Immobilization of epidermal growth factor on titanium

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Introduction: Metals such as titanium, titanium alloy, and stainless steel implants are widely used in medicine and for their biocompatibility, non-toxicity, good mechanical properties and excellent corrosion resistance. Surface properties are of prime importance in establishing the response to tissue to biomaterials with topography appearing to provide a set of very powerful signals for cells. Therefore some biological modification was investigated to induce biological activity on the surface. Covalently immobilization of biological molecules onto titan was performed by several researchers to induce specific biological responses (Ito Y., Soft Matter, 2008;4:46-56.).

We so far modified various polymeric materials with growth factors by immobilization and found that the immobilized growth factors regulated cell functions (Ito Y., Y. ″Biological Interactions on Materials Surfaces: Understanding and Controlling Protein, Cell and Tissue Responses,” D. Puleo and R. Bizios, eds., p.173-197, Springer, 2009). In the present study, we employed two methods to immobilize epidermal growth factor (EGF) on titanium as shown in Figure 1. One is covalent immobilization of EGF on dopamine-modified titanium (Method A). Another is synthesis of engineered EGF having binding affinity to titanium (Method B).

Materials and Methods: Titanium-coated plate was prepared by vacuum-deposition of pure titanium on a cleaned glass plate by electron beam with thickness of 400 nm (±25%). Method A; The titanium-coated plate was incubated in an aqueous solution of dopamine at pH 4.5 and 8.5. After the incubation, the treated plate was washed and incubated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and EGF. Method B; an active sequence of salivary statherin for apatite binding was added to the C-terminal of EGF by solid phase synthesis method. Because the sequence contains two phosphate serine residues, the addition was performed by organic synthetic process. The titanium plate was incubated in an aqueous solution of engineered EGF.

Results: Method A; When the plate was incubated at pH 8.5, the surface of plate turned to brown as reported by Lee et al. (Science, 2007;318:426-430). Comparing the water contact angle on the treated and non-treated plate, the hydrophobicity increased by dopamine treatment. The incubation pH did not affect the water contact angle. However, ESCA measurement revealed that the organic layer composed of dopamine was thicker on the plate treated at pH 8.5 than that treated at pH 4.5. It is known that the dopamine is polymerized at pH 8.5. Therefore it is considered that thick layer was formed at pH 8.5. The same phenomenon was observed suing stainless steel SUS 316.

The amount of amino groups on the dopamine-treated surface by was measured by fluoresceine isocyantate. The amount was higher on the surface treated at pH 8.5 than that at pH 4.5. Although the amino groups were considered to be spent for polymerization, the absolute amount of unreated amino groups were more at pH 8.5 than that at pH 4.5 because of the thick layer formation. EGF was immobilized using these amino groups by a coupling agent EDC. The amount of immobilized EGF was determined by using anti-EGF antibody.

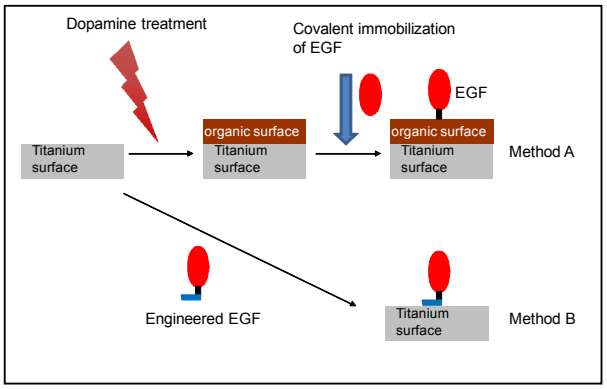

Figure 1. Immobilization of EGF on titanium by Methods A and B.

NRK cell was cultured on the EGF-immobilized surface. The growth rate was enhanced by the immobilized EGF and the effect was higher than that of soluble EGF. The reason why the immobilized EGF was so effective was considered to be the high local concentration on the surface, stabilization of EGF-EGF receptors by multivalent interaction, and the inhibition of down-regulation of cells as discussed previously (Ito Y., Soft Matter, 2008;4:46-56.).

Method B; When the circular dichroism of modified EGF was measured, the conformational change by addition of peptide was observed. In addition, the mitogenic activity of modified EGF was a little less than that of unmodified EGF.

The modified EGF adsorbed on apatite and also on the titanium plate. Because the modified EGF contains the phosphate groups which are known to have an affinity to titanium surfaces (Cantin GT et al., Anal Chem, 2007;79:4666-4673). Unmodified EGF was not bound to the titanium surface, while the modified EGF bound and retained the biological activity.

Conclusions: EGF immobilization on titanium was performed by organic layer formation on titanium or addition of affinity peptide to EGF. The immobilized EGF was effective than soluble EGF.