ADSORBED GROWTH FACTORS AS MODULATORS OF CELL BEHAVIOUR ON BIOMATERIAL SURFACES

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Introduction

Currently used or tested synthetic vascular prostheses made of ePTFE, PET or PCL have a limited patency rate for replacement of small-diameter blood vessels. This is caused mainly by poor endothelial cell adhesion and proliferation on the biomaterial surface [1]. It is generally known that a confluent mature endothelial cell layer provides the best prevention of the graft thrombosis, restenosis and failure.

In order to improve the surface properties of synthetic materials for cell colonization, it is suitable to coat the surface with growth factors exhibiting adhesion-enhancing and mitogenic effects [2,3,4].

The purpose of our study was to investigate the effect of basic fibroblast growth factor (FGF-2) and vascular endothelial growth factor A (VEGF-A_{165}) adsorbed to the plastic surface on the behaviour of two cell types which are commonly used in cardiovascular tissue engineering, namely adipose-derived stem cells (ADSCs) and human umbilical vein endothelial cells (HUVECs).

Materials and Methods

FGF-2 and VEGF-A_{165} were expressed in eukaryotic system of methyltrophic yeast *P. pastoris* (strain KM71H) using pPICZαA expression vector.

Cell adhesive properties of the growth factors were monitored with the use of xCELLigence RTCA SP sensing device. Wells in a sensory E-plate were adsorbed with FGF-2 or VEGF (concentration range from 0.01 μM to 10 μM), and the non-specific binding sites for cells were blocked with 0.5% BSA. The initial adhesion of ADSCs and HUVECs was monitored for 4 hours in a pure cultivation medium containing no supplements (DMEM for ADSCs and EBM2 for HUVECs).

To determine the effect of the adsorbed growth factors on the proliferation of HUVECs and ADSCs, wells in 96-well tissue culture plates were coated with the growth factors in concentrations from 0.01 μM to 10 μM. ADSCs were grown in DMEM medium containing 10% of fetal bovine serum (FBS). HUVECs were grown in EGM2 medium containing hydrocortisone, heparin, ascorbic acid and 2% of FBS. The metabolic activity of the cells was evaluated by a resazurin assay on day 1, 3 and 7 after cell seeding. The cells were fluorescently stained with phalloidin-TRITC to visualize the cell morphology after the initial adhesion and during long-term cultivation. The cell nuclei were counterstained with Hoechst 33258.

Results and Discussion

In FGF-2-coated wells, the initial adhesion of ADSCs was significantly elevated. Surprisingly, HUVECs showed no specific interaction with FGF-2-coated wells.

Both studied cell types showed poor adhesion to wells adsorbed with VEGF.

The proliferation and the cell number of ADSCs on immobilized FGF-2 was significantly elevated, reaching the highest values at 10 μM concentration of this factor. A similar effect was also observed in HUVECs (FIG. 1).

On the other hand, ADSCs grown on adsorbed VEGF showed only slight increase in the proliferation and the cell number. HUVECs grown on VEGF-coated wells displayed a higher metabolic activity and a higher cell number. However, the increase in the cell number was not that high when compared with FGF-2-coated wells, where the cell number showed large increase (FIG. 1).

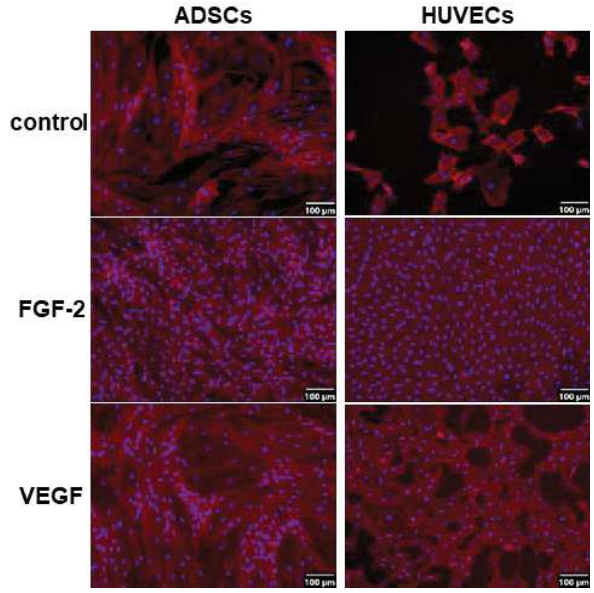

FIG. 1. The morphology of ADSCs and HUVECs 7 days after seeding in wells coated with 10 μM of FGF-2 or VEGF. Scale bar 100 μm.

Conclusions

Our study proved the bioactivity of growth factors adsorbed to plastic surface. Immobilized FGF-2 promoted the proliferation of both cell types and mediated cell type-specific adhesion. Adsorbed VEGF increased the proliferation of HUVECs and displayed poor adhesion-supporting properties. Our results suggest that the coating of the biomaterial surface with FGF-2 and VEGF can direct the adhesion and growth rate of different cell types in different ways.

Acknowledgments

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