Endothelialization of Peptide-treated ePTFE Vascular Grafts in a Circulatory Bioreactor

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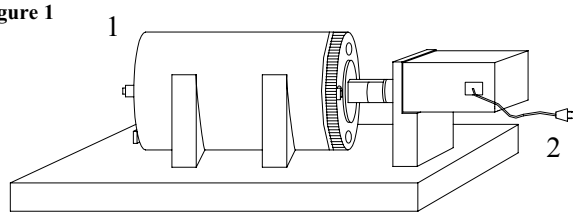
Introduction: The purpose of this study is to evaluate the effectiveness of linear and branched cell-binding peptides for promoting endothelial cell growth on ePTFE vascular grafts in a circulatory bioreactor under different pulsatile flow conditions similar to the physiological shear-stressed environments of the blood vessels. Cell-binding peptides that are immobilized on the surface of synthetic grafts allow the grafts to become more biomimetic because cell-binding peptides facilitate the interaction between the surface of the grafts and endothelial cells. The formation of the endothelium on the surface of the peptide-treated grafts reduces neointimal hyperplasia in the lumen and at the anastomosis, thus, the long term patency rates can be significantly improved.

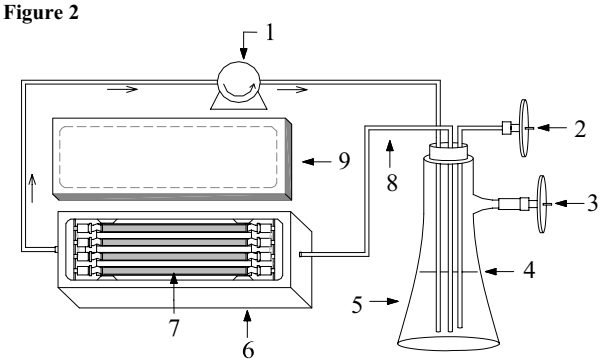
Methods: Two cell-binding peptides, P15 (linear) and MAP4 (branched), were covalently immobilized onto the surface of ePTFE grafts by a plasma/chemical treatment method. Primary human umbilical vein endothelial cells (HUVECs) were first seeded on the peptide-treated grafts in a rotating chamber (Figure 1) at a rate of 0.25 rpm for 0.5 to 4 hours in the EGM medium (Cambrex). Initial cell seeding density ranged from 30,000 to 100,000 cells/cm^2. The seeded endothelial cells were then exposed to different pulsatile flow conditions in a circulatory bioreactor (Figure 2) for 1 to 5 days. The bioreactor was placed in an incubator at 37°C, 5% CO_2 and 98% humidity. The medium in the bioreactor was also percolated with 5% CO_2 and 95% Air. Cells were counted by a hemocytometer.

Figure 1



1-Seeding Chamber. 2-Rotating Motor.

Figure 2



1-Peristaltic Pump. 2-Filter Gas In. 3-Filter Gas Out. 4-Medium Level.
5-Medium Reservoir. 6-Bioreactor. 7-Graft Samples
8-Silicone Tubing. 9-Reactor Cover.

Results and Discussion: Cell seeding on the grafts was influenced by the rotating rate of the seeding chamber and the seeding duration. Amount of cells seeded on the grafts increased significantly at a lower rotating rate or for a longer seeding duration. When the seeding duration extended from 0.5 to 4 hours at a rotation rate of 0.25 rpm, amount of cells seeded on the grafts increased by six to ten folds (Figure 3). Cells seeded on MAP4 and P15 treated grafts were 500% and 400% more respectively than on ePTFE control grafts after seeding for 4 hours. After 24 hours, at a flow rate of 50 ml/min, there were 325% and 70% more cells on MAP4 and P15 treated grafts respectively, compared to untreated ePTFE control grafts (Figure 4).

Figure 3

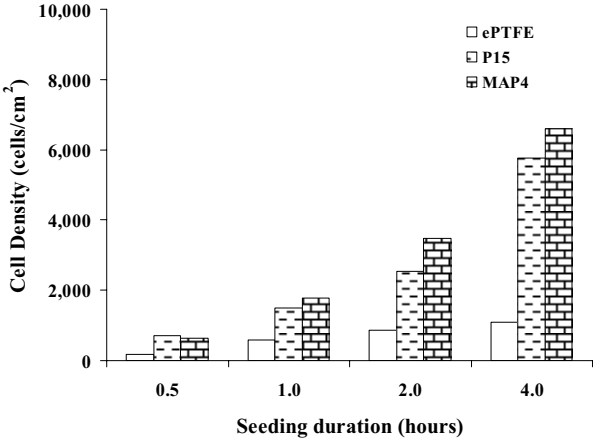
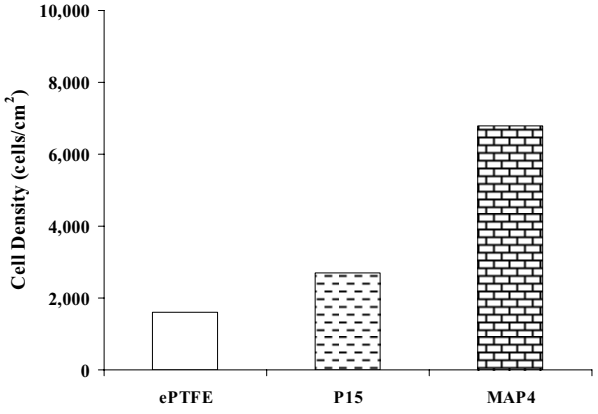


Figure 4



Conclusions: MAP is more effective than the structurally similar linear P15 for promoting endothelial cell adhesion and proliferation under both static and circulatory flow conditions[1]. The superior cell-promoting properties exhibited by MAP4 treated ePTFE grafts clinically could have better wound healing characteristics, reduced stenosis and improved long-term patency rates.

([1]Li C, J Biomed Mater Res 2004; 71A: 134-142)