

EFFECT OF FIBRIN-NANOCOATING OF NANOFIBROUS POLYMER MEMBRANES ON THE ADHESION AND PROLIFERATION OF HUMAN DERMAL FIBROBLASTS

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Introduction

Our research aims to construct appropriate scaffolds for healing the skin damage. Nanofibrous membranes seem to be promising for fabrication of advanced bilayered skin substitutes. The structure of biodegradable synthetic nanofibrous membranes well simulate natural composition of extracellular matrix and by that enhance communication between cells. Moreover, adhesion-mediating molecules can be adsorbed in a right conformation on nanostructured membranes and therefore can be better recognized by the cells integrins [1]. Our study contributes to the basic research in the field of molecular mechanisms of adhesion, proliferation and phenotypic maturation of dermal fibroblasts and the control of their behavior through the extracellular matrix, represented by fibrin.

Materials and Methods

Nanofibrous polylactid acid (PLA) membranes were prepared by needle-less electrospinning technology. The membranes were further coated with fibrin by using different preparation methods and subsequently fibronectin was attached in order to enhance cells affinity to the colonizing material. Adhesion and growth of human dermal fibroblasts, i.e. major cell type of dermis, were evaluated on these nanocoated membranes by MTS assay and by immunofluorescence staining. Relative expression of collagen I mRNA was measured by real-time PCR and also collagen I protein production was observe by immunofluorescence staining. Structure of fibrin-nanocoating was characterized by scanning electron microscopy (SEM). The quantitative data is presented as mean \pm standard deviation (S.D.) or standard error of mean (S.E.M) from three independent samples for each experimental group and time interval. Statistical significance was evaluated using the Kruskal-Wallis test. Values of $p \leq 0.05$ were considered as significant.

Results and Discussion

The results show that the fibrin-modified membranes improved adhesion and proliferation of human dermal fibroblasts. Furthermore, the morphology of fibrin modification seems to be crucial for the fibroblasts adhesion and consequently for their phenotypic maturation. Fibrin either formed coating around individual fibers in the membrane (FIG. 1A), or created thin nanofibrous mesh on the whole surface of the membrane

(FIG. 1B), which depended of the method of fibrin preparation. Fibroblasts on the membranes with fibrin distributed around the fibers remained in their typical spindle-like morphology while the cell behaviour on thin fibrin mesh on the membrane was absolutely different. The cells were more spread in all directions and moreover their proliferation was slightly higher. Fibronectin created secondary mesh on primary fibrin mash and enhanced the cell attachment and also the cell growth. Relative expression of collagen I mRNA and also protein production were higher on the fibrin mash in compared with fibrin distributed around the fibers.

Fibrin generally improved fibroblasts adhesion and proliferation which correlate with previous works [2], but the fibrin homogenous mesh probably provides better conditions for the cell attachment. This accelerates fibroblasts growth and production molecules of ECM.

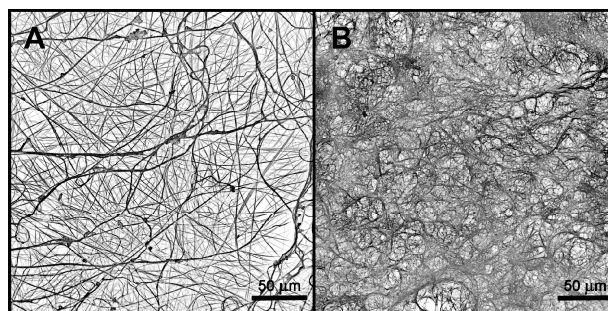


FIG. 1. Morphology of fibrin-nanocoating. Fibrin around the fibers (A) and fibrin mesh on the membrane surface (B). Fibrin was stained by immunofluorescence using primary and secondary antibody conjugated with Alexa Fluor 488. Leica TCS SPE DM2500 confocal microscope, obj. 40x/1.15 NA oil.

Conclusions

The PLA membranes modified with fibrin homogenous mesh are promising for construction of advanced bilayered skin substitutes. They enhanced the adhesion, proliferation and ECM production by dermal fibroblasts.

Acknowledgments

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