세포외기질에 부착된 혈관내피세포의 증식과 프로스타사이클린 분비에 관한 연구

GROWTH AND PROSTACYCLIN PRODUCTION OF ENDOTHELIAL CELLS ADHERED ON EXTRACELLULAR MATRIX COATED POLYURETHANE

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Introduction

The confluent endothelialization of the prosthetic grafts has been shown experimentally to reduce platelet deposition and enhance the biocompatibility of the luminal surface (1). Vascular endothelial cells form continuous monolayer that lines all blood vessels and provides a nonthrombogenic interface with the blood by the production of anticoagulation factor such as prostacyclin (PGI_2), plasminogen activator , antithrombin III, heparin/ heparan sulfate. For the rapid endothelialization on the artificial surface, various techniques of surface treatment were developed. Many investigators have used a variety of proteins including fibronectin, laminin, and collagen as coating substances for improving cell attachment onto vascular grafts (2,3). Several studies have reported that the artificial surfaces coated with the extracellular matrix(ECM), produced by confluently cultured cells, enhanced endothelialization of the surface. To enhance the efficiency of endothelialization onto the polyurethane (PU) surface, we used the ECM synthesized *in vitro* by fibroblast. In the present paper, we studied growth and prostacyclin (PGI_2) production of endothelial cells onto the polyurethane coated by lethally treated fibroblast producing ECM.

Materials and Methods

Preparation of substrates

Four types of samples were tested for the endothelial cell(EC) growth. The solution- cast smooth PU surface was used as the control surface. Fibronectin coated PU surface was also tried to test for the comparison of the EC growth. Two types of the ECM-coated PUs were produced by two detaching methods of the pre-cultured fibroblast. Human umbilical vein ECs (HUVECs) of 2-3 passage were used to seed all four experimental groups of PU surfaces. HUVECs were seeded at a density of 10,000 cells/cm^2 onto each of the four surfaces.

Preparation of extracellular matrix (ECM) from human foreskin fibroblast

To produce ECM coated PU surface , human fibroblast cells between passage 7 to 10 were seeded at a concentration 2.5 x 10^4 / cm^2 onto the smooth PU surface. The cells became confluent within 2-3 days . After confluence, the cells were left for seven to ten days.

Two different detaching methods were used. One is the hypotonic shock treatment. The cells were lysed by aspirating the culture medium and replacing it with distilled water containing ammonium hydroxide (20mM). The cells burst rapidly by osmotic shock within 3 minutes and the gelatinous debris were removed and washed with PBS.

The other is the irradiation method in which ^{60}Co(10,000 rad) γ-ray was exposed for one and half hour. The composition of ECM was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Fibronectin- coated PU surface

Fibronectin coating was performed by adding 0. 5ml of a 20 μg/ml solution of human fibronectin (Boehringer Mannheim, Germany) in PBS to PU surface.

After incubation for about 2 hrs at room temperature, the fibronectin-coated PU surface was washed with PBS.

Prostacyclin (PGI_2) assay

PGI_2 production from HUVECs cultured on nude, fibronecitn-coated and two ECM-coated PU surfaces were examined under basal condition and stimulated condition with arachidonate after *in vitro* cultivation for 7 days. PGI_2 released into the media was measured by detecting its hydrolysis product(6-keto-PGF1α)

using a commercial ^{3}H-6-keto-PGFα kit (Amersham). HUVEC monolayer was washed with serum free media and then incubated in 0.5 ml of serum free media with 20 μM arachidonate to stimulate PGI_2 formation. After incubation at 37°C for 30 minutes, the supernatant was collected and stored at -20°C. Then all cells were washed with PBS and harvested by trypsin treatment for cell counting with a Coulter counter.

Analysis of ECM components

To analyze the components of the natural ECM derived from fibroblasts on the PU surfaces, SDS-PAGE was accomplished. ECM-coated PU surface was incubated with 2 % SDS at 37°C for 2 days. Acetone precipitation was followed, and protein samples were loaded in SDS-PAGE apparatus(BIO-RAD, CA). After running, the major proteins were readily detected by staining the gel with a dye such as Coomassie blue, and minor proteins were shown in gels treated with a silver stain(BIO-RAD, CA).

Results and Discussion

PGI_2 synthesis by HUVECs cultured for 7 days on each surfaces was measured under basal condition and after stimulation with arachidonate. In the basal condition, PGI_2 production of HUVECs on the ECM-coated PU surface has slightly increased compared to the fibronectin-coated PU surface as shown in Figure 1. After stimulation with 20 μM arachidonate, however, PGI_2 amount released by HUVECs on the ECM-coated surface derived by hypotonic shock was similar to that observed in the fibronectin-coated PU surface whereas PGI_2 concentration was significantly increased on the irradiated ECM surface. To determine whether differences in cell numbers accounted for the differences observed in PGI_2 production, the cell densities of the EC-seeded PU surfaces were evaluated (Figure 2). The cell numbers in contact with ECM followed by hypotonic shock and irradiation(0.42×10^5 ECs/cm^2, 0.38×10^5 ECs/cm^2) were significantly greater than those on the other surfaces. These results showed that ECM-coated PU surface significantly enhanced HUVEC attachment and proliferation compared to PU and fibronectin-coated PU surface, however, the functional activity of PGI_2 on ECM-coated PU surface was similar to that on the fibronectin-coated PU surface. Several major bands were observed in purified ECM of precultured fibroblast on the PU surfaces after silver staining.

ECM proteins derived from fibroblast were significantly adsorbed on the PU surface after removal of fibroblasts. From these results, we concluded that a ECM-coated PU surface could be used as a substrate for endothelialization on the prosthetic grafts.

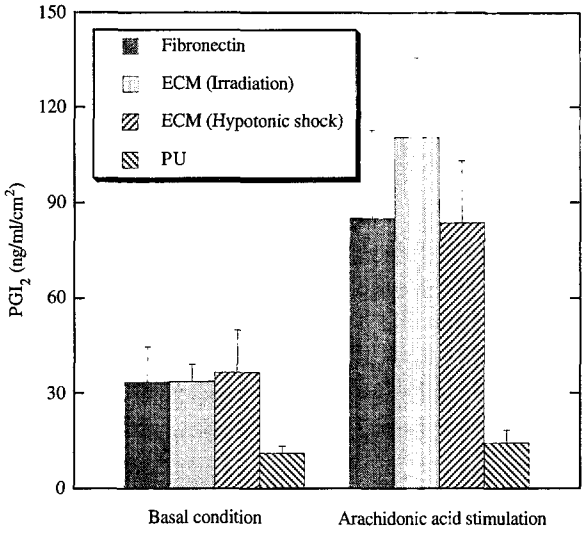

Fig.1 PGI_2 production by HUVECs cultured on noncoated and precoated PU surfaces under basal conditions and in response to stimulation with arachidonic acid

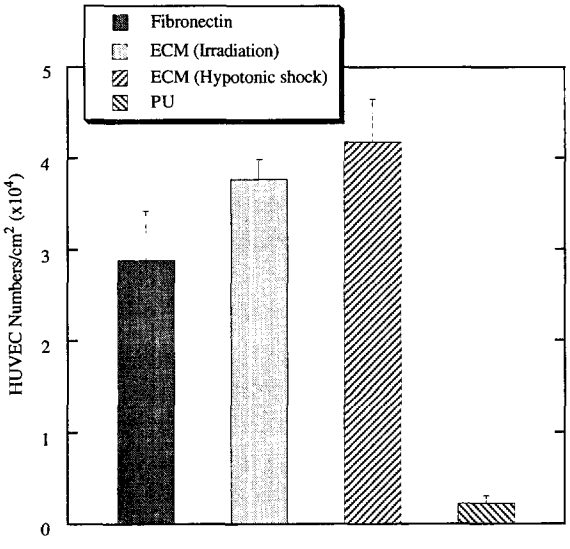

Fig.2 Densities of HUVECs cultured on noncoated and precoated PU surfaces after 7 days of in vitro cultivation

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