1-Cu have more Cu–O bonds, suggesting that more copper may have been bound to the surface by the formation of chemical compounds. Therefore, it is believed that copper is released more easily from sample 01-Cu, which results in the high apatite-forming ability. Samples 001-Zn, 01-Zn, and 1-Zn did not form apatite in SBF irrespective of the zinc concentrations. As discussed in Section 3.1, this might be because zinc exists as stable chemical compounds such as ZnTiO_3 in these samples (Figs. 2 and 3) and does not release into SBF. In the discussion about the difference in the apatite-forming ability among samples 01-Cr, 01-Al, 01-Ca, and 01-Zn in Section 3.1, it was suggested that anatase plays an important role in apatite formation. Although anatase was formed on samples 001-Zn, 01-Zn, and 1-Zn, more rutile formed than anatase or ZnTiO_3 (Fig. 2). The preferential formation of rutile and/or ZnTiO_3 might be responsible for the low apatite-forming ability of samples 001-Zn, 01-Zn, and 1-Zn. Samples 001-Ag, 01-Ag, and 1-Ag formed dense and uniform apatite layers on their surfaces irrespective of the silver concentration. The structure and

apatite-forming ability of titanium subjected to similar NaOH, AgNO₃, and heat treatments have been reported previously [9], but the concentrations of silver ions (10⁻⁵, 10⁻⁴, and 10⁻³ M) were lower than those in the present study (10⁻², 10⁻¹, and 10⁰ M). Apatite formation in SBF has been confirmed both in the present and in previous studies, but the surface structure of the samples differed slightly. That is, the silver existed as metallic colloids in both studies, but while some was incorporated into sodium titanate in the previous study [9], none was incorporated into titanate in the present study (Figs. 2 and 3). These results also support our hypothesis that the chemical states of metals control the apatite-forming ability of samples in SBF. Nevertheless, *in vitro* and *in vivo* tests should be conducted in future for the present samples, because as described in the Introduction, *in vitro* apatite-forming ability in SBF does not always lead to bioactivity of samples.

4. Conclusions

We treated NaOH-modified titanium with aqueous solutions containing different types (aluminum, calcium, chromium, copper, zinc, and silver) and concentrations (0.01, 0.1, or 1 M) of metal ions, to investigate the relationship between the amount and chemical state of doped metals and the apatite-forming ability in SBF. Chromium, copper, and silver-doped titanium formed apatite in SBF, and the apatite-forming ability can be interpreted in terms of the formation of anatase as well as the chemical states of the metals incorporated into the surface of titanium. Moreover, the chemical states of the metals can be associated with the standard electrochemical potential. *In vitro* and *in vivo* tests for the present samples should be conducted in a future study, but the present findings are anticipated to be constructive for future design of metal-doped bioactive titanium for orthopedic or dental implants.

Acknowledgments

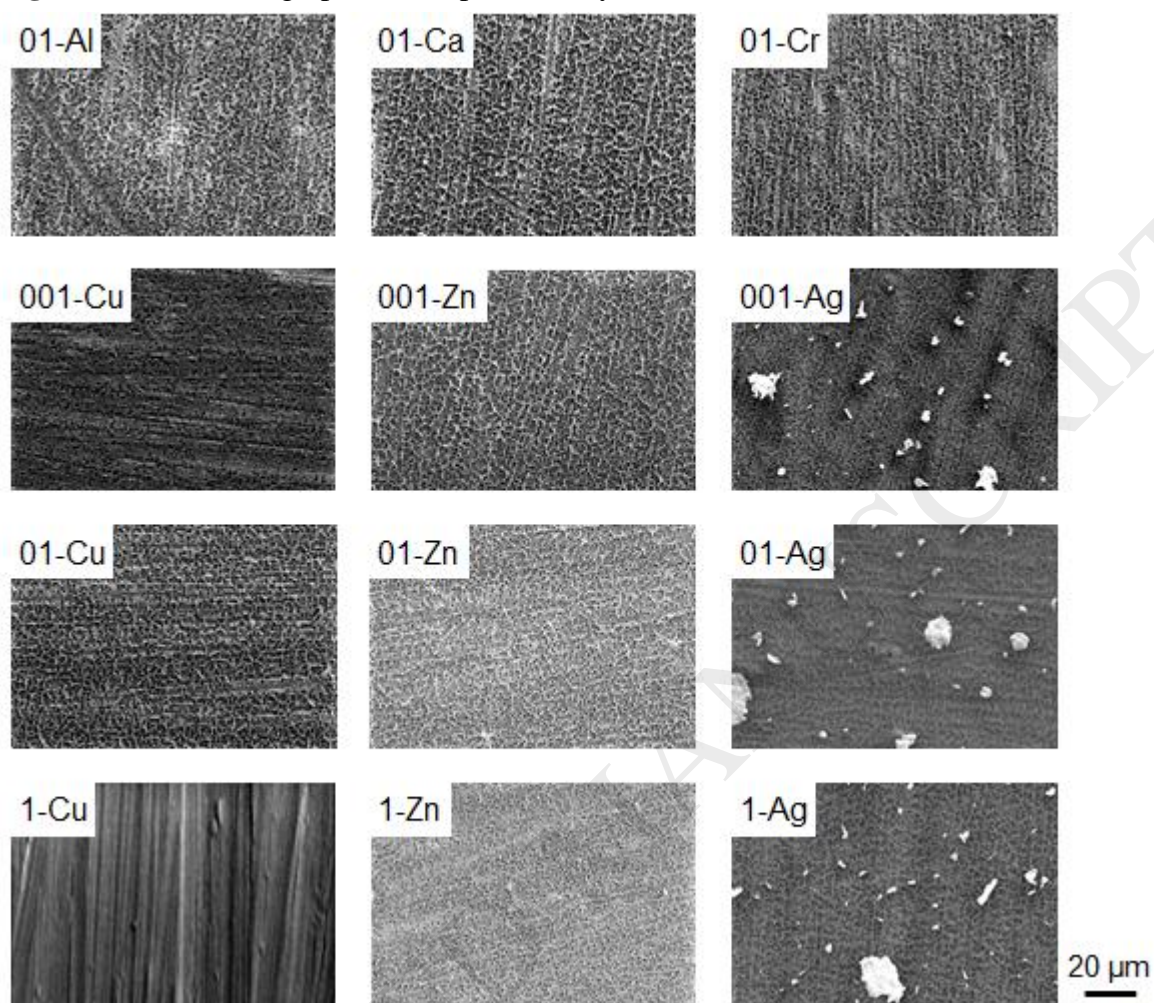
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Figure Captions**Fig. 1** SEM micrographs of samples after synthesis.**Fig. 2** TF-XRD patterns of samples after synthesis.

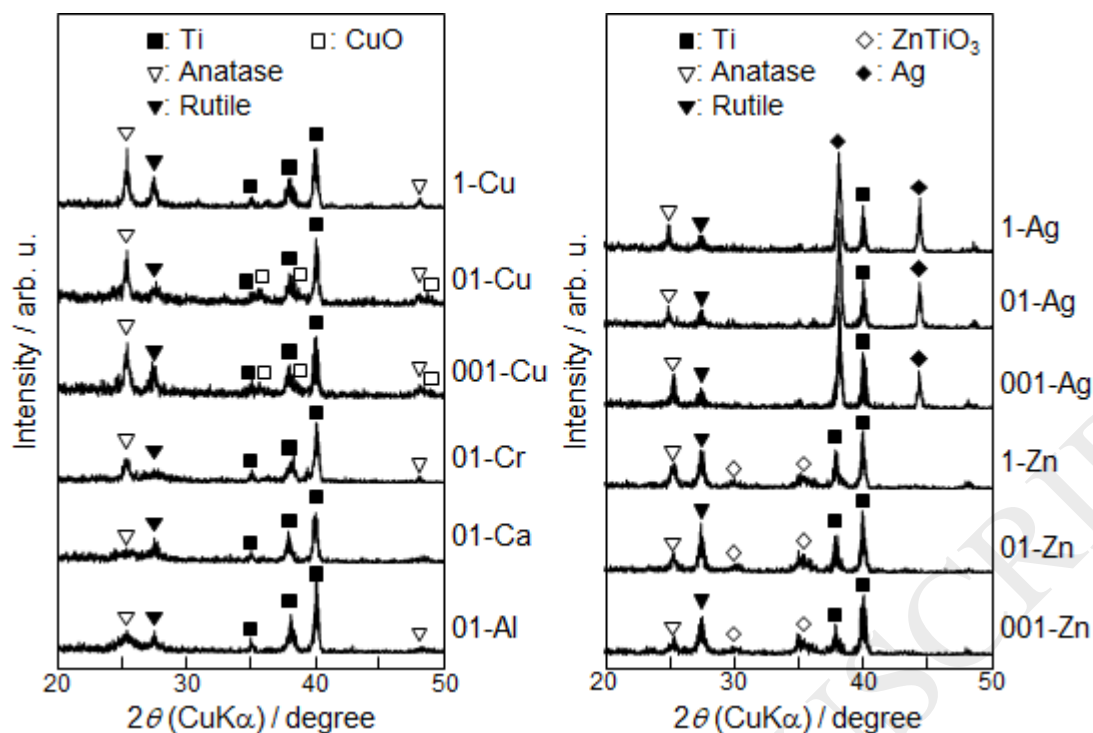


Fig. 3 Al_{2p} , Ca_{2p} , Cr_{2p} , Cu_{2p} , Zn_{2p} , and Ag_{3d} XPS spectra of samples after synthesis.

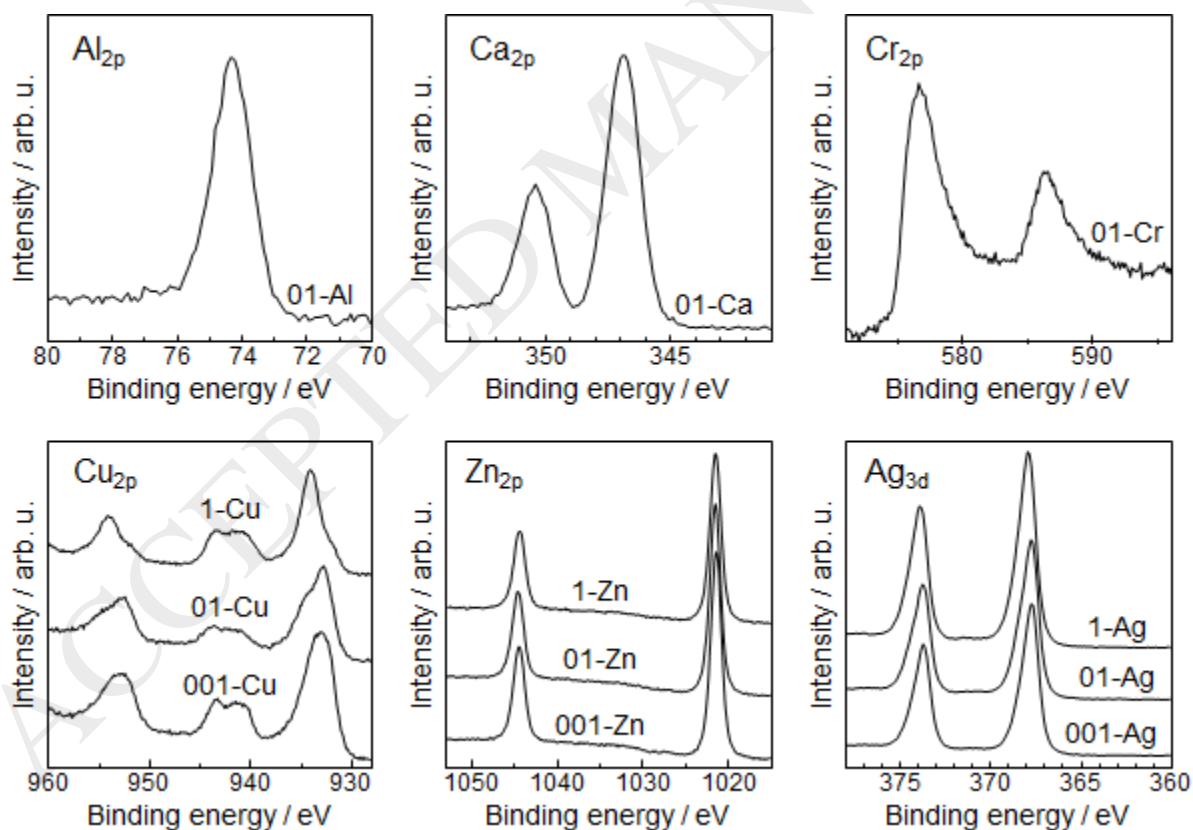


Fig. 4 Na_{KLL} XPS spectra of samples after synthesis, in comparison with those of samples subjected to NaOH treatment or NaOH and heat treatments.

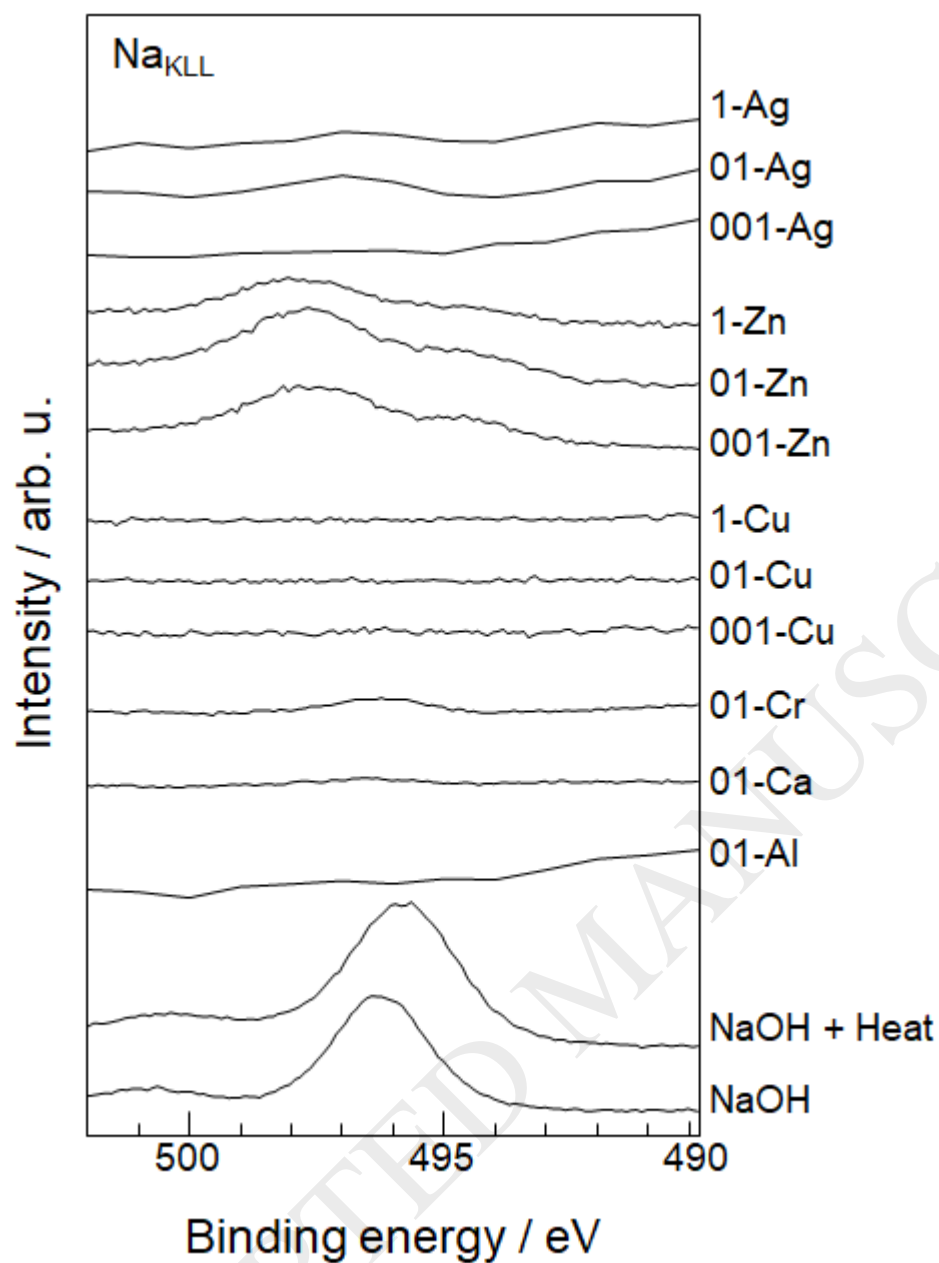


Fig. 5 SEM micrographs of samples after soaking in SBF for 7 days.

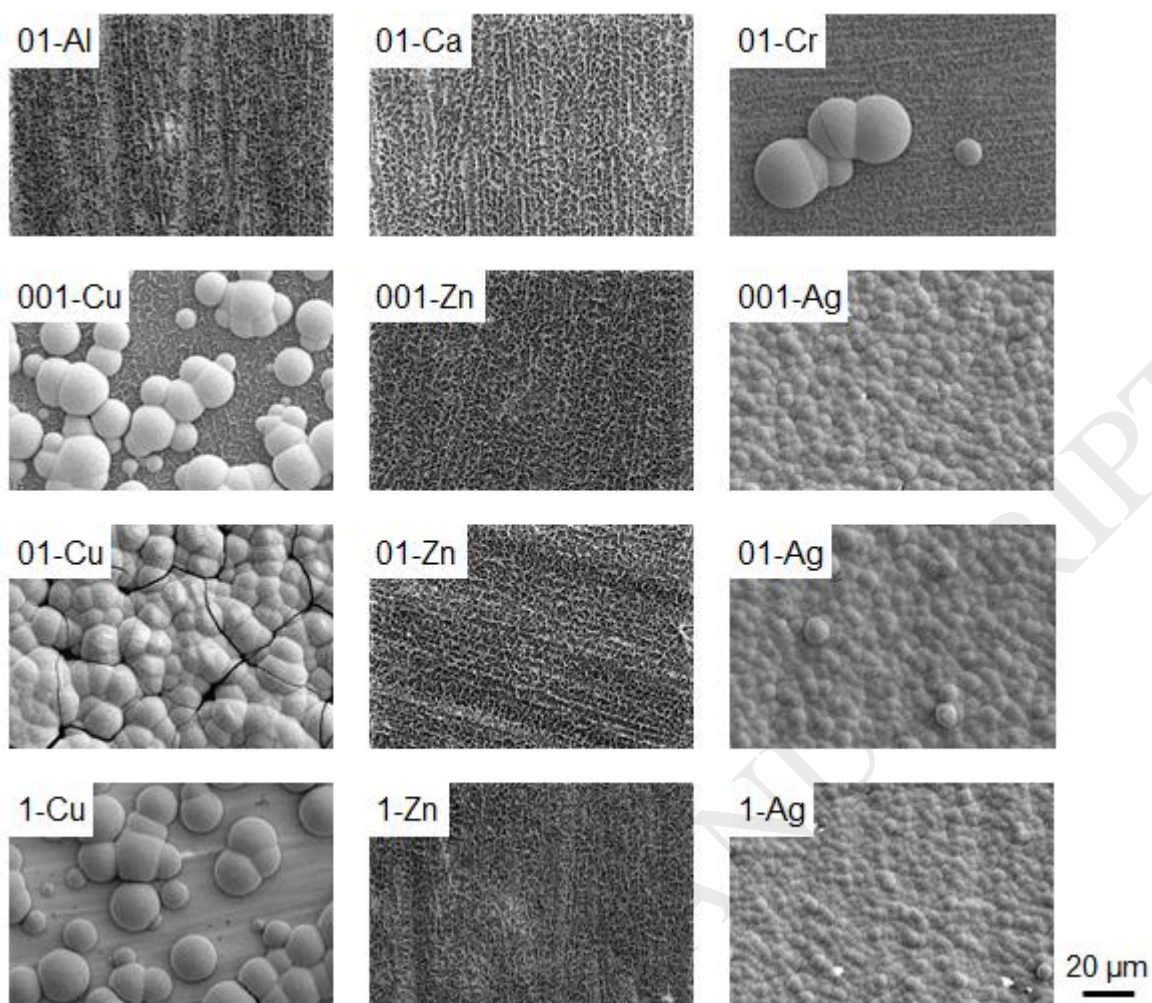


Fig. 6 TF-XRD patterns of samples after soaking in SBF for 7 days.

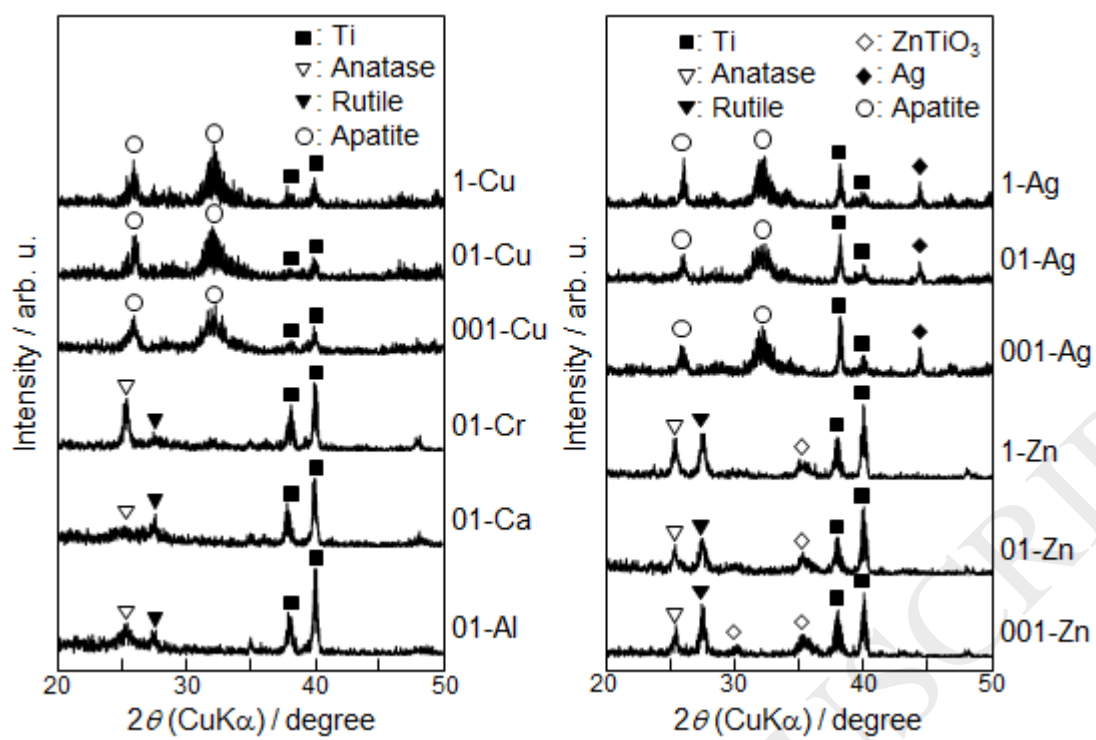


Table captions

Table 1 Sample names with the type and concentration of metal ions in solutions used in this study.

Table 2 Atomic concentrations of metals doped on the surfaces of samples.

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Table 1 Sample names with the type and concentration of metal ions in solutions used in this study.

Sample name	Metal ion	Metal ion concentration [M]
01-Al	Al ³⁺	0.1
01-Ca	Ca ²⁺	0.1
01-Cr	Cr ³⁺	0.1
001-Cu	Cu ²⁺	0.01
01-Cu	Cu ²⁺	0.1
1-Cu	Cu ²⁺	1
001-Zn	Zn ²⁺	0.01
01-Zn	Zn ²⁺	0.1
1-Zn	Zn ²⁺	1
001-Ag	Ag ⁺	0.01
01-Ag	Ag ⁺	0.1
1-Ag	Ag ⁺	1

Table 2 Atomic concentrations of metals doped on the surfaces of samples.

Sample	Surface concentration of metal [at.%]
01-Al	7.8
01-Ca	6.3
01-Cr	8.0
001-Cu	12.1 (Cu–Cu: 3.6, Cu–O: 8.5)
01-Cu	8.9 (Cu–Cu: 5.3, Cu–O: 3.6)
1-Cu	7.8 (Cu–Cu: 1.0, Cu–O: 6.8)
001-Zn	13.0
01-Zn	13.2
1-Zn	10.9
001-Ag	5.1
01-Ag	5.9
1-Ag	6.8