Simple Surface Modification Process to Produce a Transparent Apatite–Polystyrene Composite for In Situ Observation of Cell Behavior

Ayako Oyane,*[1] Hideo Tsurushima,[1] and Atsuo Ito[2]
[1]*Nanotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Central 5, 1-1-1 Higashi, Tsukuba 305-8565*
[2]*Institute for Human Science and Biomedical Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Central 6, 1-1-1 Higashi, Tsukuba 305-8566*

(Received September 4, 2006; CL-061006; E-mail: a-oyane@aist.go.jp)

An apatite layer was formed on a transparent polystyrene (PS) substrate using a simple surface modification process, in which apatite precursors were deposited on a PS substrate surface that was then immersed in a calcium phosphate solution supersaturated with respect to apatite. The apatite-coated PS substrate thus obtained was highly transparent, which enables in situ observation of cell behavior on the surface apatite layer under a transmission optical microscope.

Apatite is a major inorganic component of human hard tissues, and exhibits bone-bonding ability in vivo. Therefore, sintered apatite and its composites with other calcium phosphates or polymeric materials have been widely used in orthopedic applications. Another important application of apatite is as a cell culture substrate to study the behavior of bone cells including osteoblasts and osteoclasts on the apatite surface.[1] For in situ observation of viable cells under an optical microscope, a cell culture substrate should be highly transparent. Although various processes have been developed to prepare a transparent sintered apatite,[2] all of them involve complicated and costly procedures including heat treatment. In addition, sintered apatite has limited variety in its composition and structure, and is largely different from bone apatite. Coating a transparent and inexpensive substrate with an apatite layer using a low-temperature process would be an improvement on the approach using a sintering process. As a substrate material, polystyrene (PS) is highly appropriate, as it has long been used as a cell culture substrate in the form of dishes, flasks, plates etc., owing to its high transparency, fine mechanical and chemical properties, and economy of production. Among the various coating processes used to place an apatite layer on a polymer substrate,[3–7] a process using a metastable calcium phosphate (CaP) solution supersaturated with respect to apatite,[4–6] is intrinsically suitable to produce apatite with a desired composition and structure. By selecting various solution conditions, such as pH, reagent concentration, and temperature, the composition and structure of apatite deposited from the solution can be considerably varied. For example, bonelike apatite with a composition and structure similar to that of bone apatite can be obtained from a CaP solution with pH, ion concentrations, and temperature approximately equal to those of human blood plasma.[5] The problems of previous processes[4,5] using CaP solutions include complicated and long-term modification procedures.

The present authors have recently developed a simple and quick surface modification process to coat an apatite layer onto a polymer surface.[6] In this process, apatite precursors are deposited onto a polymer surface using plasma and a simplified alternate dipping treatment,[7] and the polymer was then immersed in a CaP solution. Although opaque poly(ε-caprolactone) (PCL) has been tested hitherto,[6] it remains unknown whether this surface modification process can be applied to PS retaining its transparency. In the present study, a PS substrate was subjected to the surface modification process described above. The specimen thus obtained was tested as a cell culture substrate for in situ observation of cells on its surface.

A PS substrate was treated with oxygen plasma.[8] X-ray photoelectron spectroscopy (XPS) revealed that oxygen-containing functional groups such as carbonyl and carboxyl groups were produced on the PS surface by the plasma treatment. Accordingly, water contact angle on the PS surface was decreased from 98° to 7° after the plasma treatment. The untreated and plasma-treated specimens were then subjected to the alternate dipping treatment[6,9] (denoted as U–PS and P–PS, respectively). According to XPS, calcium and phosphorus peaks were readily detected on the P–PS surface, but not on the U–PS surface. This result suggests that a kind of calcium phosphate was deposited only on the P–PS surface. It is considered that the functional groups formed on the plasma-treated PS surface are a prerequisite for deposition of the calcium phosphate. The calcium phosphate deposited on the P–PS surface is most likely to be amorphous calcium phosphate (ACP), according to our previous observation using a transmission electron microscope.[10]

U–PS and P–PS were subsequently immersed in the CaP solution.[11] After 24 h, a uniform layer with a water contact angle of 7° and thickness of approximately 3 μm was formed only on the P–PS surface (Figure 1). In contrast to P–PS, the surface

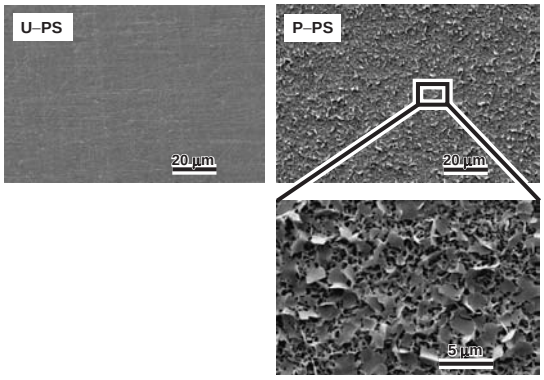

Figure 1. SEM photographs of the surfaces of U–PS and P–PS, after immersion in the CaP solution for 24 h.

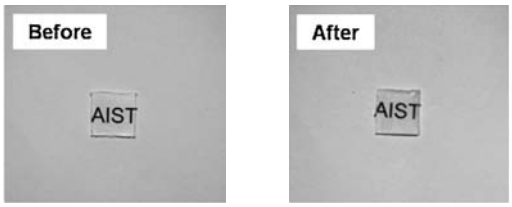

Figure 2. Photographs of the PS substrate, before and after the present surface modification process.

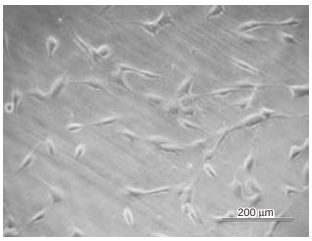

Figure 3. Transmission optical micrograph of the viable cells cultured on the apatite-coated PS substrate.

structure of U–PS was unchanged even after immersion in the CaP solution. The layer observed on the P–PS surface was identified as low-crystalline apatite from the thin-film X-ray diffraction data. It is considered that P–PS formed an apatite layer on its surface in the CaP solution using ACP as a precursor of apatite. These results are consistent with previous results obtained with PCL.[6]

Transparency of PS was successfully retained even after the present surface modification process, as shown in Figure 2. This indicates potential applicability of the resulting apatite-coated PS substrate as a cell culture substrate. Therefore, osteosarcoma cells were cultured on the apatite-coated PS substrate thus obtained.[12] A transmission optical micrograph of the viable cells after culture is shown in Figure 3. As shown in the figure, cell morphology can be clearly observed under a transmission optical microscope in situ without fixation and dying of the cells, which is required for an opaque cell culture substrate.

It should be noted that the present surface modification process is simple compared to previous processes,[4,5] and can be completed within 2 d without using any toxic reagents. Moreover, this process is applicable to commercially available PS cell culture substrates such as 12-well cell culture plates (data not shown). On these PS cell culture substrates, various types of apatite including bonelike apatite,[6] protein-immobilized apatite,[13] and antibacterial agent-immobilized apatite[14] can be formed using various CaP solutions. Hence, the present process is potentially a powerful tool for the future study of cell behavior on the surface of various kinds of apatite, as to be published elsewhere.

In summary, the present surface modification process was found to be useful to produce an apatite-coated PS substrate while retaining transparency of the PS. The resulting composite was applicable as a cell culture substrate that enabled in situ cell observation on the surface apatite layer using a transmission optical microscope.

This work was supported by an Industrial Technology Research Grant Program in 2005 from the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

References and Notes

1 J. A. Gilles, D. L. Carnes, A. S. Windeler, *J. Endodont.* **1994**, *20*, 327; K. Gomi, B. Lowenberg, G. Shapiro, J. E. Davies, *Biomaterials* **1993**, *14*, 91.
2 K. Takikawa, M. Akao, *J. Mater. Sci.: Mater. Med.* **1996**, *7*, 439; K. Uematsu, M. Takagi, T. Honda, N. Uchida, K. Saito, *J. Am. Ceram. Soc.* **1989**, *72*, 1476; K. Ioku, M. Yoshimura, S. Somiya, *J. Ceram. Soc. Jpn.* **1988**, *96*, 109.
3 H.-M. Kim, Y. Kim, S.-J. Park, C. Rey, H. Lee, M. J. Glimcher, J. S. Ko, *Biomaterials* **2000**, *21*, 1129; Y. Shikinami, H. Kawarada, *Biomaterials* **1998**, *19*, 617; E. N. Antonov, V. N. Bagratashvili, L. I. Krotova, V. K. Popov, *Key Eng. Mater.* **2000**, *192-1*, 63; T. Furuzono, K. Sonoda, J. Tanaka, *J. Biomed. Mater. Res.* **2001**, *56*, 9.
4 M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura, T. Yamamuro, *J. Am. Ceram. Soc.* **1994**, *77*, 2805; A. Oyane, M. Kawashita, K. Nakanishi, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura, *Biomaterials* **2003**, *24*, 1729; A. Oyane, M. Kawashita, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura, *J. Ceram. Soc. Jpn.* **2002**, *110*, 248.
5 H.-M. Kim, K. Kishimoto, F. Miyaji, T. Kokubo, T. Yao, Y. Suetsugu, J. Tanaka, T. Nakamura, *J. Biomed. Mater. Res.* **1999**, *46*, 228; A. Oyane, H. M. Kim, T. Furuya, T. Kokubo, T. Miyazaki, T. Nakamura, *J. Biomed. Mater. Res.* **2003**, *65A*, 188.
6 A. Oyane, M. Uchida, Y. Yokoyama, C. Choong, J. Triffitt, A. Ito, *J. Biomed. Mater. Res.* **2005**, *75A*, 138.
7 T. Taguchi, A. Kishida, M. Akashi, *Chem. Lett.* **1998**, 711.
8 A PS substrate ($1 \times 10 \times 10\,mm^3$) was treated with an O_2 plasma ($0.5\,W/cm^2$) for 30 s using a compact ion etcher.
9 The specimen was dipped in 200 mM of $CaCl_2$ solution for 10 s, dipped in ultrapure water for 1 s, and then dried. The specimen was subsequently dipped in 200 mM of $K_2HPO_4 \cdot 3H_2O$ solution for 10 s, dipped in ultrapure water for 1 s, and then dried. The above alternate dipping procedure was performed three times.
10 A. Oyane, M. Uchida, A. Ito, *Key Eng. Mater.* **2005**, *284*, 227.
11 The ACP-modified specimen was immersed in the CaP solution (142 mM NaCl, 1.50 mM $K_2HPO_4 \cdot 3H_2O$, 3.75 mM $CaCl_2$) with pH 7.40 at 25 °C for 24 h.
12 Osteosarcoma cells derived from human (5×10^4 cells/500 μL MEM supplemented with 10% FBS) were cultured on the specimen at 37 °C under 5% CO_2 for 1 d.
13 Y. Liu, P. Layrolle, J. de Bruijn, C. van Blitterswijk, K. de Groot, *J. Biomed. Mater. Res.* **2001**, *57*, 327; M. Uchida, A. Oyane, H.-M. Kim, T. Kokubo, A. Ito, *Adv. Mater.* **2004**, *16*, 1071; A. Oyane, M. Uchida, A. Ito, *J. Biomed. Mater. Res.* **2005**, *72A*, 168.
14 M. Stigter, K. de Groot, P. Layrolle, *Biomaterials* **2002**, *23*, 4143; A. Oyane, Y. Yokoyama, M. Uchida, A. Ito, *Biomaterials* **2006**, *27*, 3295.

Published on the web (Advance View) October 21, 2006; doi:10.1246/cl.2006.1300