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# **Suppression of fluoride-induced corrosion of titanium by albumin in oral modified environment**

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## **Abstract**

Although titanium was corroded by fluoride in the oral environment, influence of the protein on the corrosion was not clarified in detail. The objective of this study was to investigate suppression of fluoride-induced corrosion of titanium where albumin was either present in a solution or where albumin was pre-adsorbed on titanium. Titanium dissolution in titanium specimens and surface characterization of each specimen were determined. Dissolution in a saline solution containing both albumin and fluoride was less than that in only fluoride-containing saline solution. The titanium specimen was covered in an albumin film. The morphology of the titanium in the fluoride-containing saline solution revealed jagged edges, whereas titanium immersed in the saline solution containing albumin and fluoride showed a round and plate-like morphology. When albumin-adsorbed titanium was immersed in a fluoride-containing solution, dissolution within 6-h immersion was less than that in non albumin-adsorbed titanium. Dissolution increased with desorption of adsorbed-albumin from the titanium surface. The results suggest that albumin in a solution suppresses dissolution of titanium compounds, thus influencing their morphology. Albumin adsorbed on titanium reduces fluoride attack and suppresses dissolution.

*(181 words)*

*Keywords:* Titanium; Albumin; Fluoride; Corrosion; Surface Characterization

Running heads: Suppression of fluoride-induced corrosion of titanium by albumin

## **1. Introduction**

Dental implants, orthodontic wires, and denture bases made of titanium and its alloys are widely used in clinical dentistry. However, such devices have been reported to show discoloration or corrosion<sup>1-3</sup>. The dissolution of metal ions that such discoloration or corrosion involves can present problems in terms of aesthetic considerations or allergic disease. One reason for discoloration and corrosion is the presence of fluoride in prophylactic agents used to help prevent dental caries<sup>4-6</sup>. Titanium is generally believed to possess good biocompatibility and resistance to corrosion due to a closely packed surface oxide film<sup>7-9</sup>. However, this oxide film can be broken or corroded by fluoride. Corrosion of titanium by fluoride depends on certain conditions; such as pH, concentration of dissolved oxygen and fluoride concentration<sup>10-20</sup>.

Recently, some reports have suggested that the resistance of titanium and its alloys to corrosion by fluoride is influenced by salivary proteins such as albumin<sup>21-24</sup>, with results indicating that fluoride-induced corrosion of titanium on electrochemical corrosion behavior is suppressed by addition of albumin to fluoride-containing solutions. However, one of these studies noted that dissolution of titanium from titanium plate in a saline solution containing both fluoride and albumin was not affected by the presence of albumin<sup>21</sup>. Although this report suggested that proteins such as albumin influenced resistance to fluoride-induced corrosion, this remains to be clarified.

Elucidating the role of albumin in the dissolution of titanium would help clarify the potential degree of corrosiveness in an oral environment in which a prophylactic agent was used. Therefore, the objective of this study was to determine whether albumin enhanced resistance to fluoride-induced corrosion in titanium by investigating suppression of corrosion by albumin under two potential scenarios: one where albumin was present in a solution; and another where the albumin was adsorbed on titanium.

## **2. Experimental Procedure**

### 2.1 Specimens and solutions

Titanium specimens 13 mm in diameter and 2 mm in thickness were cut from a rod of commercially available pure titanium (Ti grade 2, Kobe Steel, Japan). The specimens were polished with the following series of silicon carbide papers: No. 120, 240, 400, and 600. They were then ultrasonically washed for 5 min 3 times in acetone and distilled water.

Three solutions were used: 1) a fluoride-containing saline solution consisting of 9.00 g sodium chloride (NaCl; Wako Chem., Osaka, Japan) and 2.00 g sodium fluoride (NaF; Wako Chem., Osaka, Japan) in 1 L distilled water; 2) a fluoride- and albumin-containing saline solution consisting of 9.00 g NaCl, 2.00 g NaF, and 1.00 g bovine serum albumin (BSA; Wako Chem., Osaka, Japan) in 1 L distilled water; and 3) a pre-treatment solution for preparation of albumin-adsorbed titanium specimens consisting of 9.00 g NaCl and 1.00 g BSA in 1 L distilled water. All solutions were adjusted to pH 5.0 using lactic acid (Wako Chem., Osaka, Japan) at 37°C.

Albumin-adsorbed titanium specimens were prepared by immersion in the pre-treatment solution at 37°C for 4 h, followed by rinsing with distilled water. Titanium and albumin-adsorbed titanium were immersed in 35 mL of each test solution at 37°C. Non albumin-adsorbed titanium specimens were immersed in the saline solution containing fluoride or the saline solution containing albumin and fluoride (denoted as NAF and AFS, respectively); the albumin-adsorbed titanium specimens were immersed in the saline solution containing fluoride (denoted as CAF). The specimen codes and preparation method are summarized in Table 1. After immersion for 0.5, 1, 3, 6, 12, or 24 h, the specimens were retrieved and washed with distilled water. Three specimens were subjected to each condition.

## 2.2 Analysis of Dissolved Titanium and Surface Characterization

The titanium concentrations in the test solutions and pH of each solution after specimen immersion were determined with an inductively coupled plasma emission spectrometer (ICP; Vista-MPX, SII, Japan) and a pH meter (BASIC, Denver Instrument, USA), respectively.

All titanium specimens were examined with an X-ray photoelectron spectroscope (XPS; SSX-100, SSI, USA) and a scanning electron microscope (SEM; JSM-6340F, JEOL, Japan) at both

before and after immersion. All XPS spectra were excited with a monochromatized Al K $\alpha$  (1486.6 eV). The take-off angle for photoelectrons was at 35° to the specimen surface. The spectrometer was calibrated using pure gold and pure copper according to the method of Asami et al.<sup>25</sup>. Binding energy and decomposition of the spectra were determined by means of empirical data<sup>25-27</sup>. The specimens observed under SEM were coated with Au-Pd alloy. Observation was performed at an accelerating voltage of 15 kV and an emission current of 12  $\mu$ A.

### 2.3 Statistical Analyses

Concentration of dissolved titanium and pH were statistically analyzed using a two-way analysis of variance (ANOVA) at a significance level of 95%. Comparison among conditions was carried out with the Scheffe test at a significance level of 95%.

## **3. Results**

### 3.1 Dissolution Behavior

Fig. 1 (a) shows amount of dissolved titanium and Fig. 1 (b) shows change in pH value after immersion, with or without albumin adsorption, at each time period. Dissolution of titanium was detected in all specimens, with amount of dissolved titanium increasing with period of immersion. Within 6-h immersion, CAF showed the lowest level of dissolution ( $p < 0.05$ ). After 12- or 24-h immersion, no significant difference was observed in amount of dissolved titanium among the solutions ( $p > 0.05$ ). Changes in pH, as shown in Fig. 1 (b), indicated a similar pattern of behavior to that of titanium dissolution, with pH value in each solution increasing with period of immersion.

### 3.2 Surface Observation

Fig. 2 (a)-(f) shows the SEM images of each specimen after 3- or 24-h immersion in each test solution. After 3-h immersion, a slight degree of pitting or initial crevice was observed in NAF and AFS, but not in CAF. After 24-h immersion, all specimens showed a rough surface over the entire specimen. The morphology of each specimen, however, differed. Dissolution was characterized by sharp jagged edges in NAF, whereas AFS showed round, plate-like substances.

On the other hand, CAF showed both sharp jagged edges, as seen in NAF, and plate-like substances, as observed in AFS.

### 3.3 XPS analysis

Figs. 3 and 4 show the C1s and N1s XPS spectra of each specimen with immersion up to 3 h. Carbon and nitrogen in NAF originated in the contaminated layer, which covered the entire surface of the specimen. The C1s spectra for AFS showed a peak and shoulder close to 288.0 and 286.3 eV, larger than those for NAF. This was attributed to peptide bonding (-CO-NH-) and amino (-C-NH<sub>2</sub>) or carboxyl groups (-C=O, -C-OH) of albumin, indicating albumin adsorption onto the specimens. In addition, the N1s spectra also suggested peptide bonding (-CO-NH-) and amino groups (-C-NH<sub>2</sub>), indicating adsorption of albumin. Peaks originating in amide of albumin were detected in CAF prior to immersion. These peaks showed a decrease at 3-h immersion.

Fig. 5 shows the O1s XPS spectra of each specimen after immersion up to 3 h. NAF showed at least 3 peaks originating in metal oxide (O<sup>2-</sup>), hydroxide or hydroxyl groups (OH), or hydrate and/or adsorbed water (H<sub>2</sub>O), with their peaks appearing close to peaks of 530.5, 532.0, and 533.5 eV, which were found in earlier studies, respectively <sup>25, 26</sup>. The spectra did not depend on immersion period. Large peaks of hydroxyl and hydrate groups were detected in AFS and CAF. These peaks may have resulted from peptide bonding and carboxyl groups of albumin. With CAF, peaks originating from carboxyl groups of albumin showed a decrease, whereas peaks originating from titanium oxide with longer immersion showed an increase.

Fig. 6 shows the F1s XPS spectra of each specimen after immersion for up to 3 h. With immersion for 0.5 h, NAF showed a peak originating from fluoride. No fluorine was detected in AFS, or in CAF at before and after 0.5-h immersion. A small peak close to 684.8 eV detected in CAF after 1-h immersion showed a clear increase at 3-h immersion. This indicated the presence of fluoride on the CAF at after 1-h immersion.

## **4. Discussion**

Fluoride-induced corrosion of titanium is caused by dissolution of the passive film on titanium by attack by hydrofluoric acid generated from fluoride ions in an acidic environment<sup>10-24</sup>. The pH (5.0) and fluoride concentration (0.2%) of solution in this study were determined according to previous studies, because titanium was progressively corroded by fluoride<sup>21,23</sup>. On the other hand, albumin in saliva, blood and body fluids bonds to titanium by electrostatic interaction, and is related to the biocompatibility of titanium alloys<sup>28,29</sup>. Equilibrium of albumin levels in the human body is achieved by repeated cycles of adsorption and desorption<sup>30-32</sup>. Therefore, albumin adsorption is potentially an important factor in the consideration of resistance to fluoride-induced corrosion in titanium.

Some reports on electrochemical corrosion have indicated that albumin in solution suppressed corrosion of titanium by fluoride<sup>21-24</sup>. Although the outermost surface of the titanium specimen was covered with albumin, dissolution was seen with immersion in a saline solution containing fluoride and albumin<sup>22,23</sup>. Saliva contains approximately 0.2% organic compounds including salivary protein of 200-500 mg/100 mL<sup>33</sup>. Albumin, which was used in this study, is mainly a typical protein in saliva, and the amount was chosen close to the amount in salivary protein. In this study, we investigated corrosion of titanium by fluoride under two scenarios: one where albumin was present in a fluoride-containing solution, and another where albumin was adsorbed onto the titanium prior to contact with the fluoride.

#### 4.1 Albumin-containing solution

According to XPS analysis, carbon, nitrogen and oxygen originating in albumin were detected on titanium after 0.5-h immersion in saline solution containing both albumin and fluoride, whereas no fluoride was detected. On the other hand, dissolution was confirmed within 3-h immersion in saline solution containing both albumin and fluoride. After 12-h immersion, rate of dissolution was almost the same, regardless of the presence of albumin. These results are partly similar to those of our previous study<sup>23</sup> showing that when titanium specimens were immersed in saline solution containing both albumin and fluoride for 3 days, the titanium surface was covered with an

albumin film.

Fukuzaki et al. suggested that proteins such as albumin had a higher adsorption rate when the pH of the surrounding solution was close to the zero point of the electric charge of the protein. The isoelectric point of bovine serum albumin is about 4.7<sup>34</sup>, indicating that the albumin is in an environment where it is easy to adsorb on titanium. One study noted a repeated cycle of protein adsorption and desorption on implanted materials in the body<sup>30</sup>. This suggests that the adsorption or bonding strength between albumin and the passive film on titanium is weak. If a 3-dimensional structure of albumin is inflexible, an adsorbed albumin film may be too rigid to prevent diffusion of ions and small molecules. Thus, hydrofluoric acid converted from fluoride ions in solution may come into contact with titanium by diffusion in the adsorbed albumin. This suggests that albumin adsorption and titanium dissolution occur simultaneously at the initial stage of immersion. As the adsorbed albumin has a flexible structure, the dissolution of titanium-fluorine compounds, such as  $TiF_3$ ,  $TiF_4$ , and  $TiOF_2$ <sup>10, 12, 14</sup>, may be delayed by the rate-determining factor.

Note the behavior of pH change in the solutions: the least change in pH was in the saline solution containing both albumin and fluoride. Bovine serum albumin partly consists of acidic amino acids such as aspartic acid and glutamic acid, and basic amino acids such as lysine, arginine, and histidine. The electrostatic point of albumin is close to that of a solution with a pH of 5.0<sup>34</sup>. This suggests that the albumin buffer electric charge in the fluoride and titanium-fluorine compounds. Consequently, this buffering effect could prevent increase in pH. Furthermore, this buffering effect may contribute to the difference in the surface morphology of the titanium after dissolution.

Albumin formed a flexible film by adsorbing on titanium. This adsorbed film may be a rate-determining factor in the dissolution reaction of titanium-fluorine compounds. Furthermore, albumin in a solution may prevent conversion of fluoride to hydrofluoric acid by the buffering effect, delaying corrosion of titanium.

#### 4.2 Albumin adsorbed on titanium

This study investigated titanium immersed in a pre-treatment albumin-containing saline solution maintained at 37°C for 4 h. The XPS analysis suggested albumin adsorption on the titanium. Immersion in the pre-treatment solution resulted in adsorption of albumin, which was then gradually released over time with immersion in fluoride-containing solution. This agreed with the results of Williams et al., which suggested that the adsorption or bonding strength between albumin and passive film on titanium was weak and was characterized by repeated cycles of adsorption and desorption<sup>30</sup>. The passive film on titanium has hydroxyl groups and hydroxide which dissociate in solution and take on electric charge. Since the isoelectric point for titanium oxide is 5.6 for rutile type and 6.1 for anatase type, oxide has a larger positive than negative charge in solution with a pH of closer to 5<sup>32</sup>. Therefore, weakness in bonding strength was derived from the interaction between the negative charges of the albumin and the positive charges of the passive film. Taken together with the release of albumin from titanium found in this study, this suggests that there is an increasing chance of contact between fluoride and titanium.

In the present study, the albumin-absorbed titanium showed the least titanium dissolution within 3-h immersion, whereas dissolution increased abruptly at 6-h immersion. The XPS analysis of the albumin-adsorbed titanium indicated that the adsorbed albumin was gradually released from the titanium surface over up to 3-h immersion. As mentioned above, titanium dissolution from albumin-adsorbed titanium may occur with release of adsorbed albumin. This suggests that albumin adsorbed on titanium can prevent titanium dissolution for up to 3-h immersion. Consequently, protein-adsorbed film on titanium may temporarily delay discoloration and corrosion by fluoride.

The results of this study do not directly predict discoloration and corrosion of titanium in an oral environment, as other factors such as inorganic ions, amino acids and proteins in saliva must also be considered. Albumin on titanium or in a solution indicates the possibility of a reduction in the corrosion of titanium by fluoride. When titanium comes into contact with fluoride under a repeated cycle of adsorption and desorption over a long period of time, however, discoloration and/or corrosion of titanium may occur.

## **Conclusions**

This study investigated suppression of fluoride-induced corrosion of titanium by albumin either included in a solution or adsorbed on titanium. The results may be summarized as follows:

1. When both albumin and fluoride were included in the saline solution, dissolution was less than that in only fluoride-containing saline solution. Albumin adsorption was confirmed in the titanium immersed in the saline solution with both albumin and fluoride.
2. When albumin-adsorbed titanium came into contact with the fluoride-containing saline solution, dissolution within 6-h immersion was less than that in non albumin-adsorbed titanium.
3. SEM observation revealed a round and plate-like morphology with both immersion in albumin-containing solution and albumin-adsorbed titanium

In conclusion, albumin may be the rate-determining factor in fluoride attack on titanium and the dissolution of titanium-fluorine compounds, and may be associated with the resulting morphology with dissolution. This suggests that albumin adsorbed-titanium suppresses corrosion by fluoride, and that this suppression may be reduced if the adsorbed albumin is released.

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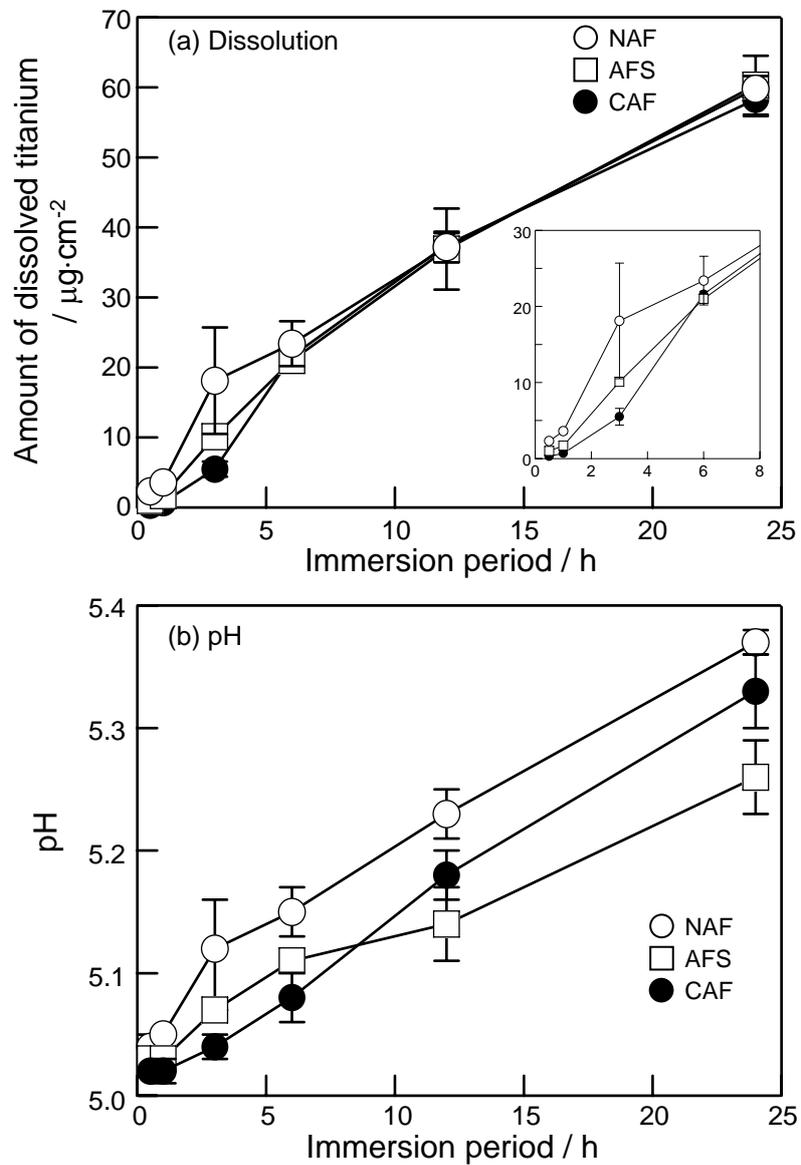


Fig. 1 Dissolution behavior in titanium specimens and changes in pH in acidic fluoride-containing saline solutions with or without albumin within 24 h.

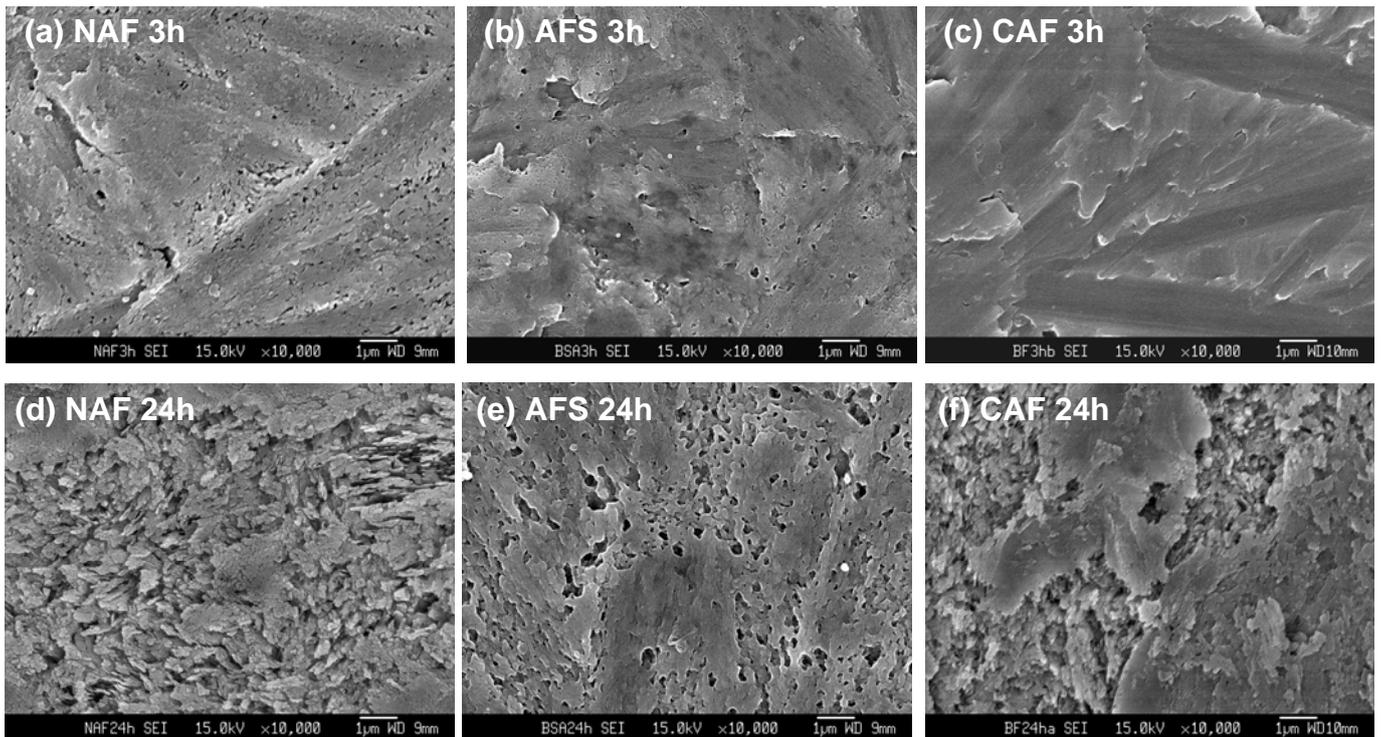


Fig. 2 SEM images of titanium specimens immersed in acidic fluoride-containing saline solutions with or without albumin. (a)~(c) after 3 h-immersion, (d)~(f) after 24 h-immersion

## C 1s

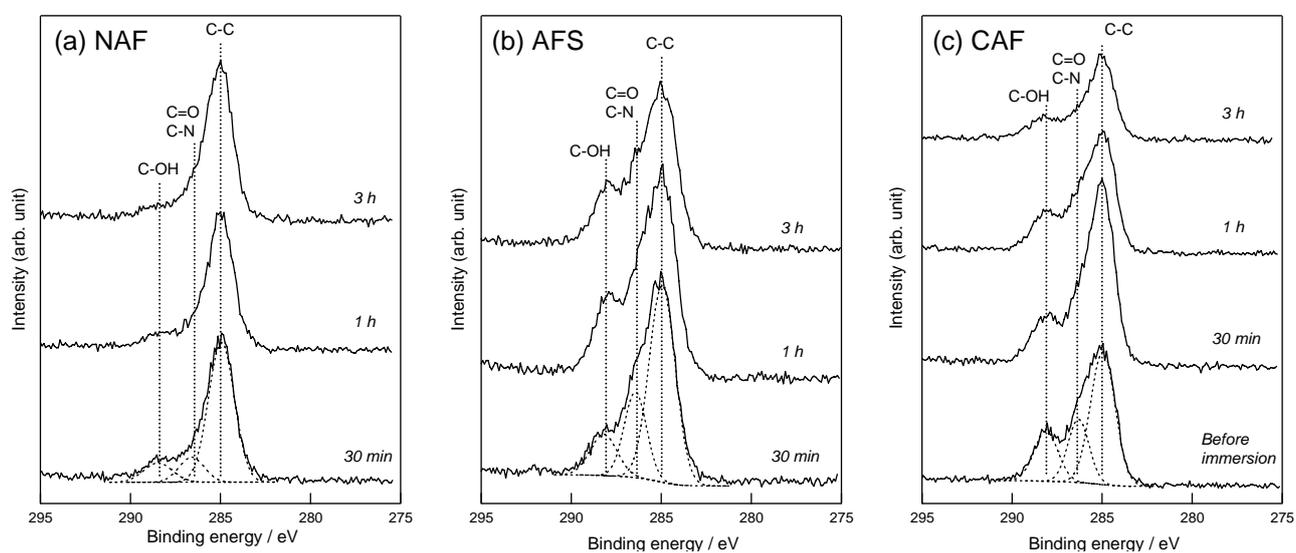


Fig. 3 C 1s XPS spectra of titanium specimens after immersion in acidic fluoride-containing saline solutions with or without albumin.

## N 1s

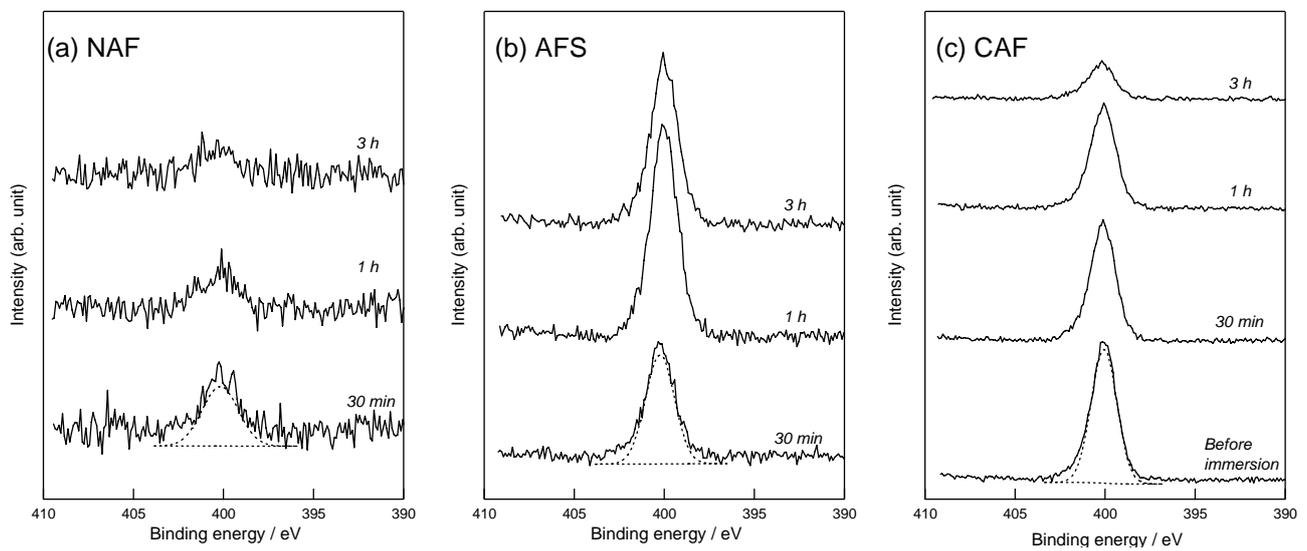


Fig. 4 N 1s XPS spectra of titanium specimens after immersion in acidic fluoride-containing saline solutions with or without albumin.

## O 1s

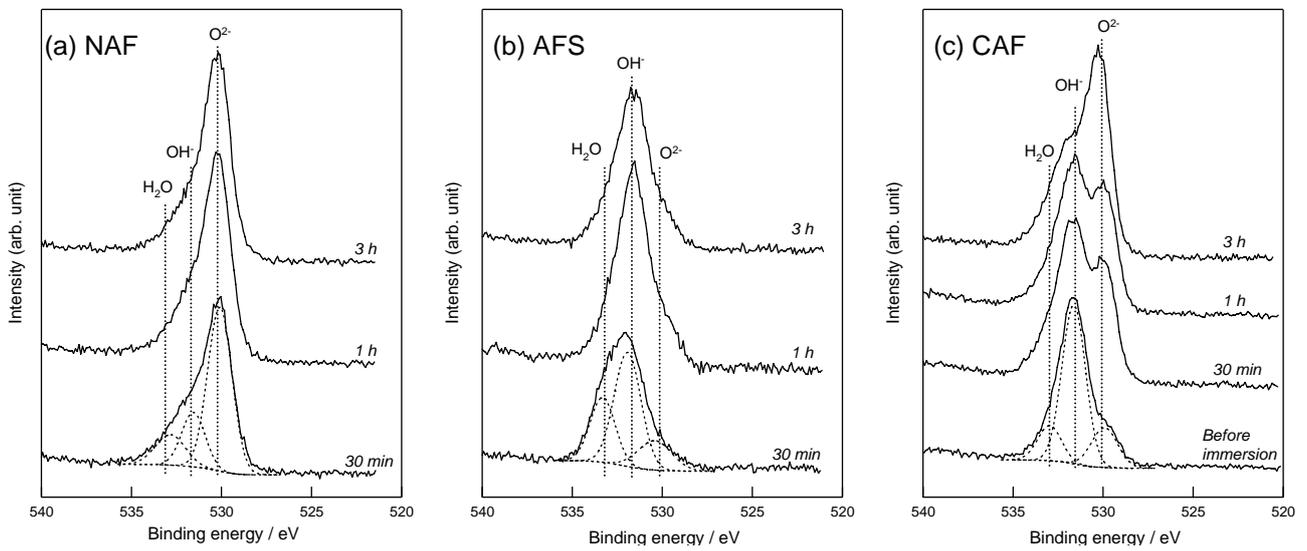


Fig. 5 O 1s XPS spectra of titanium specimens after immersion in the acidic fluoride-containing saline solutions with or without albumin.

## F 1s

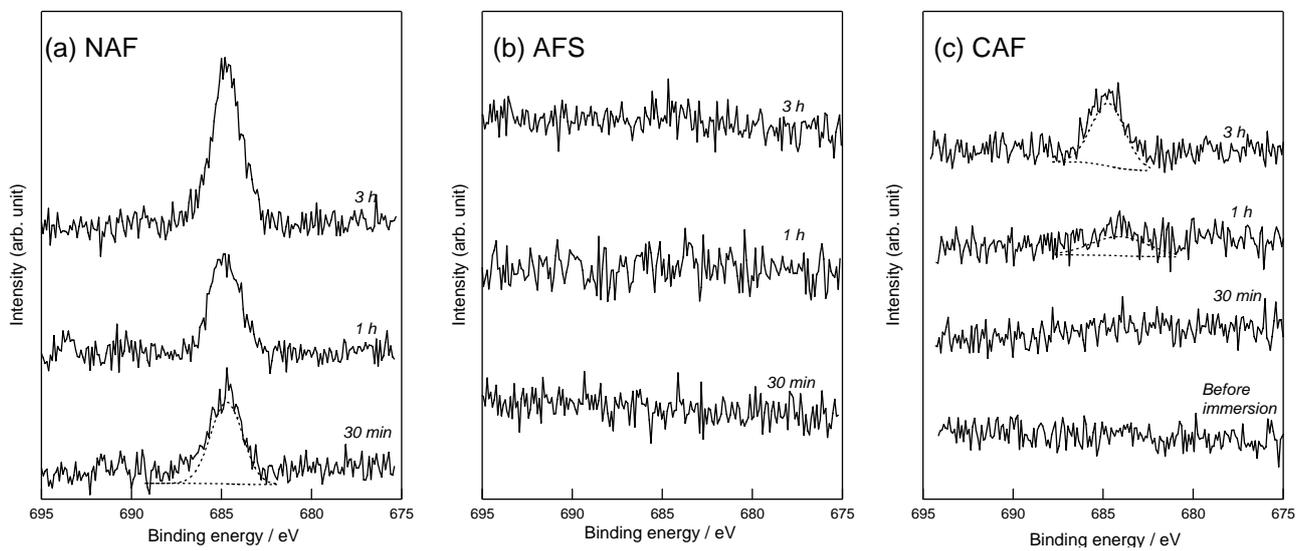


Fig. 6 F 1s XPS spectra of titanium specimens after immersion in acidic fluoride-containing saline solutions with or without albumin.