Development of Tissue Engineered Blood Vessel

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Purpose :

To investigate the bioartificial vessel that can be used for vascular grafts, we studied the cell-polymer constructs from vascular smooth muscle cells(VSMCs).

Materials and methods :

VSMCs were isolated from canine external jugular vein. The primary culture of VSMCs were accomplished by explant-derived method. To investigate the effects of PDGF on VSMC proliferation, the VSMCs were incubated in M199 media that contained 10% FBS or 20% FBS and various concentrations of PDGF(0.1-10 ng/ml). Proliferative response was determined with Alama Blue assay. Cultured VSMCs were seeded into the patch type scaffold and cultured with different methods ; Group 1 - dynamic seeding, dynamic culture, Group 2 - static seeding, static culture, Group 3 - dynamic seeding, static culture, Group 4 - static seeding, dynamic culture. Each group was divided according to the supply of the platelet derived growth factor (PDGF). The Alama Blue assay was done to determine the proliferation of VSMCs on polymer after two weeks incubation. The cell-polymer construct was implanted into the canine iliac vein. One week after implantation, the implanted construct was patency and the cell-polymer constructs was harvested and examined by histochemical method and scanning electron microscopy.

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Results :

VSMCs which obtained by explant-derived method were confirmed with immunohistochemical staining using monoclonal anti-α smooth muscle actin. The effect of PDGF on the VSMCs proliferation was summarized in Table 1 and Fig. 1. In Group 2, four weeks after the VSMCs seeded into the scaffold, hematoxylin-eosin stain showed VSMCs infiltration into the scaffold wall and the scanning electron microscope showed the tissue like VSMCs mass in the scaffold surface. The results of Alama Blue assay are summarized in Fig. 2. The results of in vivo experiment will be discussed during the Congress.

Conclusions :

This is a pilot study for constructing artificial vessels using tissue engineering. The construction of the ideal scaffold for vessel and the improvement of culture methods in vitro are the most important parts in this field.

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Table 1. Alama Blue assay for various concentrations of FBS and platelet derived growth factor (PDGF). Unit : Fluorescent Intensity at 530 nm

PDGF (ng/ml)	0 % FBS	10 % FBS	20 % FBS
0	0	177	214
0.1	0	177	168
1	0	201	186
5	0	266	241
10	79	394	354

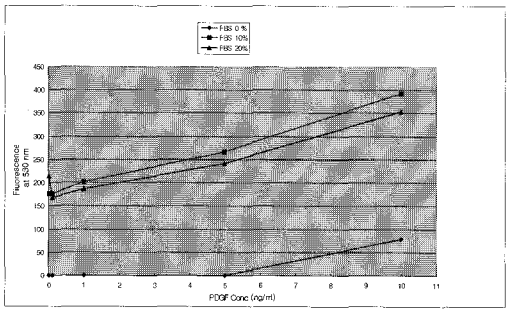

Fig 1. Alama Blue assay for various concentration of FBS and platelet derived growth factor (PDGF).
FI : Fluorescent Intensity at 530 nm

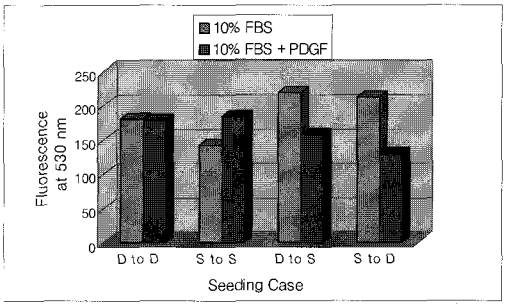

Fig 2. Alama Blue assay for various types of seeding and culture methods.
FI : Fluorescent Intensity at 530 nm