Osteoconductivity Control Based on the Chemical Properties of the Implant Surface

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Abstract

Metallic materials, such as Ti, Zr, Nb, Ta, and their alloys, and also stainless steels are widely attractive as osteoconductive materials in the dental and orthopedic fields. Ceramics and polymers are also commonly used as biomaterials. However, they do not have high osteoconductivity in their pure form, and surface coatings with bioactive substances, such as hydroxyapatite or TiO_2, are needed before implantation into the bone. Many reports claim that the surface chemical properties of implants, in particular, hydrophilicity and hydrophobicity, strongly affect the biological reactions. However, the effect of surface properties on osteoconductivity is not clear. In this review, we focus on the relationship between the surface hydrophilicity of metallic implants and osteoconductivity using *in vivo* evaluation, and the control of the osteoconductivity is discussed from the viewpoint of protein adsorption in implants.

Keywords

Hydrophilicity, Hydrophobicity, Osteoconductivity, Protein Adsorption, Surface Modification

1. Introduction

Valve metals, such as Ti, Zr, Nb, Ta, and their alloys and also stainless steels are widely used in orthopedic and dental implants. Ceramic and polymer materials are also commonly used as biomaterials, as they have high corrosion resistance in saltwater environments and high chemical stability in the body. They also have good biocompatibility, and their long-term success rates are well documented [1] [2]. However, when used in living bodies, Ti in its pure form does not always encourage hard-tissue growth onto its surface; the same is true for sintered ceramics and polymers. Therefore, the development of appropriate surface treatments

to enhance the bone-forming characteristics, such as hydroxyapatite (HAp) and other calcium phosphate coatings [3] [4], has been studied extensively using hydro- and pyro-processing (e.g., the methods of cathodic electrolysis [5] [6] [7], electrophoresis [8] [9], thermal substrate [10]-[16], plasma spraying [17] [18], sol-gel [19] [20], electron beam sputtering [21], and ion beam sputtering [22]). Similar to HAp, TiO_2 is also important as an osteoconductive material because it has been shown to exhibit strong physicochemical fixation with living bone, even though it is not a component of natural bone [23].

Coating materials and coating processes designed to improve osteoconductivity have received special attention, but the chemical characteristics of the surface of these coatings have been largely ignored in manufacturing and clinical practice, despite the fact that the surface characteristics usually affect the biological response at the implant-body interface [24]-[29]. Therefore, specific control of the surface properties, not the actual implant coating material leads directly to control of osteoconductivity. The surface bioactivity of such implants must be maintained until the actual surgical procedure. In the present circumstances, a bioactivation treatment can be performed just before clinical application, such as the irradiation with ultraviolet (UV) light [30].

In this paper, we focus on the relationship between the surface properties of metallic implants and osteoconductivity using *in vivo* evaluation of the implants. Hydrophilicity/hydrophobicity, *i.e.* the water contact angle (WCA), was picked up as one of the overall parameters for evaluating the surface properties of implants, as numerous factors affect the surface characteristics simultaneously. That is to say, WCA, as an overall factor, includes all the surface characteristics. The influence of the hydrophilicity/hydrophobicity on the osteoconductivity is discussed from the viewpoint of protein adsorption in implants.

2. Factors That Affect the Osteoconductivity

It is well known that numerous factors affect the osteoconductivity of implants, such as surface roughness and morphology [25] [31] [32] [33], film thickness [34], crystal structure [35], crystallinity, and hydrophilicity [36]. However, researchers in the field have arrived at different conclusions based on *in vivo* and *in vitro* evaluations, and agreement has not been reached. Many studies have focused on the chemical substance coatings of the surface or their mechanical and physical properties, and the chemical properties of the implant surface have been almost ignored. Our previous *in vivo* testing in relation to osteoconductivity using TiO_2 films on Ti implants revealed the following [33] [37] [38] [39].

1) The crystal structure of TiO_2 (anatase and rutile) did not affect osteoconductivity [37].

2) The osteoconductivity of thermally oxidized anatase and rutile samples was as low as that of as-polished samples [37].

3) The surface roughness (Ra; arithmetic average roughness) [40] of TiO_2 has been reported to affect osteoconductivity, but this tendency was not seen in the

region 0.3 < Ra/μm < 1.5. The osteoconductivity within this Ra region was as low as that of an as-polished sample [33]. On the other hand, the samples with Ra/μm < 0.3 had a higher osteoconductivity of nearly 40% after anodizing in H_2SO_4 aqueous solution. This tendency, however, was not seen in the thermally oxidized and as-polished samples [37].

4) There was not always a correlation between the crystallinity of the TiO_2 films and osteoconductivity. However, the osteoconductivity in amorphous anatase films formed by anodizing in an aqueous solution with high H_3PO_4 content was very high, the maximum value of BIC in cortical bone part was ca. 50% for the 14 days implantation in the rat tibia) [38].

5) Anions and cations, included in the anodizing bath and evident in the resulting TiO_2 films, did not have an effect on osteoconductivity [39].

As described above, we cannot predict the osteoconductivity of implants, by controlling only the above conventional factors, such as surface roughness and morphology, film thickness, crystal structure, and crystallinity. There is almost no doubt that numerous complex and interrelated factors influence osteoconductivity. For strict control of the osteoconductivity, other general factors including conventional influencing factors have to be introduced. We focused on the hydrophilicity, which was the WCA of the implant surface for the evaluation of the osteoconductivity.

3. Bioactivation by the Surface Treatment (Control of the Surface Hydrophilicity of Implants)

There are not many reports on changes in osteoconductivity by controlling surface chemical properties. Ogawa *et al.* recovered the wettability of blood on hydrophobic Ti implant surface by UV light irradiation [30], and have already commercialized a device that can be used in the operating room. Yoshinari *et al.* reported on improvement in osteoconductivity using the hydrophilicity control by plasma irradiation of Ti implants (*in vitro*) [36]. Both latter authors aimed at the formation of a superhydrophilic Ti implant surface, and did not examine the effect of the hydrophobic surface (WCA > 80˚). In addition, they did not evaluate other materials. The present authors produced several metallic implants over a wide range of WCA values, from hydrophilicity to hydrophobicity, using hydrothermal treatment, anodizing and high-temperature oxidation other than UV light and plasma irradiation, and evaluated the implants using *in vivo* testing. WCA was measured after 10 s from putting a 2 μL droplet of distilled water, at three different points on each sample and the average value was used as the WCA value.

4. Hydrophilization of Metallic Materials and Maintenance

In general, the hydrothermal treatment in distilled water removes adsorbed contaminants (mainly hydrocarbons) from the TiO_2 surface, and this cleaning effect creates superhydrophilic surfaces on the TiO_2 films. Irradiation using UV light or

atmospheric plasma are other alternative methods for surface cleaning [41] [42]. In this study, these processes were used to obtain TiO_2 films with superhydrophilic surfaces [43]. The change in WCA over the processing period is shown in **Figure 1**. For the TiO_2 samples produced by anodizing Ti substrates in 0.1 M H_2SO_4 at 100 V, followed by sterilization by autoclaving, the initial WCA was about 30°. This value changed to less than 15° by using hydrothermal treatment (at 180°C), UV irradiation (Hg-Xe lamp, wavelength: ~250 nm), and atmospheric plasma irradiation (operating gas: N_2, 500 W), although the rate of change varied for each technique. In particular, atmospheric plasma irradiation quickly changed the WCA to less than 10°. X-ray photoelectron spectroscopy (XPS) analysis revealed that not all processes introduced hydrophilic functional groups, such as −OH, and the amount of adsorbed hydrocarbon decreased [43]. The surface-cleaning effect created the hydrophilic surface. In this study, it was found that the hydrothermal treatment could be used to generate uniform hydrophilicity over an entire implant surface, which can have complex shapes and topographies. After superhydrophilic surfaces are prepared, it is important to maintain the surface properties until implantation, because surface hydrophilicity can be lost easily over time, as reported by Att *et al.* [44]. Therefore, the cleaned hydrophilic surfaces should be kept in an environment that does not contain hydrocarbons, such as under vacuum or in an aqueous solution. Keeping in mind that the implants were handled in air in the surgical procedure, the samples were kept in an aqueous environment
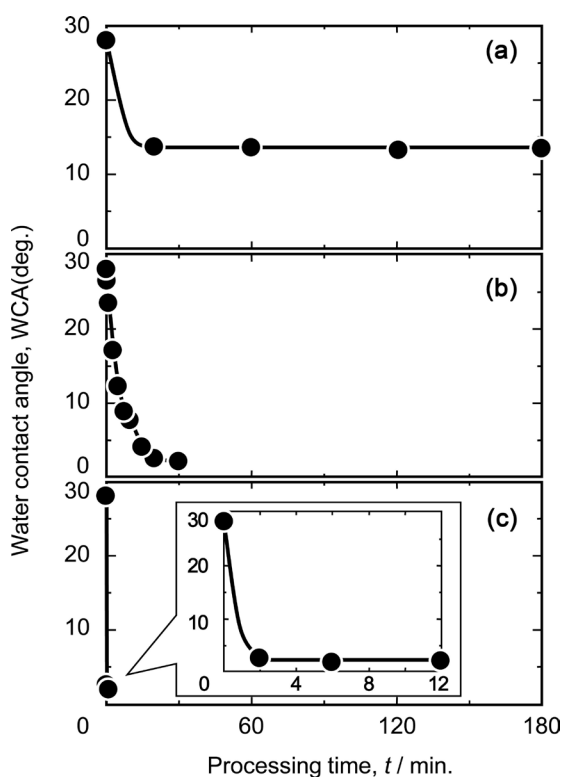
Figure 1. Changes in WCA with processing time of anodized TiO_2 in 0.1 M H_2SO_4 at 100 V. (a) Hydrothermal treatment (180°C) in distilled water; (b) UV light irradiation; and (c) Atmospheric plasma irradiation.

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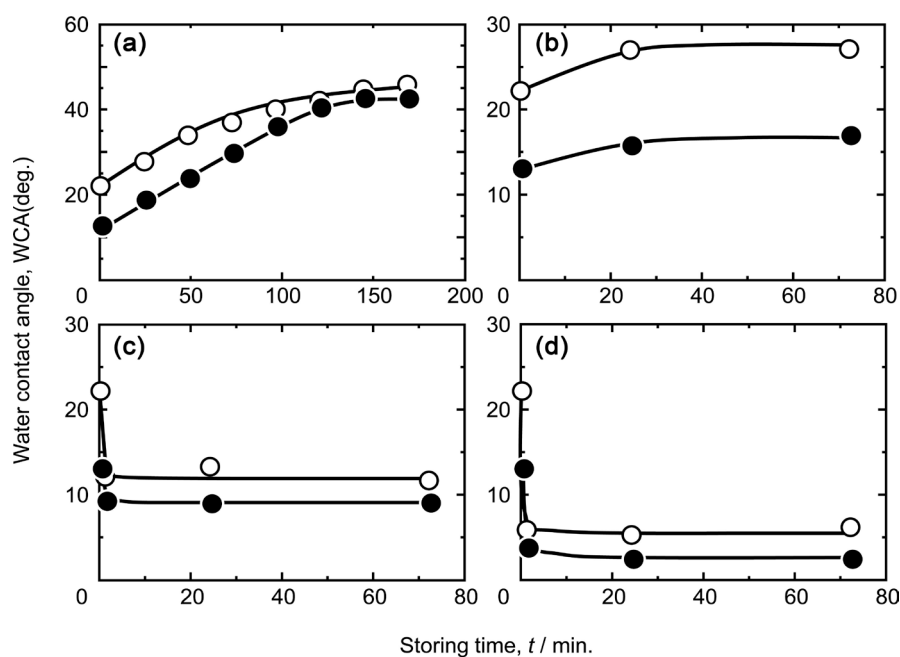
Figure 2. Changes in WCA on Ti samples processed with different surface modification techniques (○: as-anodized; ●: hydrothermally treated at 180°C for 180 min. in distilled water) and then stored under different conditions for different periods: (a) In air; (b) In distilled water; (c) In 1× PBS(−) solution; and (d) In 5× PBS(−) solution.

in this study. It can be expected that storage in an aqueous solution containing a high concentration of anions and cations will maintain hydrophilicity, and this also encourages the adsorption of these ions in preference to any hydrocarbons that may be present. **Figure 2** shows the effects of different storage environments on the variation in WCA over time for TiO_2 samples hydrothermally treated at 180°C for 180 min. The reasons for selecting hydrothermal treatment as the processing conditions for these tests were as follows. 1) Hydrothermal treatment at a temperature above 150°C but below 210°C for less than 180 min resulted in a WCA of 15°; 2) Hydrothermal treatment for more than 180 min did not result in any further decrease in the WCA. In addition, we confirmed that general sterilization using an autoclave (121°C, 20 min) could not achieve these WCA values. The WCA values of the samples varied greatly according to the storage conditions and the period of storage. Regardless of whether a sample was hydrothermally treated, storage in air caused a continuous increase in the WCA as the storage period increased, resulting in similarly high WCA values for all samples after 168 h (**Figure 2(a)**). Storage in distilled water also caused the WCA to increase slightly (**Figure 2(b)**). However, storage in a PBS(−) (8 gL^{-1} NaCl, 0.2 gL^{-1} KCl, 1.44 gL^{-1} NaH$_2$PO$_4$, 0.24 gL^{-1} KH$_2$PO$_4$, pH 7.4) solution, which provides the same wet environment as distilled water, reduced the WCA of as-anodized Ti (marked "○" in **Figure 2(c)** and **Figure 2(d)**). This tendency was enhanced when the sample was hydrothermally treated and when it was stored in a higher concentration of 5 times the PBS(−) solution (marked "●" in **Figure 2(c)** and **Figure 2(d)**). When samples were stored in concentrations higher than five times PBS(−), the WCA values did not decrease

any further. Na$^+$ and Cl$^-$ ions, which are main components of PBS($-$), were detected on the surfaces of the samples stored in the PBS($-$) solution. All these solute ions were adsorbed on the surfaces of the samples, regardless of the type of ion or the pH of the solution, consequently reducing the WCA values. There were no differences between the types of solute ions in their capacity to reduce the WCA, but when the samples were immersed in five times PBS($-$) solution, the Na$^+$ and Cl$^-$ ions were adsorbed more markedly on the surfaces of the samples because their concentrations in the solution were high. In general, it was found that storing samples in five times PBS($-$) solution effectively maintained the superhydrophilic surface for a long time. After the hydrophilization, we obtained the samples, controlled the WCA of their surface by retaining the samples for the predetermined time in the several environments (five times PBS($-$) or air at room temperature, air at 200°C, distilled water at room temperature, etc.).

5. Evaluation of Osteoconductivity

In vivo testing was selected for the evaluation of the osteoconductivity. We used the bone-implant contact ratio (BIC) as the osteoconductive index [14] [15] [16] [33], based on the observation of bone tissue on the implants using an optical microscope, to assess samples 14 days after implantation in the tibiae of 8-week-old male rats. The BIC was determined by the linear measurement of bone in direct contact with the implant surface. The sum of the length of the bone formation on the implant surface was measured, and the BIC was expressed as a percentage of the total implant length in the cancellous and cortical bone regions [14] [15] [16].

$$BIC(\%) = \frac{\text{sum of the length of the part of bone formation on the implant surface}}{\text{total implant length}} \times 100 \quad (1)$$

6. Osteoconductivity of WCA-Controlled Samples

Before surgery, all the implants were cleaned in normal saline solution and immediately implanted in the tibiae of 8-week-old male rats. To examine the surface chemical characteristics of hydrophilicity and hydrophobicity, the BIC in the cortical bone region was plotted against the WCA of TiO$_2$ films (**Figure 3**) [37] [39]. In this figure, the as-polished Ti sample is marked as "■". To perform the WCA measurements, a 2-μL water droplet was used. Samples underwent TiO$_2$ coating and autoclave sterilization (121°C, 20 min.). The BIC had a minimum value at ca. 65°, and samples that not only had a more hydrophilic surface (smaller value of WCA) but were also more hydrophobic (bigger value of WCA) had a higher BIC. The hydrophilic samples, hydrothermally treated, showed quite high BICs, up to 58%, *i.e.*, about four times higher than the BIC for the as-polished surface (marked "■" in **Figure 3**). This indicates that the hydrophilic surface had significantly higher osteoconductivity. In particular, the very high BIC in the sample with WCA < 10° deserves special mention.
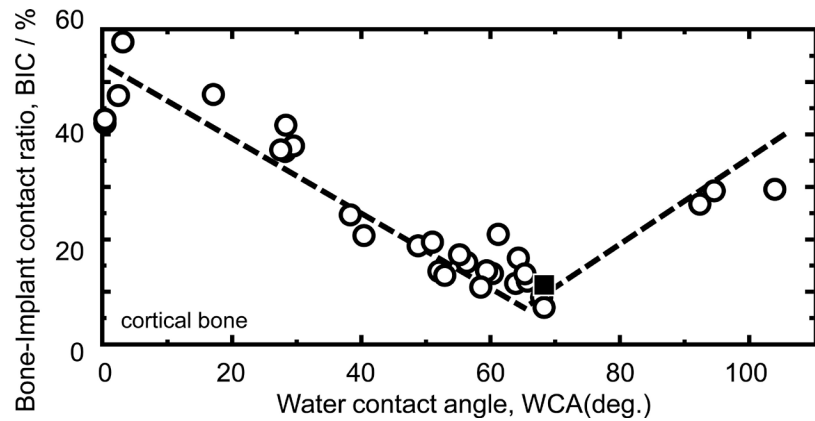
Figure 3. Relationship between the WCA and BIC of anodized TiO$_2$ in several aqueous solutions (○). ■: as-polished Ti.
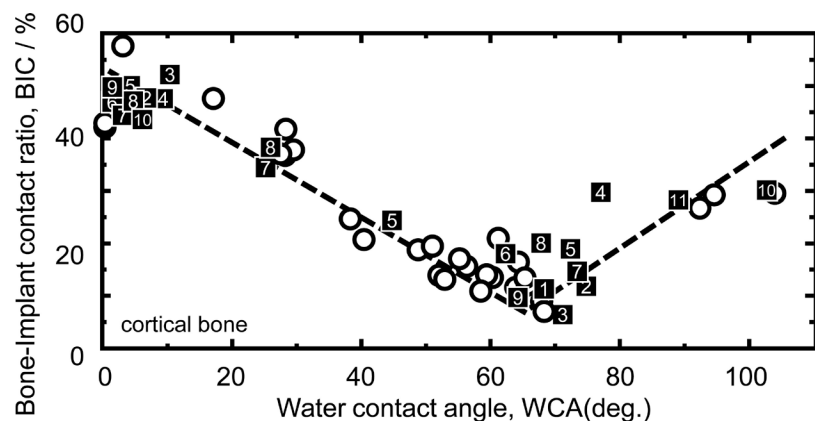
Figure 4. Relationship between the WCA and BIC of several metals and alloys (■: polished and hydrothermally treated at 180°C for 180 min. in distilled water and then stored in the several environments; ○: anodized TiO$_2$). (1) Ti, (2) Nb, (3) Ta, (4) Zr, (5) Ti-29Nb-13Ta-4.6Zr, (6) Ti-13Cr-1Fe-3Al, (7) Ti-6Al-4V, (8) Ti-6Al-7Nb, (9) Zr-9Nb-3Sn, (10) stainless steel 304, and (11) stainless steel 316 L.

We also examined whether the hydrophilic surfaces of other metals and alloys had high osteoconductivity, regardless of the presence or absence of coatings. Valve metals (Ti, Nb, Ta, and Zr), their alloys (Ti-6Al-4V, Ti-6Al-7Nb, Ti-29Nb-13Ta-4.6Zr, Ti-13Cr-Fe-Al, and Zr-9Nb-3Sn) and stainless steel (304 and 316L) were selected and their WCAs were controlled using several surface treatments, such as hydrothermal treatment and storing conditions [45] [46] [47] [48]. That is to say, these samples did not have any surface coating films, except for the surface oxide films naturally formed during storing in air and the surface treatment. Their BIC values are shown in Figure 4. These metals and alloys presented a similar osteoconductive dependency on the WCA as the TiO$_2$ films, and their BIC values could be plotted on the V-shaped BIC line determined for the TiO$_2$ films. There was no dependency on the kind of metals and alloys. The BIC values of hydrophilic surface were also as high as those of TiO$_2$. Therefore, hydrophilic treatments could improve the osteoconductivity of valve metals dramatically, and osteoconductive coatings (such as HAp, TCP (tricalcium phosphate), and TiO$_2$) are not re-

quired.

## 7. Protein Adsorption of WCA-Controlled Samples

The reason for the high osteoconductivity of hydrophilic or hydrophobic surfaces is unclear. However, we think that this is reasonable to assume, based on the following facts on the protein adsorption on implants. 1) It is easy for cell-adhesive proteins (such as fibronectin and decorin) to adhere to a surface with high hydrophilicity; 2) A surface that has proteins adhered to it encourages osteoblast cell attachment [36] [49] [50].

Protein adsorption testing was carried out on WCA-controlled Ti samples [51]. Fibronectin and decorin were determined to be cell-adhesive proteins, and albumin was not. The proteins were separately dissolved in distilled water. The protein content was 0.5 mg mL^{-1} in fibronectin, 0.5 mg mL^{-1} in decorin, and 25 mg mL^{-1} in albumin, considering the protein content in the human body. A drop of the aqueous solution with one kind of protein (40 μL) was dripped on the sample surface and was maintained at 37˚C for up to 3.6 ks. Next, the samples were washed using the ultrasonic cleaning equipment without the scrub down. Fourier transfer infrared (FT-IR) analysis (in attenuated total reflection (ATR) mode) determined the concentration of adsorbed proteins on the sample surface using the peak area at 1650 cm^{-1} (C=O stretch) in FT-IR spectra. At first, the change in time of the amount of adsorbed albumin and fibronectin (A) and the WCA value (B) was examined in the difference original WCA samples (**Figure 5**). The WCA values became constant within 50 s regardless of the original WCA and the kind of protein; however, the amount of adsorbed protein increased even after that time. It is thought that the protein adsorbed on the outermost surface up to ca. 50 s and the protein continued to pile up after that. The piled-up protein was removed partially during the ultrasonic cleaning, but not all the adsorbed protein desorbed. It was not clear whether after cleaning the protein that remained was still present on the surface as a monolayer. Therefore, it took more than 3.6 ks, by more than ca. 50 s, for the amount of protein on the surface to reach the maximum value. The maximum amount of adsorbed protein, fibronectin, decorin, and albumin, with respect to the WCA value of the Ti samples [51] is shown in **Figure 6**. From this figure, the maximum amount of adsorbed protein depends on the WCA and on the kind of protein, and the adsorbed protein content had a minimum at ca. 48˚ in fibronectin, 65˚ in albumin and 72˚ in decorin, and samples with a more hydrophilic surface (smaller WCA) but also more hydrophobic (larger WCA) had a higher concentration as a boundary of those WCA with minimum adsorption, in the same manner as BIC with respect to WCA. Also, after the proteins were adsorbed on the hydrophilic and hydrophobic surfaces, there was a change in the WCA value with which the least protein was adsorbed on the surface. There was no difference in this tendency, in any kind of proteins. Furthermore, the distribution of albumin concentration with respect to WCA was similar to that of BIC.
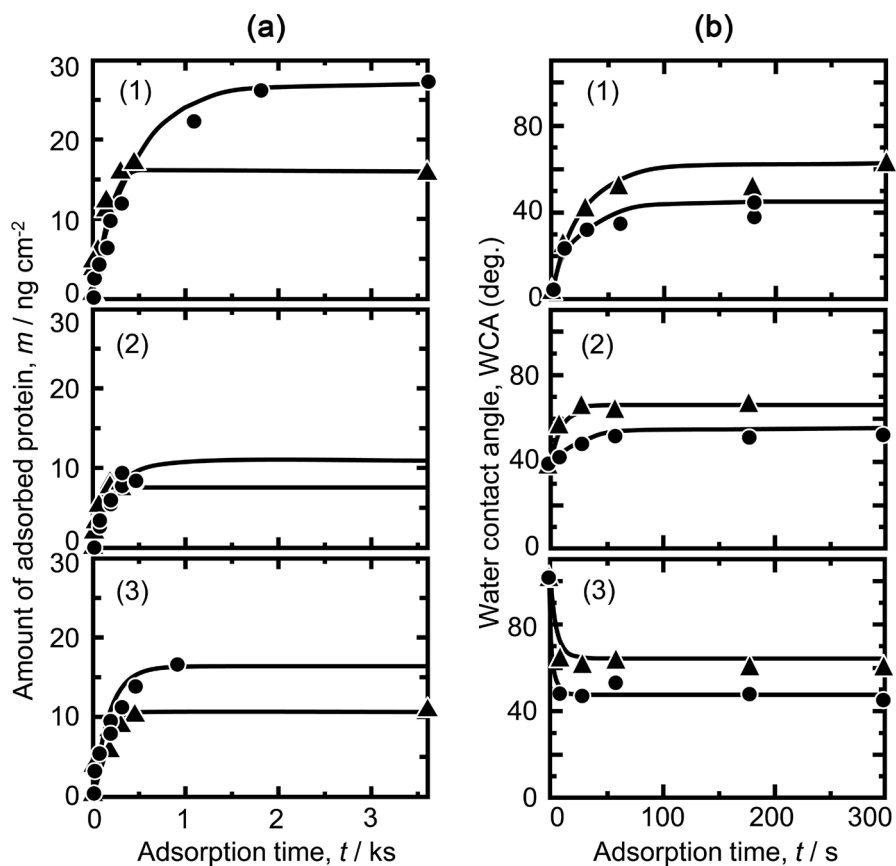
Figure 5. Changes in the amount of adsorbed protein with time (a) and WCA (b) on the WCA-controlled samples ((1) WCA = 0˚, (2) WCA = 35˚, and (3) WCA = 100˚) (●: 0.5 mg mL^{-1} fibronectin aqueous solution and ▲: 25 mg mL^{-1} albumin aqueous solution).
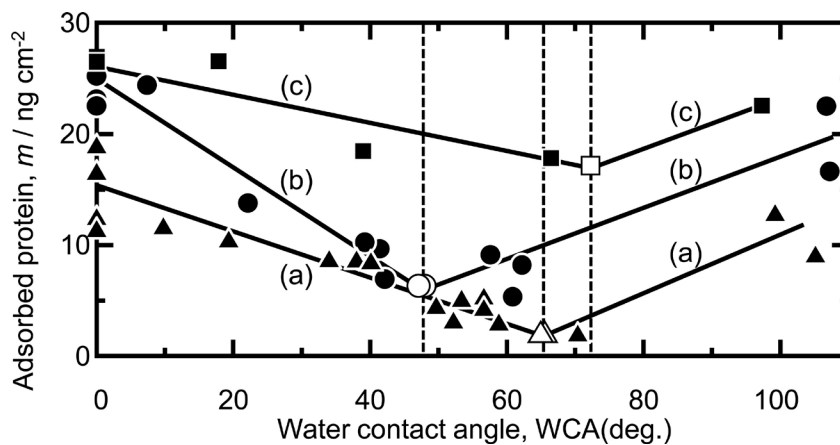
Figure 6. Relationship between the original WCA of Ti and the amount of adsorbed protein in (a) 25 mg mL^{-1} albumin aqueous solution, (b) 0.5 mg mL^{-1} fibronectin aqueous solution, and (c) 0.5 mg mL^{-1} decorin aqueous solution.

8. Osteoconductivity of Protein Adsorbed Samples

Protein-adsorbed samples were subjected to *in vivo* evaluation (**Figure 7**) [51]. The samples were hydrophilic and hydrophobic Ti surfaces, and fibronectin and albumin were selected as a protein. After the protein adsorption described
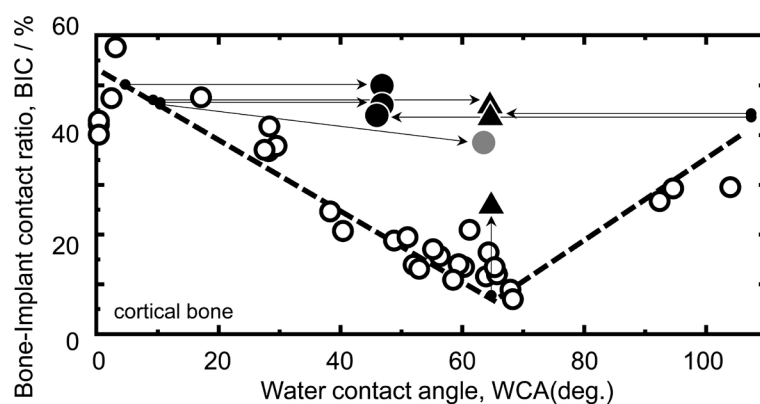
Figure 7. BIC and WCA change by the adsorption of proteins (●: fibronectin adsorption; ▲: albumin adsorption; ●: stored in air after fibronectin adsorption).

above, the WCAs changed to ca. 48˚ in fibronectin and ca. 65˚ in albumin. The BIC values of samples that absorbed a lot of protein reached about 50% and came to about the same BIC value as the original WCA value before protein adsorption. The BIC value of the sample with very little albumin (original WCA = ca. 65˚) increased slightly, not so drastically. The BIC value of the contaminated sample (WCA = ca. 65˚) decreased slightly by the adsorption of hydrocarbon in air after fibronectin adsorption (ca. 48˚) on the original hydrophilic surface (ca. 10˚). From these facts, it is clear that WCA did not have an effect on the osteo-conductivity and the effect is induced by the substance adsorbed on the outer-most surface. Therefore, the protein adsorption on the outermost surface plays the most important role in osteoconductivity. Furthermore, it is very interesting to note that the kind of the adsorbed protein (cell adhesive or not) did not affect the osteoconductivity. Moreover, protein adsorbed samples had almost the same BIC value irrespective of whether the original surface was hydrophilic or hydro-phobic, although the conformation of the adsorbed protein must be different. It is well known that many kinds of protein do not exist individually in the solu-tion, and form associated molecules. Fibronectin and albumin were no excep-tion, examination of the protein adsorption using a quartz crystal microbalance (QCM) revealed that they formed associated molecules. There are many substances, other than protein, in the body. Therefore, it is thought that although the above results were built under a very limited situation, they were, however, worthy to note.

9. Conclusion

Osteoconductivity control was discussed based on the hydrophilicity/hydrophobicity of the implant surface. It is clear that the chemical properties of implant surface influence the biocompatibility. However, our research results were limited, and we need to press forward further research. For the further advancement of bio-materials and their clinical application, many severe problems must be solved such as not only the discovery of new bioactive substances, but also the combi-nation of organic and inorganic materials, the development of the new surface

modification techniques, alloy designs for the implants and so on. And also the collaboration with the engineering and medical and dental science is more important than ever. We think that engineering, in particular materials engineering, has an important role to play in the development of advanced biomaterials that will fulfil a wide variety of medical and dental requirements.

Acknowledgements

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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