Comparative study of seeding and culture methods to vascular smooth muscle cells on the biodegradable scaffold

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1. Introduction

One of the more recent applications of absorbable polymeric biomaterials is in the growing field of tissue engineering research (1-2). The shortage of donor organs and high cost as well as possible complications of transplant surgery have driven the need for an alternate mammalian tissue source. Absorbable materials are a potential option. They can be sculpted into a particular tissue shape and can be used as a delivery vehicle on which mammalian cells can be seeded in vitro before transplantation. The material offers not only a guiding shape, but also the mechanical structure to help induce the development of appropriate tissue structure. Once implanted, the polymer gradually absorbs as the new tissue develops.

This research addressed optimizing the method of distributing cells onto the matrix to maintain polymer integrity and maximize cellular adhesion and proliferation.

2. Material & Methods

The primary culture of VSMCs obtained from canine external jugular vein was accomplished by explant-derived method. Cultured VSMCs were seeded into the scaffold and cultured with different methods; static or dynamic seeding, static or dynamic culture. The difference in the proliferative response of VSMCs was analyzed with Alamar Blue assay. Cell-polymer construct was examined by histochemical method and scanning electron microscopy (SEM). Mesh type scaffold (sized 10 x 10 x 0.4mm) was made of PGA (polyglycolic acid) suture thread, Surgisorb, obtained from Samyang (Taecheon, Korea). The PGA mesh type scaffold was 45% in porosity, and 0.03gm in weight.

The alamar blue assay results showed optimal cell seeding density. Optimum cell density of the static seeding is 0.1-1 x 10^7 cells/scaffold, dynamic seeding is 0.1-5 1 x 10^7 cells/scaffold. Two types of SMC seeding onto polymer matrices were first examined to optimize cell seeding. Dynamic seeding condition yielded a small number of cells adherent to the matrices (Fig. 1c,d). In the static seeding condition, a cell suspension (0.1ml, 0.5-1 x 10^7 cells/scaffold) was injected into the PGA scaffolds. This condition yielded high numbers of cells adherent to the scaffolds (Fig. 1a,b), probably due to quiescent cell maintained on the surface of the scaffolds.

Most cells likely stayed on the surface of the scaffold and did not contact inter-PGA fiber space in the low

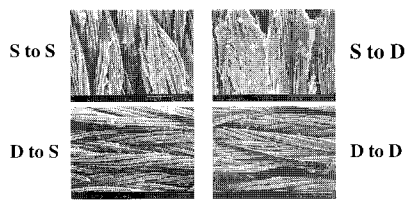

Fig. 1 Scaning electro microscopes of cell adherent on the scaffold.

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porous scaffolds (approximately 45%). Scanning electron microscopic examination of samples indicated that static seeding method resulted in a significantly higher number of adherent cells compared to the dynamic seeding method (Fig. 1) Individual cells attached to the polymer fiber and cell clusters filling the inter connecting spaces on the surface of the polymer matrices could be visualized when the static method was used.

The number of adherent cells as a function of cell concentration in the seeding solution with all two seeding conditions was subsequently quantified using the alamar blue assay (Fig. 2). The number of adherent cells per scaffold increased with cell concentration in the seeding solution for all seeding conditions, but more cells adhered with the static seeding condition than the dynamic condition. H&E staining indicated that 2-week cell-polymer constructs seeded with the static condition contained more cells than those seeded with the static condition (Fig. 3a-d).

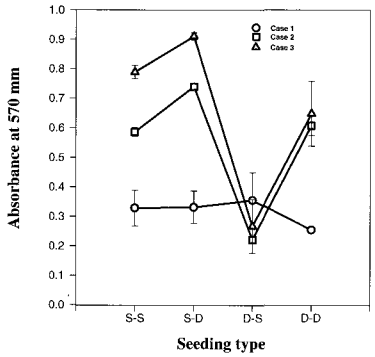

Fig. 2 The number of adherent cells as the cell scaffold constructs was quantified using the alamar blue assay

Fig. 3 Histological cross section of cellular construct demonstrating the appearance of cells within the scaffold, hematoxylin and eosin stain.

Furthermore, cells in cell-polymer constructs seeded with static condition were distribution more uniformly throughout the constructs compared to those seeded with the dynamic condition (Table I).

Table I Ranks among the cases

	Surface cellular distribution and attachment	Attachment within matrix	Metabolic activity	Average rank
S to S	2	2	2	2
S to D	1	1	1	1
D to S	3	3	4	3
D to D	4	3	3	3

3. Results

1) Static seeding and dynamic culture condition of these PGA scaffold produced the best results based on metabolic activity, cellular attachment, and cellular proliferation.
2) This is a pilot study for constructing artificial vessels using tissue engineering.
3) The construction of an ideal scaffold for vessel and the improvement of culture methods in vitro are the most important parts in this field.

References

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