IMPROVED ADHESION AND GROWTH OF VASCULAR SMOOTH MUSCLE CELLS ON POLYCAPROLACTONE NANOFIBROUS MEMBRANES MODIFIED BY AMINE-RICH PLASMA

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Introduction

Plasma polymerization of amine rich coatings is a promising technique for preparing cell carriers for tissue engineering. This technique can be used for modifying a wide range of biomaterials, such as synthetic polymers, ceramics and metals [1, 2], and has been shown to improve the adhesion and growth of various cell types, e.g. osteoblasts [1] and vascular endothelial cells [2]. In this study, we have explored plasma polymerization of cyclopropylamine (CPA), i.e. a promising isomer of allylamine, on electrospun polycaprolactone (PCL) nanofibrous membranes. The modified membranes were then tested with vascular smooth muscle cells (VSMC), i.e. an important cell type used in advanced vascular tissue engineering aiming at the reconstruction of the *tunica media* in vascular replacements.

Materials and Methods

Plasma polymers were deposited from CPA in low pressure radio frequency (RF) discharges, as described earlier [3]. The average power ranged from 9.9 W to 150 W. The freshly modified nanofibrous membranes were cut into square samples 1x1 cm in size, fixed in CellCrown inserts (Scaffdex), and inserted into polystyrene 24-well cell culture plates (TPP). The membranes were then seeded with VSMCs (isolated by explantation from the thoracic aorta of young male Wistar rats) in a density of 17,000 cells/cm², and in 1.5 ml of DMEM medium with 10% foetal bovine serum (Gibco). The cells were cultured for 1, 3 or 7 days, and were counted on microphotographs. For each experimental group and time interval, three samples were used.

Results and Discussion

The numbers of initially adhered VSMCs on day 1 after seeding ranges from 1550 ± 150 to 6200 ± 650 cells/cm², and were higher on all modified membranes than on pure PCL membranes. This trend remained the same for the rest of the experiment. The cells on the modified PCL membranes were similar in shape to the cells on the control polystyrene wells, i.e. they were spindle-shaped and polygonal, while the cells on the pure PCL membrane were mostly rounded (FIG. 1). The number of VSMCs was rising continuously with the time of the experiment. We also observed a positive correlation of the cell number with the average power. On day 7, the lowest cell number (3350 ± 460 cells/cm^2), which was only slightly higher than that on unmodified PCL (2930 ± 200 cells/cm^2) was obtained on the sample modified at the average power of 9.9 W, while the highest cell number (7200 ± 860 cells/cm^2) was achieved on the sample modified at the average power of 150 W. This result was rather surprising, because the content of amine groups, which are known to improve cell adhesion, was inversely correlated with the average power. However, the samples modified at the highest average power are more crosslinked, i.e. more stable and less soluble in the water environment [3]. The amine groups improve the cell adhesion mainly by their positive charge, which promote the adsorption of cell adhesion-mediating molecules, e.g. fibronectin and vitronectin, in appropriate geometrical conformations, which increases the accessibility of specific amino acid sequences in these molecules for adhesion receptors on cells [4]. Similar results were obtained after grafting silicon substrates with CPA using ultraviolet light, which improved the adhesion of epithelial cells of three lines [5].

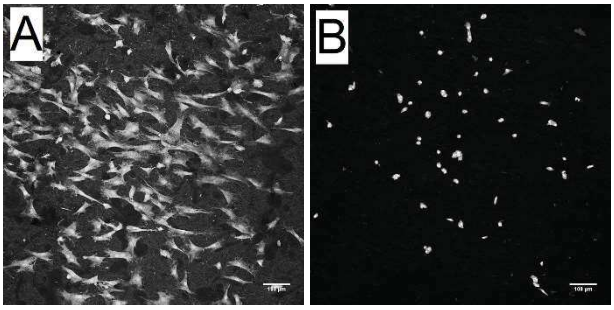

FIG. 1. Morphology of vascular smooth cells on day 7 after seeding on sample modified at average power of 33 W (A) or pure PCL membrane- (B). The cells were stained with Texas Red C2-maleimide and Hoechst #33342. Leica TCS SPE DH 2500 confocal microscope, obj. 10.0x0.30, bar = 100 µm.

Conclusions

The modification of PCL nanofibrous membranes by plasma polymerization of CPA had positive effect on the adhesion and growth of VSMCs. This result indicates that this technique is perspective for modification of the materials used for construction of vascular replacements.

Acknowledgments

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