In vitro osteoconductivity evaluation of alumina treated hydrothermally in CaCl_2 solution

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Alumina (Al_2O_3) is classified as bioinert materials and thus, no direct bonding to bone was observed when implanted. In the present investigation, possibility of osteoconductive Al_2O_3 was evaluated based on hydrothermal treatment in calcium chloride (CaCl_2) solution. Based on X-ray photoelectron spectroscopy measurement, Ca_{2p} peaks were detected on Al_2O_3 after hydrothermal treatment with 10 and 50 mmol/L CaCl_2 solution at 125°C. The Ca_{2p} peak intensity for Al_2O_3 treated with 50 mmol/L CaCl_2 was higher than that treated with 10 mmol/L CaCl_2 . Then, *in vitro* osteoconductivity screening evaluation was made using simulated body fluid (SBF). After being soaked in SBF for 14 days, calcium phosphate deposition could be observed on Al_2O_3 treated with 10 and 50 mmol/L CaCl_2 solution at 125°C for 7 days whereas no deposition was observed on Al_2O_3 treated at 150 and 200°C as well as non-treated Al_2O_3 . We concluded, therefore, osteoconductive Al_2O_3 could be fabricated based on the hydrothermal treatment of bioinert Al_2O_3 with CaCl_2 .

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1. Introduction

Alumina ceramics has been used as biomaterials due to their attractive mechanical properties. They are used in several orthopedics and maxillofacial application due to its excellent wear resistance, fracture toughness and very high compressive strength.^{1)–2)} They have been also used both as a pure form and associated with other biomedical materials such as polymethyl methacrylate (PMMA) cement in hip joint prosthesis and dental implants.^{3)–5)} However, alumina is classified as bioinert and has no direct bonding ability to living bone. Therefore, the alumina ceramic devices cannot avoid loosening in long periods when clinically used as bone substitutes for the reconstruction of bone defect. Fibrous tissue layer which reduce bonding strength is formed at the alumina/bone interface.^{6)–7)} On the other hand, an intermediate apatite layer is formed at the interface between a living bone and bioactive ceramics such as Bioglass[®] and Cerabone A-W[®] when implanted. The formed apatite layer plays as a “glue” to attach to living bone directly.^{8)–17)} Therefore, it has been proposed that the prerequisite for implants with bone-bonding ability is the formation of a biologically active bone-like apatite layer on its surface under the body environment.¹⁸⁾ Coating techniques of bioactive materials such as bioactive glasses and glass-ceramics^{19)–21)} have been developed for alumina substrates in order to induce their osteoconductivity. However, the coating layer might be peeled off from the surface during implantation. Therefore, a novel technique for surface modification to make alumina osteoconductive has been desired.

Kokubo and his colleagues have reported that calcium ions firstly adsorbed to the negatively charged surface, then, subsequently phosphate ions adsorbed on the surface when alkali and heat treated Ti was soaked in SBF. Amorphous calcium phosphate was then formed and it transformed into bone-like apatite.²²⁾ This alkali and heat-treated Ti and Ti alloy showed direct bonding to bone when implanted.²³⁾ If this bone-bonding mechanism of bioactive materials is correct, Ca-attached material should show osteoconductivity.

On the basis of this idea, titanium, that is not osteoconductive but osteointegrative material, was hydrothermally-treated with calcium chloride (CaCl_2) solution in our previous study.^{24),25)} After the treatment, titanium surface was found to be modified by Ca. Also, low crystalline apatite crystal was formed on the surface of Ca-treated titanium when it was soaked in simulated body fluid.²⁴⁾ Moreover, our *in vivo* studies further demonstrated that the Ca-treated titanium bond to bone without having connective tissue between the titanium and bone indicating osteoconductivity whereas connective tissue was observed between non-treated titanium and bone.²⁵⁾ These results suggested that the hydrothermal treatment with CaCl_2 could be an effective method for materials to acquire osteoconductivity. In this study, this method was applied to bioinert alumina substrates and we investigated whether the modified alumina could show osteoconductivity *in vitro*.

2. Materials and methods

2.1 Calcium modification of Al_2O_3

Commercially obtained alumina substrate (Al_2O_3) (Nikkato Co., Osaka, Japan) was used in this study. Square substrates of $5 \times 5 \times 0.5 \text{ mm}^3$ in size were employed for SBF test (described

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Fig. 1. Ca_{2p} XPS spectra for Al_2O_3 substrates treated at 125 (a), 150 (b) and 200°C (c) under the hydrothermal condition in the presence of different CaCl_2 concentration (0, 10 and 50 mmol/L).

later). All the substrates were polished with 0.50 and 0.25 μm diamond slurry followed by washing with distilled water and ethanol in an ultra sonic cleaner and dried in the room temperature. The Al_2O_3 substrates were soaked in 10 or 50 mmol/L of CaCl_2 aqueous solution in Teflon vessel with stainless steel jacket and then it was kept at 125, 150 or 200°C for 7 days under the hydrothermal condition. After the treatment, the substrates were sufficiently rinsed by distilled water. For comparison, Al_2O_3 substrates treated with distilled water under the same hydrothermal condition mentioned above and Al_2O_3 substrates without any treatment were also prepared. Surface element analysis of treated Al_2O_3 substrates was performed by X-ray photoelectron spectrometer (XPS) (ESCA 750; Shimadzu Co., Ltd, Japan) at 2 kV, 20 mA under the pressure of 5×10^{-4} Pa. In order to investigate surface corrosion on Al_2O_3 substrates during hydrothermal treatment, CaCl_2 solution after the hydrothermal treatment was analyzed by inductively coupled plasma (ICP) emission spectroscopy (Optima 7300DV, PerkinElmer, USA).

2.2 In vitro SBF screening test

A simulated body fluid (SBF) was used to estimate osteoconductivity *in vitro*. The Al_2O_3 substrates were soaked in an acellular SBF having an inorganic ion concentration nearly equal to that of human blood plasma. The SBF was prepared by dissolving high purity reagents in distilled water. The pH value of the SBF solutions was adjusted at pH 7.40 by adding tris(hydroxymethyl)aminomethane and hydrochloric acid. The preparation method in detail was reported by Kokubo et al.²⁶⁾ The untreated and treated Al_2O_3 substrates were soaked in 20 mL SBF in a polyethylene bottle at 37°C, without stirring. After 14 days, the substrates were removed from the solution, then gently rinsed with distilled water, and dried in room temperature. The surfaces of the Al_2O_3 substrates with and without the hydrothermal treatment and before and after being soaked in SBF were analyzed by scanning electron microscopy (SEM) (JSM-5400LV, JEOL Ltd., Japan) and energy dispersive X-ray analysis (EDX) (Genesis XM4, EDAX, USA) at 15 kV of accelerating voltage. The surface was also examined by thin-film X-ray diffraction (TF-XRD) (Rint2500, Rigaku, Japan; $\text{Cu K}\alpha$, $\lambda = 1.5406 \text{ \AA}$, 40 kV, 200 mA). ICP was used to investigate Ca ion release from Ca-modified Al_2O_3 after being soaked in SBF.

3. Results and discussion

Figure 1 shows Ca_{2p} XPS spectra for Al_2O_3 substrates treated at 125, 150 and 200°C under the hydrothermal condition in the presence of different CaCl_2 concentration (0, 10 and 50 mmol/L). When treated at 125°C, the Ca_{2p} peak intensity increased with CaCl_2 concentration as shown in Fig. 1(a). However the peak intensities of Ca_{2p} of Al_2O_3 treated at higher temperature, 150 and 200°C, were much weaker when compared to those at 125°C. The Ca_{2p} peaks did not disappear even after rinsed with distilled water preceded XPS measurement. Moreover, Cl_{2p} peaks could not be detected for all Al_2O_3 substrates treated at present experimental condition (not shown here). Although there is still unknown how Ca ions can be immobilized on the surface in the case of treatment at 125°C, a hypothesis can be explained based on the present results. In the Ca-treated Al_2O_3 surface, calcium had no chance to exist as CaCl_2 because Cl_{2p} peaks could not be detected simultaneously. Furthermore, a plenty of hydroxyl groups formed on Al_2O_3 surface during hydrothermal treatment may be employed for Ca-modification on the surface. Ca ions may attach to hydroxyl groups on Al_2O_3 treated at 125°C. Of course, the reaction rate becomes higher with increasing treatment temperature. At the high temperature such as 150 and 200°C, however, effect of surface corrosion can't be ignored. Based on the ICP analysis, actually, CaCl_2 solution after the hydrothermal treatment at 200°C contained Al ions (110 ppb) whereas Al ion concentration in CaCl_2 solution after the hydrothermal treatment at 125°C was below detection limit of ICP. Besides, the modified Ca ions could not be detected by EDX and XRD analyses, that is, it was only detected by XPS as shown in Fig. 1. These results indicate that only a small amount of Ca can be immobilized on Al_2O_3 substrates by the present treatment, that is, the Ca-modified layer is extremely thin.

Figure 2 shows SEM images for non-treated and hydrothermally-treated Al_2O_3 substrates with different CaCl_2 concentration (0, 10 and 50 mmol/L) at 125°C after being soaked in SBF for 14 days. No morphological change was observed on the non-treated and hydrothermally-treated Al_2O_3 with distilled water as shown in Figs. 2(a) and (b). On the other hand, the deposition of spherical particles was observed on Al_2O_3 treated with CaCl_2 at 125°C (Figs. 2(c) and (d)). Amount of depositions on Al_2O_3 treated with 50 mmol/L CaCl_2 was much higher than

Fig. 2. SEM images for non-treated (a) and hydrothermally-treated Al_2O_3 substrates with different CaCl_2 concentration (0 (b), 10 (c) and 50 mmol/L (d)) at 125°C after being soaked in SBF for 14 days.

Fig. 3. SEM images for hydrothermally-treated Al_2O_3 substrates with 50 mmol/L CaCl_2 solution at 150°C (a) and 200°C (b) after being soaked in SBF for 14 days.

that treated with 10 mmol/L CaCl_2 . Moreover, the morphology of depositions was similar to that of low crystalline apatite observed on bioactive glass and glass ceramics after being soaked in SBF.¹⁷⁾ **Figure 3** shows SEM images for hydrothermally-treated Al_2O_3 substrates with 50 mmol/L CaCl_2 solution at 150°C and 200°C after being soaked in SBF for 14 days. No deposition was observed for Al_2O_3 when treated at 150°C or 200°C . To understand the chemical composition of the deposit formed on the surface of Al_2O_3 , EDS spectra were measured for non-treated and hydrothermally-treated Al_2O_3 substrates with different CaCl_2 concentration (0, 10 and 50 mmol/L) at 125°C after being soaked in SBF for 14 days (**Fig. 4**). Two peaks assigned to Al and O due to Al_2O_3 were detected for all specimens. No other peak was detected for non-treated Al_2O_3 and Al_2O_3 hydrothermally treated with distilled water. In contrast, new peaks assigned to Ca and P were observed in the case of Al_2O_3 hydrothermally treated with CaCl_2 solution after SBF soaking. This result indicated that precipitates observed in Figs. 2(c) and (d) were composed of Ca and P. To further identify the precipitate, TF-XRD patterns were measured as shown in **Fig. 5**. Peaks for Al_2O_3 (JCPDS: 43-1484) were observed for all specimens. No other peaks were observed for non-treated (**Fig. 5(a)**) and hydrothermally-treated Al_2O_3 with distilled water (**Fig. 5(b)**). In order to confirm possible apatite formation, enlargement was also prepared as a right figure in **Fig. 5**. Small broad peak around 32° in 2θ was observed for Al_2O_3 substrates treated hydrothermally at 125°C in 50 mmol/L CaCl_2 (**Fig. 5(d)**). However, the peak intensity was too weak to

Fig. 4. EDS spectra for non-treated (a) and hydrothermally-treated Al_2O_3 substrates with different CaCl_2 concentration (0 (b), 10 (c) and 50 mmol/L (d)) at 125°C after being soaked in SBF for 14 days.

Fig. 5. TF-XRD patterns for non-treated (a) and hydrothermally-treated Al_2O_3 substrates with different CaCl_2 concentration (0 (b), 10 (c) and 50 mmol/L (d)) at 125°C after being soaked in SBF for 14 days.

identify apatite (JCPDS: 72-1243). Besides, typical peak for apatite around 26° in 2θ was blinded by overlapping with peak for Al_2O_3 . Therefore, precipitation observed in Figs. 2(c) and (d) was defined as amorphous calcium phosphate.

It was clear that Ca-modified Al_2O_3 substrate induced calcium phosphate precipitate on its surface when soaked in SBF. It has been reported that apatite formation for almost bioactive glass and glass ceramics is started from initial Ca ion release or increment of pH after SBF soaking. Because both factor affect increment of supersaturation with respect to apatite in SBF. In the present study, however, the attached Ca could not be released from Al_2O_3 surface during SBF test which was judged by ICP analysis of SBF after 1 day soaking. Besides, pH of SBF was also constant even after 1 day soaking. Therefore, calcium phosphate formation mechanism of Ca-modified Al_2O_3 is thought to be different from that of alkali and heat-treated titanium²³⁾ which is the most typical example of providing materials with osteoconductivity by using surface modification. Strong relationship was found between the presence of Ca on the Al_2O_3 surface and calcium phosphate formation. In other words, Ca-modified Al_2O_3 elicited the formation of calcium phosphate precipitation on its surface when soaked in SBF. Also, the induction ability of calcium phosphate precipitate was higher when the amount of modified Ca was larger. These phenomena are consistent with our previous results with respect to the

Ca-modification of titanium.²⁴⁾ Amount of the precipitate after immersion of Ca-modified specimens was larger in the case of titanium when compared to Al_2O_3 . Although no detailed mechanism has been clarified in this study, it may be caused by difference between titanium oxide and alumina oxide on basic potential of calcium phosphate formation under SBF.

It should be noted that osteoconductive Al_2O_3 is useful for medical use aimed for the reconstruction of bone defect. Further studies are awaited based on the results found in this initial investigation.

4. Conclusions

It was demonstrated that Al_2O_3 could be modified with Ca when hydrothermally treated with CaCl_2 solution at 125°C . Ca-modified Al_2O_3 elicited the formation of calcium phosphate precipitate when soaked in SBF. Fabrication of Ca-modified Al_2O_3 and calcium phosphate precipitate formation in SBF along with our previous titanium results demonstrated that hydrothermal treatment with CaCl_2 has good potential value to alter the non-osteoconductive materials to osteoconductive materials.

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