

Reduction of surface roughness of a laminin–apatite composite coating via inhibitory effect of magnesium ions on apatite crystal growth

Ayako Oyane^{a,*}, Yuusuke Ishikawa^b, Atsushi Yamazaki^b, Yu Sogo^c, Katsuko Furukawa^{d,e}, Takashi Ushida^{d,e}, Atsuo Ito^c

^a *Nanotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Central 4, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan*

^b *Department of Resources and Environmental Engineering, Waseda University, 3-4-1 Ohkubo, Shinjuku, Tokyo 169-8555, Japan*

^c *Institute for Human Science and Biomedical Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan*

^d *Department of Bioengineering, Graduate School of Engineering, University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-8656, Japan*

^e *Center for NanoBio Integration, University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-8656, Japan*

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Abstract

An artificial material coated with a laminin–apatite composite layer on its surface would be useful as a percutaneous device with cell-adhesion properties and good biocompatibility. The present authors recently prepared such a composite by a liquid-phase coating process in which a surface-modified material was immersed in a laminin-containing calcium phosphate solution (LCP solution) supersaturated with respect to apatite. As a result of this coating process, however, the material increased in surface roughness, which may have an adverse effect on the prevention of bacterial infection. In the present study, a reduction of the surface roughness of the laminin–apatite composite layer was attempted by adding MgSO₄ to the LCP solution as an inhibitor of apatite crystal growth. The surface roughness, Ra, of the resulting laminin–apatite composite layer decreased from approximately 0.43 to 0.31 μm upon increasing the MgSO₄ concentration in the LCP solution from 0 to 1.5 mM, while retaining its cell adhesion characteristics. The thus-obtained material, having a laminin–apatite composite coating with reduced surface roughness, has potential as a material for percutaneous devices with improved resistance to bacterial infection through the interface between the device and the skin.

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1. Introduction

An essential requirement for percutaneous devices such as catheters and external bone fixation devices is the effective prevention of bacterial infection through the interface between the device and the skin. Therefore, a percutaneous device must adhere firmly to skin tissue and prevent epidermal downgrowth. In the early 1990s, Aoki et al. produced a

percutaneous device composed of dense sintered hydroxyapatite (sHA) [1,2]. Because of the good biocompatibility of apatite [3,4], the sHA percutaneous device demonstrated long-term prevention of epidermal downgrowth [1,2]. In terms of epithelial tissue adhesion, however, a laminin–apatite composite is more effective than sHA, because laminin is a cell-adhesion molecule that is present in the basement membrane and interacts with integrins and non-integrin receptors on the cell surface [5–7].

On the basis of this background, the present authors have recently prepared an ethylene–vinyl alcohol copolymer (EVOH) [8,9] and a titanium metal [10] that were coated with a laminin–apatite composite layer on their

* Corresponding author. Tel.: +81 29 861 4693; fax: +81 29 861 3005.
E-mail address: a-oyane@aist.go.jp (A. Oyane).

surfaces. Both materials enhance epithelial cell adhesion onto their surfaces [8,10], and hence have great potential as materials for new percutaneous devices with improved adhesiveness to skin tissue. The coating of a laminin–apatite composite layer is carried out by the following process. First, an EVOH or titanium plate is modified on its surface with apatite precursors [8,9] or functional groups [10] that are effective in inducing apatite nucleation. Second, the surface-modified material is immersed in a laminin-containing calcium phosphate solution (LCP solution) that is supersaturated with respect to apatite [8–10]. As a result of this coating process, however, the material increases in surface roughness [11], which may have an adverse effect on the prevention of bacterial infection. In general, the higher the surface roughness of a percutaneous device, the higher the risk of interfacial infection by bacteria.

In the present study, reduction of surface roughness of the laminin–apatite composite layer was attempted by adding MgSO_4 as an inhibitor of apatite crystal growth [12–15] to the LCP solution. As a substrate material, an EVOH with high chemical stability and mechanical strength was employed. The physicochemical properties as well as cell adhesion properties of the laminin–apatite composite layer formed on the EVOH surface are discussed in terms of MgSO_4 concentration in the LCP solution.

2. Materials and methods

2.1. Preparation of the EVOH plates

A 1 mm thick EVOH plate was prepared by hot-pressing EVOH pellets with a quoted ethylene content of 32 mol% (generously supplied by Kuraray Co. Ltd., Japan) at a temperature of 210 °C. The EVOH plate, cut into $10 \times 10 \text{ mm}^2$ squares, was abraded on one side using SiC paper (average grain size = 7.6 μm), ultrasonically washed with acetone and ethanol, and then dried at a temperature of 100 °C under vacuum for a period of 24 h.

2.2. Modification of the EVOH surface with amorphous calcium phosphate

The EVOH plates were subjected to an alternating dipping treatment in calcium and phosphate ion solutions [16–18]. First, the sample was dipped in 20 ml of 200 mM aqueous CaCl_2 (Nacalai Tesque Inc., Japan) solution for a period of 10 s, then dipped in ultrapure water for a period of 1 s and dried. The sample was subsequently dipped in 20 ml of 200 mM aqueous $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ (Nacalai Tesque Inc., Japan) solution for a period of 10 s, then dipped once again in ultrapure water for a period of 1 s and dried. The above alternating dipping procedure in calcium and phosphate ion solutions was repeated three times. It has already been reported that nanoparticulates of amorphous calcium phosphate (ACP), which is a precursor of apatite, are deposited onto an EVOH plate as a result of this treatment [19].

2.3. Coating of a laminin–apatite composite layer

The ACP-modified EVOH plate was subsequently immersed in 3 ml of an LCP solution or one supplemented with 0.5, 1.0, 1.5, 3.0 or 5.0 mM of MgSO_4 (Wako Pure Chemical Industries Ltd., Japan) at 25 °C for a period of 24 h. The LCP solution [8–10] was prepared by adding 40 $\mu\text{g ml}^{-1}$ of laminin (Sigma-Aldrich Co., USA) to an aqueous calcium phosphate solution. The aqueous calcium phosphate solution was prepared by dissolving NaCl (142 mM), $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ (1.50 mM) and CaCl_2 (3.75 mM) (Nacalai Tesque Inc., Japan) in ultrapure water and buffering the solution to pH 7.4 at 25 °C using tris(hydroxymethyl)aminomethane (at a final concentration of 50 mM) and 1 M aqueous HCl solution (Nacalai Tesque Inc., Japan). The EVOH plate removed from the LCP solution was gently washed with ultrapure water and then dried in air. The EVOH plate immersed in the LCP solution is denoted as M00, and the plates immersed in the LCP solution supplemented with 0.5, 1.0, 1.5, 3.0 and 5.0 mM of MgSO_4 are denoted as M05, M10, M15, M30 and M50, respectively.

2.4. Surface analysis of the samples

The samples' surfaces were characterized by scanning electron microscopy (SEM; Model XL30, FEI Japan Ltd., Japan), thin-film X-ray diffraction (TF-XRD; Rint-Ultima X, Rigaku Co., Japan) employing Cu K_α X-rays, and X-ray photoelectron spectroscopy (XPS; Model Quantum-2000, ULVAC-PHI Inc., Japan) employing Al K_α X-rays. The glancing angle of the sample in the TF-XRD measurements was set at 1° against the direction of the incident beam. The photoelectron takeoff angle in the XPS measurements was set at 45°. The surface roughness of the samples was measured by a surface roughness tester (VF-7500, KEYENCE Co., Japan). The surface roughness measurements were performed at six different points on each type of sample to obtain an average value and a standard deviation.

The thickness of the coating layers formed on the samples' surfaces was measured by the following procedure. The sample after the coating process was embedded in an epoxy resin (Agar 100®, Agar Scientific Ltd., UK), abraded vertically to the surface layer with SiC paper with a grain size of 130 μm , then polished with diamond abrasive films with a grain size of 30, 15, 9, 3 and finally 1 μm to finish. The cross-section of the sample was observed by SEM to measure the thickness of the surface layer. More than 12 points were measured for each type of sample to obtain an average value and a standard deviation.

2.5. Analysis of the solutions

Changes in calcium and phosphorus concentrations of the LCP solution due to immersion of the sample were measured using inductively coupled plasma atomic emission

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