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Surface modification of poly(L-lactic acid)-vaterite composites on a zirconia substrate by imogolite coating

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Zirconia ceramics were coated with poly(L-lactic acid)-vaterite composite for introducing osteoconductivity. Aluminum silicate (imogolite) nanotubes were successfully applied to the composite coatings via a dipping process and subsequent heat treatment for the enhancement of cell attachment, using the hydrophilicity of imogolite. The heat treatment improved the adhesive strength between the imogolite and the composite coatings. Osteoblast-like cell cultures on the samples showed that the imogolite coating enhanced the cell attachment.

Key-words : Imogolite, Coating, Zirconia, Poly(lactic acid), Composite, Vaterite

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1. Introduction

Zirconia ceramics have attracted considerable attention in recent years as a potential material for dental implant applications because of their excellent mechanical properties and biocompatibility.[1)–3)] They are bioinert ceramics, which show no bonds directly at interfaces between the bone and the material after implantation. Various modifications of the surface of zirconia ceramics have been attempted to prepare a bioactive layer on the surface, using techniques such as micro-arc oxidation, chemical etching, and calcium phosphate ceramics or bioactive glass coating.[4)–7)]

Synthetic biodegradable polymers such as poly(lactic acid) and poly(ε-caprolactone) have become very important biomaterials. These polymer coatings have been widely applied to enhance the resistance of magnesium metals and its alloys to corrosion, which originates from a reaction with water.[8),9)] However, some problems have been encountered regarding the use of these polymers because of their poor biocompatibility compared to ceramics, such as hydroxyapatite, for biomedical applications.[10)] We previously succeeded in preparing poly(L-lactic acid) (PLLA) composites containing vaterite, which is one of the polymorphs for calcium carbonate, with oseteoconductivity.[11),12)] The composites are expected to be used in potential coatings on zirconia ceramics for biomedical applications.

The initial attachment, proliferation and differentiation of bone-forming cells at the interface between an implant and bone have important roles in the early stage of osseointegration. Both the surface topography and physicochemistry (such as surface wettability) of materials influence the behavior of bone-forming cells.[13),14)] Imogolite is a poorly crystalline hydrous aluminum silicate with nano-sized tubular morphology (external tube diameter: ~2.3 nm, internal tube diameter: ~1.0 nm) and its average length is 100 nm.[15)] Imogolite has been reported to show hydrophilicity due to numerous hydroxyl groups on its surface, and to have a high specific surface area.[16)] Human osteoblast-like cells (Saos-2) and mouse osteoblast-like cells (MC3T3-E1) showed high affinity to imogolite, compared to a conventional culture dish.[17),18)]

We found previously that imogolite can be successfully coated on an electrospun fibermat consisting of PLLA-based composite microfibers containing siloxane-doped calcium carbonate particles by electrophoretic deposition.[19)] Imogolite could not be deposited on a PLLA substrate in our preliminary experiment. Imogolite is the material of choice to be used in the surface modification of biodegradable polymers containing vaterite to enhance its biocompatibility for example, initial attachment of bone-forming cells. In the present study, we coated zirconia ceramics with a PLLA-vaterite composite to introduce bioactivity, and we attempted the surface modification of the composite coatings using imogolite to enhance the biocompatibility.

2. Experimental procedure

2.1 Preparation of zirconia substrates

Zirconia substrates in a rectangular shape were prepared by a cold isostatic pressing of the powders (TZ-3Y-E, Tosho, Tokyo, Japan), which were pre-heated at 1000°C for 1 h. The substrate was prepared by sintering at 1350°C for 2 h. After grinding and polishing, the substrates were heated again at 1350°C for 1 h to remove the surface stress. The resultant substrates were 10.0 × 5.0 × 0.9 mm in size. The substrates were soaked in separate plastic tubes filled with 10 mL of 0.01 mmol/L HCl at pH 5.0 at room temperature for 12 h to be decontaminated.

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We determined the concentration of HCl by an optimization process based on a trial-and-error approach to achieve satisfactory results in terms of preventing transformation of the tetragonal phase.

2.2 PLLA/vaterite composite coating on the zirconia substrates

The zirconia substrate was dipped into dichloromethane containing 10 mass % PLLA (LACIA®, Mitsui Chemicals, Tokyo, Japan). It was drawn up at a speed of 3.0 mm/s and then dried at room temperature in air. In a preliminary experiment, the tensile strength between zirconia and PLLA could be improved by heat treatment around the melting point temperature of PLLA when a zirconia substrate was coated with PLLA. The substrate coated with PLLA was heated at 200°C for 30 min.

For the introduction of vaterite into the PLLA coating, the substrate was dipped into a suspension containing 15 mass % vaterite (Yabashi Industries, Gifu, Japan) in methanol. The substrate was withdrawn at a velocity of 3.0 mm/s, and subsequently dried at room temperature in air. To anchor the vaterite particles in the PLLA coating, the substrate was pressed at 10 MPa for 1 min, and then heated at 200°C for 30 min.

2.3 Surface modification of the composite coating using imogolite

We pretreated the composite coating with ethanol at room temperature for 10 min. A similar pretreatment procedure has been used to coat biodegradable polymers with inorganic materials.[20),21)] Here, the pretreated composite coating was dipped into an imogolite aqueous solution containing 8.7×10^{-2} mass % imogolite at pH = 3.8. It was drawn up at a speed of 3.0 mm/s and then dried at room temperature in air. The imogolite aqueous solution was obtained from the National Institute of Advanced Industrial Science and Technology (Applied Technology with Traditional Ceramics Group, Chubu Center). The details of imogolite synthesis were described.[22),23)] Finally, the composite coating was heated at 110°C for 30 min.

2.4 Evaluation of the composite coatings

The samples were coated with amorphous osmium by plasma chemical vapor deposition and then observed by scanning electron microscopy (SEM). Detachment tests using Scotch® tape (CC1820-Bx-J) were carried out to evaluate the adhesive strength between the composite coatings and the imogolites. Concentrations of calcium elements for soaking the samples in 5 mL of Tris-buffer solution included 50 mM of $(CH_2OH)_3CNH_2$ and 45 mM of HCl at pH 7.4 at 37°C were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) to measure the amount of Ca^{2+} ion released from the samples.

Osteoblast-like cells (MC3T3-E1) were used to examine the initial cell attachment on the composite coating with and without imogolite. One-milliliter of a cell suspension at a density of 5.0×10^4 cell/mL was seeded onto the samples in a 24-well tissue culture plate. All cultures were supplemented with an α–minimum essential medium with 10% fetal bovine serum and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 6 h. After the culturing period, the samples were fixed with 2.5% glutaraldehyde in phosphate buffer for 40 min at 4°C. After being rinsed several times in the same buffer, the cells were dehydrated through a series of increasing concentrations of ethanol, and finally dried with hexamethyldisilazane. The morphology of the cells on the surface of the samples was observed by SEM, and the area of attached cells was measured for at least 30 cells by image-editing software: ImageJ, obtained from National Institutes of Health.

3. Results and discussion

Figure 1 shows SEM micrographs of the composite coatings before and after the surface modification by imogolite. Dispersed vaterite particles approx. 0.5 μm in diameter were embedded into a PLLA matrix before the treatment [Fig. 1(a)]. We were able to successfully coat the composite on the zirconia substrate by this method. After the treatment, an uneven surface was observed [Fig. 1(b)]; the PLLA matrix and dispersed imogolite nanotubes cannot be seen clearly. This result implies that the composite coating was covered with imogolite aggregates.

The detachment tests were carried out to clarify the importance of the heat treatment for imogolite-modification of the composite coating. **Figure 2** shows SEM micrographs of surface morphologies of the composite coating modified by imogolite prepared with and without the heat treatment after the detachment test. PLLA and vaterite particles were clearly seen at the surface of the samples prepared without the heat treatment. In contrast, the surface morphology of the samples prepared with the heat treatment was almost the same, as shown in Fig. 1(b), suggesting that the heat treatment improves the adhesive strength between imogolite aggregates and the composite coating. The zeta potential of the composite coating exhibited highly negatively charge at pH = 7.4 due to the dissociation of protons from carboxy groups of PLLA.

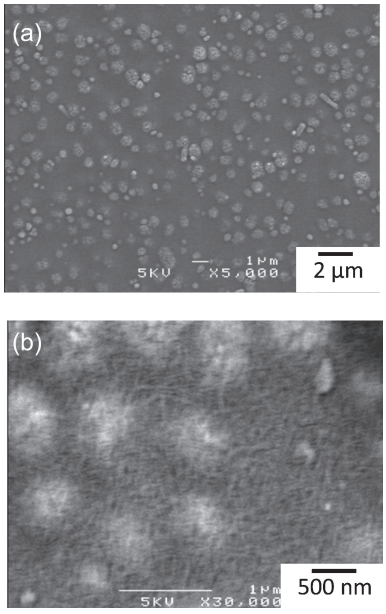

Fig. 1. SEM micrographs of the samples. (a) Composite coating and (b) composite coating modified by imogolite.

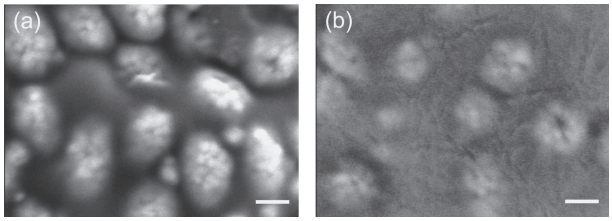

Fig. 2. SEM micrographs of the surface modification of the composite coatings prepared (a) without and (b) with the heat treatment after the detachment test. Scale bar = 500 nm.

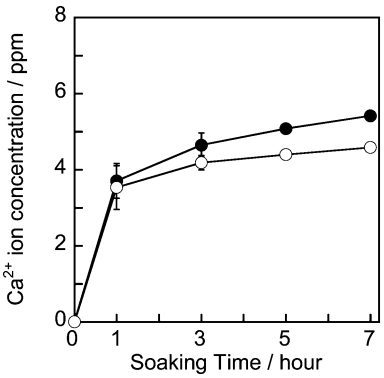

Fig. 3. Ca^{2+} ion concentrations released from the samples after the soaking. (●) Composite coatings and (○) composite coatings modified by imogolite.

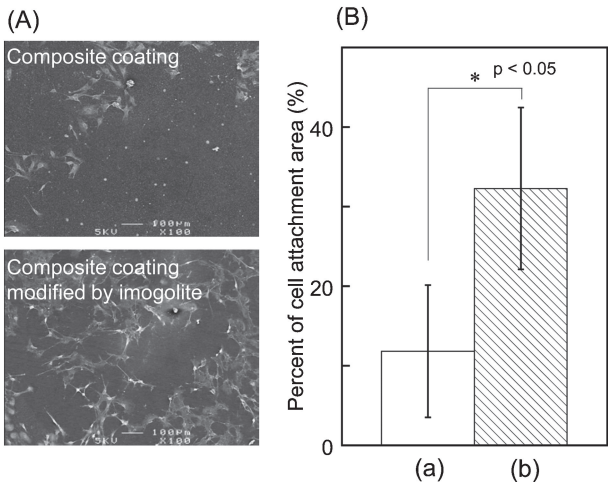

Fig. 4. (A) SEM micrographs of cells attached on the sample surface after the cell culture. (B) Cell-attached area ratios on the surface calculated by imaging software. (a) The blank represents the composite coatings, and (b) oblique lines represent the composite coatings modified by imogolite.

The zeta potential of imogolite in the solution used in the present work showed positive charged values (32.0 ± 0.9 mV). One possible mechanism for the imogolite deposition originates from an electrostatic intermolecular interaction between the composite coating and the imogolite. The thermogravimetric analysis of imogolite illustrated that a significant loss (about 25%) of weight occurs up to 100°C due to physisorbed water at their surfaces. The heat treatment at 110°C causes removal of the adsorbed water, providing a large amount of free hydroxyl groups at the surface of the imogolite. The increase in the electrostatic interaction is thought to improve the adhesive strength.

Figure 3 shows the Ca^{2+} ion concentrations released from the samples after they were soaked in Tris-buffer solution for various periods. There was almost no difference in the release behavior of Ca^{2+} ions among the samples, but, the amount of ions released from the composite coating modified by imogolite was slightly lower than that from the composite coating without imogolite-modification. The imogolite on the composite coating may suppress the release of Ca^{2+} ions from the vaterite particles in the coating, which was covered with imogolite as shown in Fig. 1(b).

Figure 4(A) shows SEM micrographs of the samples after 6 h of cell culture. The cell numbers on the composite coating modified by imogolite were larger than those on the composite coating; the imogolite enhanced the initial cellular attachment on the composite coating without imogolite-modification. The cell-attached area calculated by imaging software on the composite coating modified by imogolite was significantly higher than that on the composite coating without imogolite-modification [Fig. 4(B)]. This implies that the imogolite modification made it easier for the cells to spread.

Numerous reports have pointed out a more rapid cell response (such as attachment) to rough surfaces compared to smooth surfaces in culture tests.[24] As shown in Fig. 1, the modification by imogolite aggregates caused a change in the surface texture compared to the composite coating. These results showed that the imogolite modification enhanced the cell attachment.

4. Conclusions

Zirconia substrates were successfully coated with a PLLA/vaterite composite and imogolites by a dipping process and subsequent heat treatment. The composite coatings modified by imgolite prepared with the heat treatment showed stronger adhesion compared to those prepared without the heat treatment. The Ca^{2+} ions released from the coatings were slightly suppressed by the imogolite aggregates. The surface modification of the composite coatings by imogolite led to the enhancement of the initial cellular attachment. Surface modification using imogolite is a suitable option for improving the cellular compatibility of polymer-based matrices containing vaterite.

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References

1) C. Piconi and G. Maccauro, *Biomater.*, 20, 1–25 (1999).
2) Y. Yang, J. L. Ong and J. Tian, *Biomater.*, 24, 619–627 (2003).
3) J. Chevalier and L. Gremillard, *J. Eur. Ceram. Soc.*, 29, 1245–1255 (2009).
4) Y. Han, Y. Y. Yan, C. G. Lu, Y. M. Zhang and K. W. Xu, *J. Biomed. Mater. Res., Part A*, 88A, 117–127 (2009).
5) M. Uchida, H. M. Kim, T. Kokubo, M. Nawa, T. Asano, K. Tanaka and T. Nakamura, *J. Biomed. Mater. Res.*, 60, 277–282 (2002).
6) M. Stefanic, K. Krnel, I. Pribosic and T. Kosmac, *Appl. Surf. Sci.*, 258, 4649–4656 (2012).
7) M. N. Rahaman, Y. Li, B. S. Bal and W. Huang, *J. Mater. Sci.: Mater. Med.*, 19, 2325–2333 (2008).
8) J. Gray and B. Luan, *J. Alloys Compd.*, 336, 88–113 (2002).
9) L. Xu and A. Yamamoto, *Colloids Surf., B*, 93, 67–74 (2012).
10) L. G. Griffith, *Acta Mater.*, 48, 263–277 (2000).
11) H. Maeda, T. Kasuga and M. Nogami, *Mater. Trans.*, 45, 989–993 (2004).
12) H. Maeda, T. Kasuga, M. Nogami and M. Ueda, *Sci. Technol. Adv. Mater.*, 6, 48–53 (2005).
13) T. G. Van Kooten, J. M. Schakenraad, H. C. Van der Mei and H. J. Busscher, *Biomater.*, 13, 897–904 (1992).
14) L. Montanaro, C. R. Arciola, D. Campoccia and M. Cervellati, *Biomater.*, 23, 3651–3659 (2002).
15) S. Mukherjee, V. A. Bartlow and S. Nalr, *Chem. Mater.*, 17, 4900–4909 (2005).
16) K. J. D. Mackenzie, M. E. Bowden, I. W. M. Brown and R. H. Meinhold, *Clays Clay Miner.*, 37, 317–324 (1989).

17) K. Ishikawa, S. Abe, Y. Yawaka, M. Suzuki and F. Watari, *J. Ceram. Soc. Japan*, **118**, 516–520 (2010).
18) M. Suzuki, H. Sato, C. Ikeda, R. Nakanishi, K. Inukai and M. Maeda, *J. Clay Sci. Soc. Japan*, **46**, 194–199 (2007) in Japanese.
19) S. Yamazaki, H. Maeda, A. Obata, K. Inukai, K. Kato and T. Kasuga, *J. Nanomater*, 463768 (2012).
20) H. Maeda, V. Maquet, Q. Z. Chen, T. Kasuga, H. Jawad and A. R. Boccaccini, *Mater. Sci. Eng., C*, **27**, 741–745 (2007).
21) J. A. Roether, A. R. Boccaccini, L. L. Hench, V. Maquet, S. Gautier and R. Jérôme, *Biomater.*, **23**, 3871–3878 (2002).
22) M. Suzuki, F. Ohashi, K. Inukai, M. Maeda and S. Tomura, *J. Clay Sci. Soc. Japan*, **40**, 1–14 (2000) in Japanese.
23) M. Suzuki, K. Inukai and M. Maeda, *J. Vac. Soc. Japan*, **49**, 29–33 (2006) in Japanese.
24) D. D. Deligianni, N. D. Katsala, P. G. Koutsoukos and Y. F. Missirlis, *Biomater.*, **22**, 87–96 (2001).