Electrodeposition of Collagen on Titanium to Add Hard and Soft Tissue Compatibilities

T. Hanawa[1], H. Kamata[2], J. Choi[1], K. Oya[1], Y. Tsutsumi[1], H. Doi, N[1]. Nomura[1], K. Moriyama[2]
[1] Institute of Biomaterials and Bioengineering, Tokyo Medical and Dentals University, Tokyo, Japan
[2] Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

Statement of Purpose: According to increasing demand for biofunctions of metals, we have developed an electrodeposition technique of poly(ethylene glycol), PEG, to metal surfaces. PEG-immobilized titanium (Ti) with electrodeposition inhibited the adsorption of protein, adhesion of cells, adhesion of platelet, and adhesion of bacteria [1-4]. On the other hand, calcification on Ti was accelerated with immobilization of a cell-adhesive Arg-Gly-Asp, RGD, peptide through PEG zwitterion with -NH_2 and -COOH [5, 6]. The advantages of these techniques are as follows: (1) The technique is applied to all metallic materials, (2) complex surface morphologies, and (3) all molecules containing positive and /or negative charges. On the basis of these techniques, we attempted the electrodeposition of collagen to Ti surface to add hard tissue compatibility and soft tissue compatibility. The performance of the materials was evaluated with cell culture test.

Methods: Commercially pure Ti disks with grade 2 (8 mmϕ×1.5 mm in thickness) were mirror-polished and ultrasonically rinsed in acetone for 10 min. Type I collagen from bovine serum was dissolved into 0.9mass%NaCl aqueous solution with a concentration of 10 mg mL^{-1}. The titration curve of the collagen was obtained, because the pH influences the dissociation of side-chains of collagen and active hydroxyl groups on Ti. According to the result, the best pH for electrodeposition was determined as 5. The pH was adjusted to with a 0.1 mol L^{-1} NaOH solution. The collagen was electrodeposited on the whole area of the Ti surface using the following process. The cathodic or anodic potential was charged from open circuit potential to -1.0 V or +0.1 vs. SCE, respectively, at 25°C and maintained at this potential for 30 and 1800 s (DC-cathod or DC-anode). On the other hand, alternating current between -1 and +1 V vs. SCE with 1 Hz was charged for 30 s and 1800 s (AC), according to a previous study [7]. After electrodeposition, specimens were ultrasonically rinsed in water for 15 min. The surface was characterized using scanning probe microscopy (SPM) and X-ray photoelectron spectroscopy (XPS). The thickness of the immobilized collagen layer was determined with ellipsometry. The specimens were shacked in water for 72 h to evaluate the durability of the immobilization. Cell culture test using MC3T3-E1 and HaCAT cells were performed for hard and soft tissue compatibilities, respectively.

Results: Fig.1 shows the thickness of the immobilized collagen layer before and after shacking in water. The layer was much larger in AC than that in DC-cathode. In DC-anode, the thickness contained that of the surface oxide film on Ti, confirmed with XPS. Collagen molecules have both positive and negative side-chain. The positive sites were attracted when cathodic potential was charged; the negative sites were attracted when anodic potential was charged. Therefore, the most proper condition was AC electrodeposition which generated both cathodic and anodic potentials. Fig.2 shows SPM image of immobilized collagen by AC electrodeposition at pH 5. Collagen fibers formed a network on Ti. Cells were well attached and expanded on AC electrodeposited collagen than on untreated Ti. In addition, calcification was accelerated by network-like immobilized collagen on Ti.

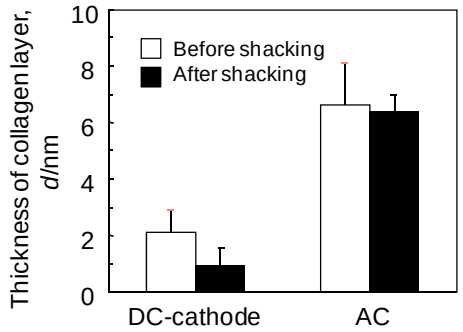

Figure 1. The thickness of collagen layer immobilized with DC-cathode and AC for 1800 s before and after shacking in water.

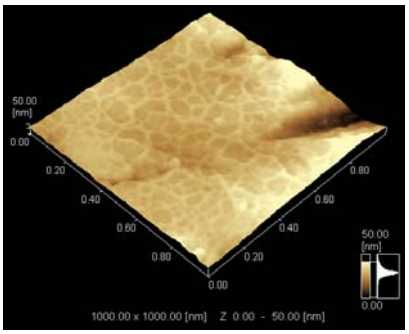

Figure 2. SPM image of collagen immobilized on Ti with AC electrodeposition.

Conclusions: AC electrodeposition is the most effective to immobilize collagen to Ti surface and the collagen forms a network-like layer. The immobilized layer gives both hard and soft tissue compatibilities to Ti.

References:

[1] Tanaka Y et al., J Mater Sci Eng C 27:206 (2007)
[2] Tanaka Y et al., Mater Trans 48:287 (2007)
[3] Tanaka Y et al., Mater Trans 49:805 (2008)
[4] Tanaka Y et al., J Biomed Mater Res (2009) in press
[5] Tanaka Y et al., J Colloid Interface Sci 330:138 (2009)
[6] Oya K et al., Biomaterials 30, 1281 (2009)
[7] Tanaka Y et al., J Mater Sci Mater Med 18: 797-806 (2007)