Effect of detachment method of pre-cultured fibroblast for endothelial cell growth on polymer

Jin Hee Kim, Jongwon Kim, Kyu Back Lee, Gyu Ha Ryu,
Yun Shin Lee, and Byounggoo Min

Dept. of Biomedical Engineering and Institute of Biomedical Engineering
College of Medicine, Seoul National University

The confluent endothelialization of the prosthetic graft has been shown experimentally to reduce platelet deposition and enhance the biocompatibility of the luminal surface [1]. In order to achieve efficient seeding of endothelial cells (ECs) onto the artificial surface, various techniques were developed [2-3]. Many investigators used fibronectin as the coating substance for improving cell attachment onto vascular graft materials [4-6]. Several reports on using the extracellular matrix (ECM) synthesized in vitro by cells in tissue culture as the coating substance for improving cell attachment were published [7-9].

In this paper, we tried to find the effect of detachment methods of the pre-cultured fibroblast on the EC growth. Two methods were tested to detach the pre-cultured fibroblast onto the polyurethane (PU) surface.

Materials and Methods

Four types of the sample surfaces were tested for the EC growth. The solution-cast smooth PU surface was used as the control surface. Fibronectin-coated surface was also tried to test for the comparison of the EC growth with others' results. Two types of the ECM-coated PU were produced by two detaching methods of the pre-cultured fibroblast.

Fibronectin-coated PU surface

Fibronectin coating were performed by adding 0.5 ml of a 20 ug/ml solution of human fibronectin in phosphate-buffered saline solution (PBS) to the PU surface for 2 hours at room temperature, and followed by twice extensive washing with PBS.

ECM-coated PU surfaces

To produce ECM-coated PU surface, fibroblast derived from human embryo skin between passage 7 - 10 were seeded onto the smooth PU surface as described previously [11]. Two different methods were adopted for the pre-cultured fibroblast. One is the irradiation of Co^{60} (10000 rad) for one and half hour. The other is the hypotonic shock by exposing to 0.02 M NH4OH in distilled water for 5 minutes [9].

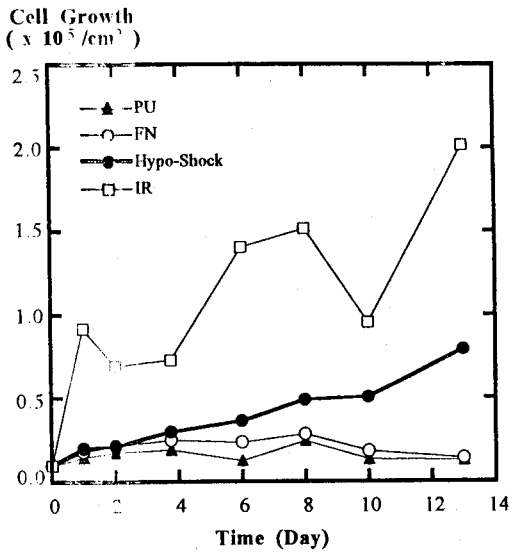

Figure 1. EC growth curves on the four test surfaces (PU: polyurethane, FN: Fibronectin-coated Polyurethane) (Hypo-Shock: hypotonic shock method, IR: irradiation method)

EC growth on four test surfaces

Human umbilical vein ECs (HUVEC) were harvested from the confluently cultured tissue flask after 2-6 passages by treatment with 0.125% trypsin / 0.01% EDTA. ECs were seeded at the density of 10^4 cells/cm^2 onto each of the four test surfaces. The complete medium composed of M199 medium, 20% fetal bovine serum, 100 units/ml penicillin, 100 ug/ml streptomycin, 2 mmol/l glutamine was changed every two days. Cell growth was monitored as follows; for each surfaces, cells were trypsinized and counted using the coulter counter at every other day until the14th day after seeding. The morphology of the cells was observed by light microscope and scanning electron microscope.

Results and Discussion

The cell growth curves of each surfaces are shown in Figure 1. The ECM-coated PU surface detached by irradiation shows the highest growth speed. In this case, all of the pre-cultured fibroblast was not detached,however, as shown in Figure 2. Therefore, the counted number by the coulter counter included other cells and/or particles such as fibroblast, debris. This might be caused from the nonuniform irradiation of the Co^{60}. The growth curve of irradiation is also very rugged, which also might be caused from the variance of the irradiation dose on the culture well. The ECM-coated PU surface by the hypotonic shock shows the higher growth speed than other two surfaces. No remnant fibroblast was observed on this surface.

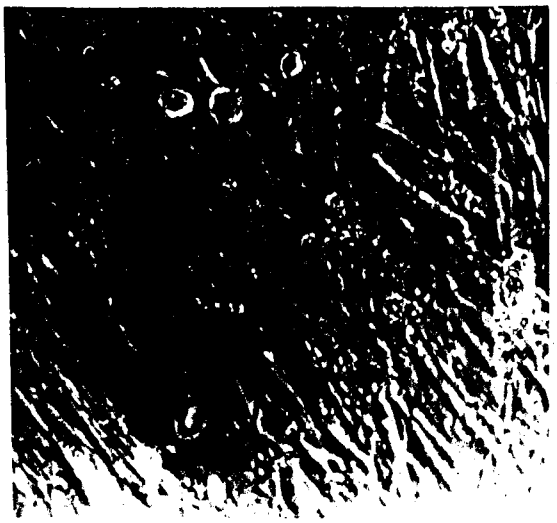

Figure 2. LM picture of the EC and Fibroblast cultured on the ECM-coated PU surface detached by irradiation

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