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# Chemical Interaction between Titanium Implant Surface and Amino Acids

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The purpose of this study was to investigate the chemical interaction between titanium implant surface and amino acids. Pure titanium disks were pretreated with 10 N HCl and ultrapure water at room temperature for 30 minutes each. Disks were then modified with one of the three amino acids－L-aspartic acid, L-serine, or L-threonine－at 37℃ for 12 hours. Modification with oxalic acid was used as a control. By means of X-ray photoelectron spectroscopy (XPS), amino acid powders and the modified surfaces without or with ultrasonic water rinsing were chemically analyzed. It was revealed that the N 1s peak which originated from amino acids was not or hardly detected in the wide scan spectra of amino acid-modified surfaces. Moreover, the $COO^-$ peak which originated from oxalic acid could hardly be detected in the narrow scan spectrum of the C 1s region of oxalic acid-modified surface with ultrasonic water rinsing. Based on the results of this study, it was concluded that amino acids could not chemically bond to the titanium surface.

Keywords: Titanium implant; Amino acid; Chemical interaction

## INTRODUCTION

Biochemical surface modification of titanium implants offers the advantage of improving surface biocompatibility without adversely affecting the bulk properties of the system. Herein is therefore a new concept of controlling and guiding the cell behavior of an ultra-thin layer of bioactive molecules of a biomimetic surface[1]. In the same vein, one approach to improve osseointegration is to modify implant surface with extracellular matrix components such as collagen, fibronectin, laminin, and vitronectin. This is to promote specific cell-extracellular matrix interaction. Likewise, implant surface should also be modified with growth factors to facilitate differentiation of osteoblasts[2,3].

However, surface immobilization of poly (ethylene glycol) (PEG) or peptide[4-7] involving silanized functionalities is generally unstable and subjected to hydrolytic degradation over time when in contact with aqueous fluids[1]. Moreover, seemingly promising molecular surface modification methods were limited either by the stability of PEG-modified surface or the availability of peptides because such adsorption was reversible and adsorbed peptides could be easily washed away with fresh buffer or replaced by other molecules in solution[8,9]. As for surface modification with the peptide sequence, arginine-glycine-aspartic acid-cysteine (RGDC), using gold-thiol chemistry[10], it is a complex procedure. Further, owing to the gold coating on titanium surface－by virtue of gold-thiol chemistry, the well-known biocompatibility of titanium would fail to be applied.

Healy and Ducheyne[11] demonstrated that phosphate has a high affinity for titanium oxide surface. Viornery *et al.*[12] suggested that phosphonic acid molecules covalently attached on a titanium surface might form a scaffold for new bone formation, ultimately leading to interfacial bonding between implant and host tissue. As for the proposal of chemically bonding amino acid as a low-mass molecule to titanium surface by means of phosphorylation of amino acid, Abe *et al.*[13] verified that *o*-phospho-L-threonine chemically and stably bonded to the titanium surface treated with HCl. On the other hand, glycine mono- and multilayers adsorbed on a titanium surface were stable up to above room temperature and did not seem to be significantly affected by the presence of water[14]. In other words, by modifying the physicochemical properties of titanium surface, it might be possible to alter the adsorption behavior of human plasma fibronectin and hence optimize cell attachment[15].

By structural definition, amino acid is a molecule that contains both amine and carboxyl functional groups. In the context of biochemical surface modification, it is necessary to aim at the carboxyl group, -COOH, which is an excellent ligand for metal ions. Aspartic acid is one of the amino acids of the RGD (arginine-glycine-aspartic acid) peptide. The former is a carboxylic acid which is a typically weak acid, while the latter is the most investigated peptide

sequence as a bridging unit between cell receptors and titanium surface[16]. In the human body, neutral amino acids—namely L-serine and L-threonine—are targets of phosphorylation or dephosphorylation. Oxalic acid is a dicarboxylic acid which is a relatively strong organic acid. The purpose of this study was to investigate, by means of X-ray photoelectron spectroscopy (XPS), the chemical interaction between titanium implant surface and amino acids such as L-aspartic acid, L-serine, and L-threonine, and with oxalic acid acting as a control.

## MATERIALS AND METHOD

*Specimen preparation*

Pure titanium disks (diameter: 5.8 mm, thickness: 2 mm; JIS 2; GC, Tokyo, Japan) were decontaminated by the following means of surface decontamination: ultrasonic treatment with 10 N hydrochloric acid (HCl; Katayama Chemical, Osaka, Japan) for 30 minutes, followed by ultrasonic rinsing with ultrapure water (milliQ water: >18 MΩcm) for 30 minutes[17]. Then, these disks were modified with one the following three amino acids at 37℃ for 12 hours: 50 mM L-aspartic acid $C_4H_7NO_4$ (Asp; Lot No. ELH7185, Katayama Chemical, Osaka, Japan), 50 mM L-serine $C_3H_7NO_3$ (Ser; Lot No. 28－0610－2, Sigma-Aldrich, St. Louis, Mo, USA), or 50 mM L-threonine $C_4H_9NO_3$ (Thr; Lot No. 32K0896, Sigma-Aldrich, St. Louis, Mo, USA). For the control, disks were modified with 50 mM oxalic acid $C_2H_2O_4$ (Oxa; Lot No. 28－0890, Katayama Chemical, Osaka, Japan) (Fig. 1).

For disks modified with Asp, Ser, or Thr, some were kept intact as they were prepared before XPS examination as modified surfaces without ultrasonic water rinsing, whereas the rest were followed by ultrasonic rinsing with milliQ water for 10 minutes. As for Oxa-modified disks, they were subjected to ultrasonic rinsing with either acetone (>99.5%; Katayama Chemical, Osaka, Japan) or milliQ water for 10 minutes.

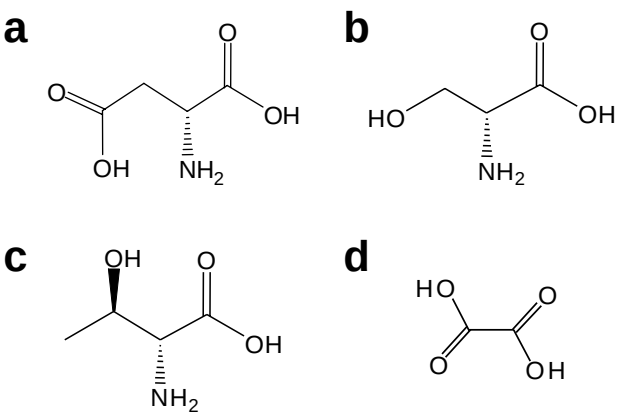


Fig. 1 Molecular structures of (a) L-aspartic acid (Asp); (b) L-serine (Ser); (c) L-threonine (Thr); and (d) Oxalic acid (Oxa).

*X-ray photoelectron spectroscopy (XPS)*

Surfaces of the modified titanium disks, which were mounted on a stub with insulating tape, were chemically analyzed using an XPS instrument (AXIS-HS, Kratos, Manchester, UK). XPS measurements were performed in a vacuum of less than $10^{-7}$ Pa. Al-K$\alpha$ monochromatic X-ray with a source power of 150 W was employed. Charge compensation was achieved with an electron flood gun equipped with the AXIS-HS instrument. Wide and narrow scan spectra were acquired at pass energies of 80 and 40 eV respectively. Peak positions were calibrated by referencing a value of 284.6 eV for the peak of C-C, C-H in the C 1s spectrum. Smoothing of narrow scans was done, and a straight-line background (for C 1s, N 1s, and O 1s) and Shirley-type background (for Ti 2p) were applied in the quantification. The relative sensitivity factors used to calculate the atomic ratios from the peak area ratios were 1.0 for C 1s, 1.68 for N 1s, 2.64 for O 1s, and 7.20 for Ti 2p. Reproducibility was guaranteed by taking nine measurements per experimental variable.

## RESULTS

*Titanium surface modified with L-aspartic acid (Asp)*

Figure 2 shows the XPS wide scan spectra of (a) Asp powder; (b) titanium exposed to Asp without rinsing; and (c) titanium exposed to Asp after being ultrasonically rinsed with milliQ water. In the wide scan spectrum of Asp powder, O, N, and C were detected (Fig. 2a), while O, Ti, N, and C were detected in the wide scan spectrum of Asp-modified titanium without rinsing (Fig. 2b). However, the N 1s peak which originated from Asp was not detected in the wide scan spectrum of Asp-modified titanium with rinsing (Fig. 2c). Figure 3 shows the XPS narrow scan spectra of the C 1s region of (a) Asp powder; (b) titanium exposed to Asp without rinsing; and (c) titanium exposed to Asp after being ultrasonically rinsed with milliQ water. The C 1s peaks for Asp powder (Fig. 3a) and Asp-modified titanium without rinsing (Fig. 3b) were attributed to three peaks at 288.4 eV ($COO^-$), 286.0 eV (C-O, C-N), and 284.6 eV (C-C, C-H). The C 1s peak for Asp-modified titanium with rinsing (Fig. 3c) was attributed mainly to the 284.6 eV (C-C, C-H) peak which originated from organic contamination, and the 288.4 eV ($COO^-$) and 286.0 eV (C-O, C-N) peaks were weaker compared to those of non-reacted Asp and non-rinsed Asp-modified titanium.

*Titanium surface modified with L-serine (Ser)*

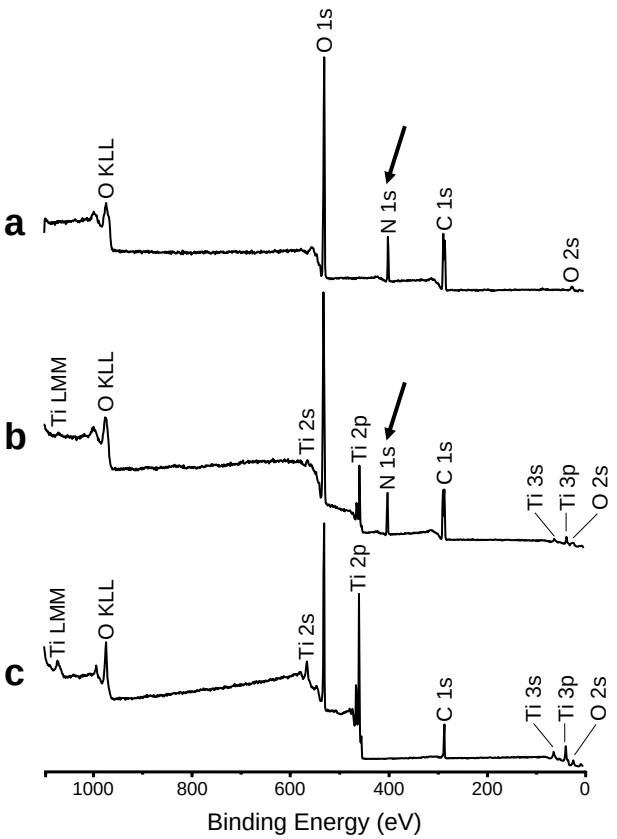


Fig. 2 XPS wide scan spectra of (a) L-aspartic acid (Asp) powder; (b) titanium exposed to Asp without rinsing; and (c) titanium exposed to Asp after being ultrasonically rinsed with milliQ water.

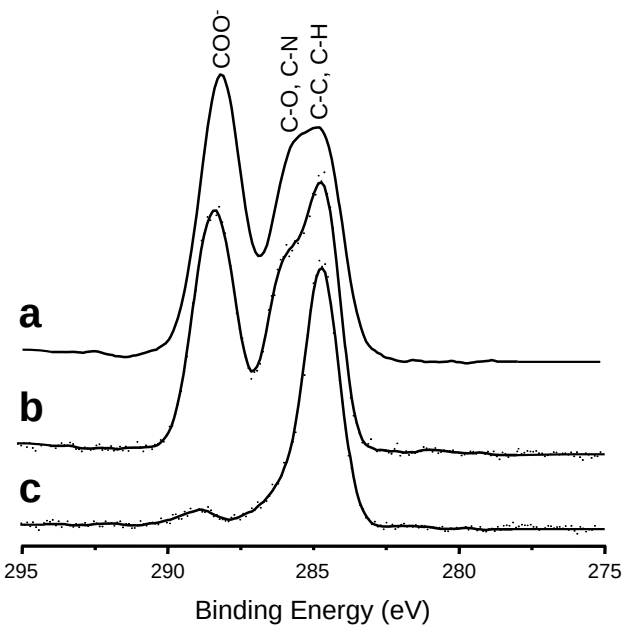


Fig. 3 XPS narrow scan spectra of the C 1s region of (a) L-aspartic acid (Asp) powder; (b) titanium exposed to Asp without rinsing; and (c) titanium exposed to Asp after being ultrasonically rinsed with milliQ water.

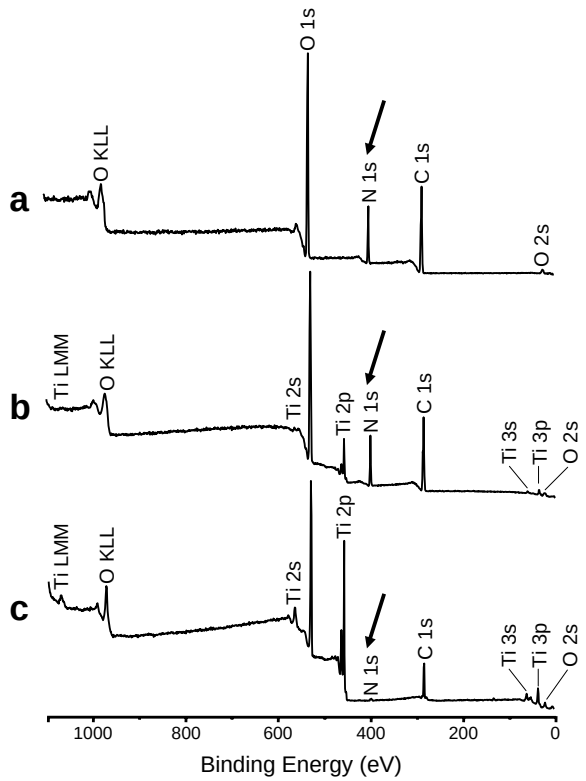


Fig. 4 XPS wide scan spectra of (a) L-serine (Ser) powder; (b) titanium exposed to Ser without rinsing; and (c) titanium exposed to Ser after being ultrasonically rinsed with milliQ water.

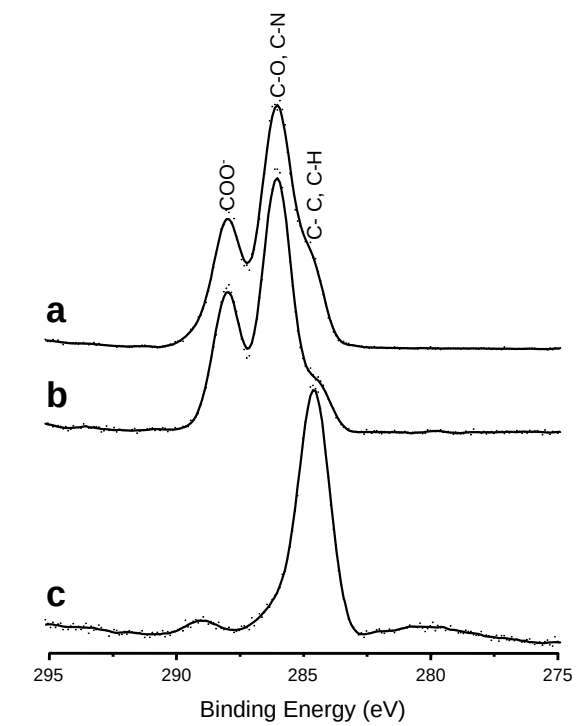


Fig. 5 XPS narrow scan spectra of the C 1s region of (a) L-serine (Ser) powder; (b) titanium exposed to Ser without rinsing; and (c) titanium exposed to Ser after being ultrasonically rinsed with milliQ water.

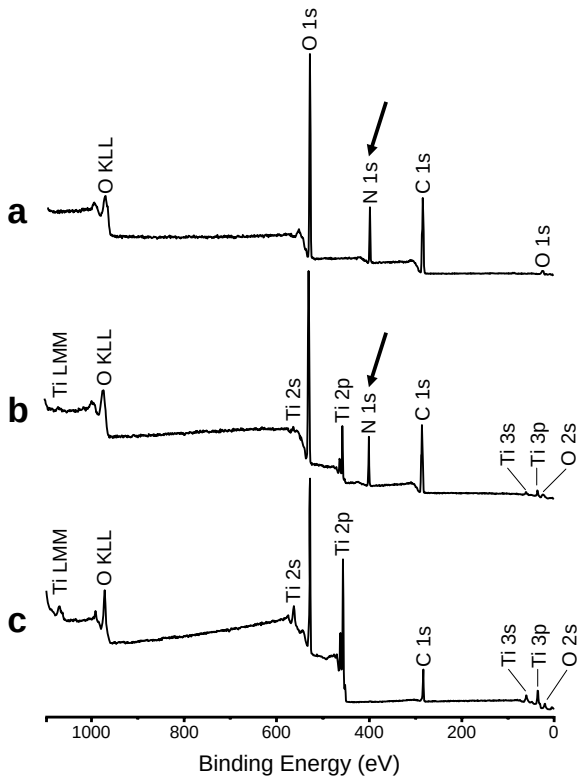


Fig. 6 XPS wide scan spectra of (a) L-threonine (Thr) powder; (b) titanium exposed to Thr without rinsing; and (c) titanium exposed to Thr after being ultrasonically rinsed with milliQ water.

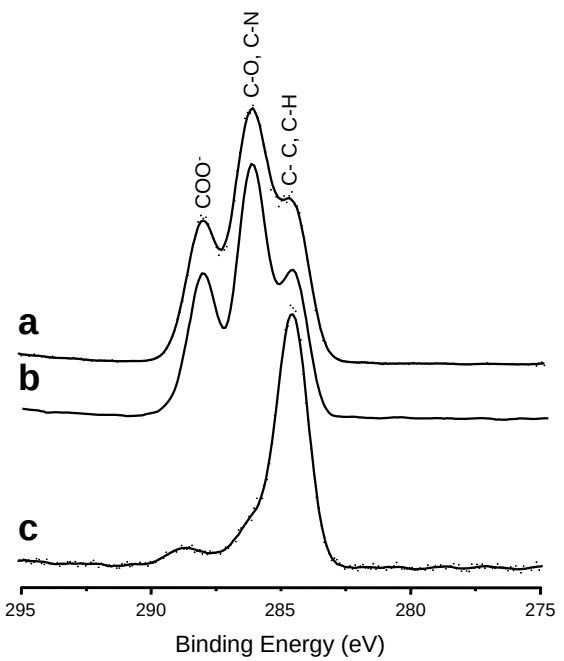


Fig. 7 XPS narrow scan spectra of the C 1s region of (a) L-threonine (Thr) powder; (b) titanium exposed to Thr without rinsing; and (c) titanium exposed to Thr after being ultrasonically rinsed with milliQ water.

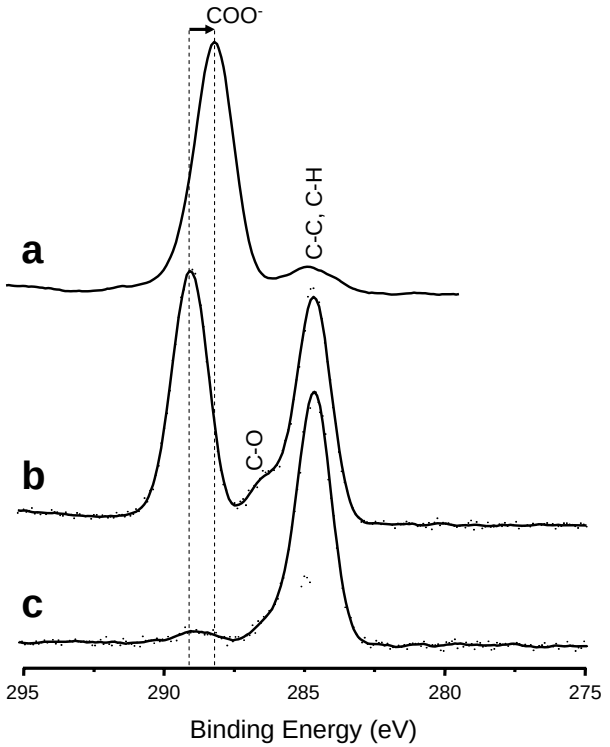


Fig. 8 XPS narrow-scan spectra of the C 1s region of (a) hydroxyapatite exposed to Oxalic acid (Oxa) after being ultrasonically rinsed with milliQ water (Yoshida *et al.*[18]); (b) titanium exposed to Oxa after being ultrasonically rinsed with acetone; and (c) titanium exposed to Oxa after being ultrasonically rinsed with milliQ water.

Figure 4 shows the XPS wide scan spectra of (a) Ser powder; (b) titanium exposed to Ser without rinsing; and (c) titanium exposed to Ser after being ultrasonically rinsed with milliQ water. In the wide scan spectrum of Ser powder O, N, and C were detected (Fig. 4a), while O, Ti, N, and C were detected in the wide scan spectrum of Ser-modified titanium without rinsing (Fig. 4b). However, a very weak N 1s peak, which came from traces of contamination, was detected in the wide scan spectrum of Ser-modified titanium with rinsing (Fig. 4c). Figure 5 shows the XPS narrow scan spectra of the C 1s region of (a) Ser powder; (b) titanium exposed to Ser without rinsing; and (c) titanium exposed to Ser after being ultrasonically rinsed with milliQ water. The C 1s peaks for Ser powder (Fig. 5a) and Ser-modified titanium without rinsing (Fig. 5b) were attributed to three peaks at 288.4 eV ($COO^-$), 286.0 eV (C-O, C-N), and 284.6 eV (C-C, C-H). The C 1s peak for Ser-modified titanium with rinsing (Fig. 5c) was attributed mainly to the 284.6 eV (C-C, C-H) peak which originated from organic contamination, and the 288.4 eV ($COO^-$) and 286.0 eV (C-O, C-N) peaks were weaker compared to those of non-reacted Ser and non-rinsed Ser-modified titanium.

*Titanium surface modified with L-threonine (Thr)*
Figure 6 shows the XPS wide-scan spectra of (a) Thr powder; (b) titanium exposed to Thr without rinsing; and (c) titanium exposed to Thr after being ultrasonically rinsed with milliQ water. In the wide scan spectrum of Thr powder, O, N and C were detected (Fig. 6a), while O, Ti, N, and C were detected in the wide scan spectrum of Thr-modified titanium without rinsing (Fig. 6b). However, the N 1s peak which originated from Thr was not detected in the wide scan spectrum of Thr-modified titanium with rinsing (Fig. 6c). Figure 7 shows the XPS narrow scan spectra of the C 1s region of (a) Thr powder; (b) titanium exposed to Thr without rinsing; and (c) titanium exposed to Thr after being ultrasonically rinsed with milliQ water. The C 1s peaks for Thr powder (Fig. 7a) and Thr-modified titanium without rinsing (Fig. 7b) were attributed to three peaks at 288.4 eV ($COO^-$), 286.0 eV (C-O, C-N), and 284.6 eV (C-C, C-H). The C 1s peak for Thr-modified titanium with rinsing (Fig. 7c) was attributed mainly to the 284.6 eV (C-C, C-H) peak which originated from organic contamination, and the 288.4 eV ($COO^-$) and 286.0 eV (C-O, C-N) peaks were weaker compared to those of non-reacted Thr and non-rinsed Thr-modified titanium.

*Titanium surface modified with oxalic acid (Oxa)*
Figure 8 shows the XPS narrow scan spectra of the C 1s region of (a) hydroxyapatite exposed to Oxa after being ultrasonically rinsed with milliQ water as a reference data (Yoshida *et al.*[18]); (b) titanium exposed to Oxa after being ultrasonically rinsed with acetone; and (c) titanium exposed to Oxa after being ultrasonically rinsed with milliQ water. Yoshida *et al.*[18] demonstrated that the narrow scan spectrum in Fig. 8a revealed that the major peak at 288.2 eV must be attributed to the formation of an ionic bond between the carboxyl group of Oxa and Ca of hydroxyapatite, and weaker 286.0 eV (C-O) and 284.6 eV (C-C, C-H) peaks which originated from traces of organic contamination on the initial surface of hydroxyapatite were detected. The C 1s peak for Oxa-modified titanium after rinsing with acetone (Fig. 8b) were attributed to three peaks at 288.4 eV ($COO^-$), 286.0 eV (C-O), and 284.6 eV (C-C, C-H). The C 1s peak for Oxa-modified titanium after rinsing with water (Fig. 8c) was attributed mainly to the 284.6 eV (C-C, C-H) peak which originated from organic contamination, and the 288.4 eV ($COO^-$) and 286.0 eV (C-O) peaks were weaker compared to those of Oxa-modified titanium after rinsing with acetone.

## DISCUSSION

XPS spectra of the modified titanium surfaces revealed that any amino acid such as Asp, Ser, or Thr could not chemically bond to the surface treated with HCl and milliQ water, and that Oxa could not do as well too.

The decontamination method using HCl in this study should serve to facilitate a stable chemical modification of the titanium surface and promote a progressive binding of amino acids to the modified surface[17]. On this note, Krozer *et al.*[19] reported that the bonding of amino-alcohol—applied for cleaning contaminated implant surfaces—was stronger to an acid-treated titanium surface than an anodically oxidized titanium surface. However, this study indicated that any N 1s peak which originated from Asp, Ser, or Thr was not detected or hardly detected in the wide scan spectra of modified titanium surfaces rinsed with water. Moreover, the 288.4 eV ($COO^-$) and 286.0 eV (C-O, C-N) peaks for the modified titanium with water rinsing were weaker compared to those of non-reacted amino acids and the non-rinsed modified titanium. Besides, shift of peak at 288.4 eV ($COO^-$) between non-reacted amino acid and non-rinsed modified surface also failed to be detected.

Oxalic acid (Oxa), as a dicarboxylic acid, is one of the strongest organic acids. It shows the same chemical behavior and reactivity as monocarboxylic acids. The ionization of the second carboxyl group occurs less readily than the first one. This is because more energy is required to separate a positive hydrogen ion from a doubly charged anion than from a single charged anion. Yoshida *et al.*[18] illustrated that the peak at 288.2 eV ($COO^-$) must be attributed to the formation of an ionic bond between the carboxyl group of Oxa and Ca of hydroxyapatite. In this study, the peak of $COO^-$ for Oxa-modified titanium after rinsing with acetone was detected at 288.4 eV—not at the lower binding energy of 288.2 eV ($COO^-$) for Oxa-modified hydroxyapatite (Fig. 8). This meant that Oxa could not chemically bond to titanium, and that it was easily washed away with water. At this juncture, it should be mentioned that it is undoubtedly necessary to analyze pure Oxa by XPS. However, it was impossible to do so because pure Oxa sublimates easily in high vacuum.

Based on the results obtained concerning chemical interaction between titanium and carboxyl groups of amino acids, it was suggested that amino acids—such as Asp, Ser, and Thr—were only adsorbed on the titanium surface but not chemically bonded to the surface. This would imply that titanium implants modified using amino acids would be unstable. On the other hand, phosphorylation of amino acids—as recommended by Abe *et al.*[13]—is an available means to acquire stable and reliable bonding of amino acids to titanium.

## CONCLUSION

The focus of this study was to investigate the chemical interaction between titanium implant surface and amino acids. It was concluded that amino acids which contain a carboxyl group—an excellent ligand for metal ions—could not chemically bond to the titanium surface.

## ACKNOWLEDGEMENTS

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