

Adhesion, proliferation and differentiation of primary keratinocytes on chemically functionalized nano- and microstructured surfaces

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INTRODUCTION: Tissue Engineering is an interdisciplinary research field with the goal to manufacture *in vitro* tissues and organs. But so far only a few 3D-tissues have been successfully developed. Culture of primary cells is challenging, since especially most human cells are fully differentiated in the adult organism and can therefore be poorly proliferated *in vitro*. They are prone to loss of function or initiation of apoptosis during isolation and cultivation. Therefore, new cell culture systems have to be created, featuring structurally and functionally tailored substrates that meet the needs of primary cells^{1,2,3}.

To gain basic insight into the impact of non-biological features on cells' behaviour, primary keratinocytes were cultured on chemically functionalized substrates with planar, nano- and microstructured surfaces. Adhesion, proliferation and differentiation have been evaluated by immunohistochemical staining and scanning electron microscopy (SEM).

METHODS: Glass-slides were modified with ultra-thin coatings of polyelectrolyte-films resulting in planar surfaces displaying positive and negative surface-charges as well as different organic functionalities e.g. amino-, carboxyl- and sulfuric groups. Additionally, amino- and carboxyl modified nano- and microparticles were deposited, to gain functionalized nano- and microscopic rough surfaces. For analyzing the percentage of surface coverage by particles the program "analySIS" from Soft Imaging System GmbH was used.

Cells were isolated from human skin and cultured at 37°C and 5% CO₂. Adhesion, proliferation and differentiation of primary keratinocytes on various surfaces were evaluated e.g. with microscopic examinations and immunohistochemical staining against specific Cytokeratins.

RESULTS: The polyelectrolyte-films were verified by ellipsometry and a surface coverage with particles between 70% and 90% was achieved. The kind of differentiation of primary keratinocytes range between progenitor-like and late differentiated depending on structural and functional modification of the substrate. Also

strong distinctions in cell's spreading time were noticed. Surface adhesion properties were illustrated by staining the actin-cytoskeleton of primary keratinocytes. Direct interaction of filopodia-like structures and nanoparticles was observed with SEM (Fig.1).

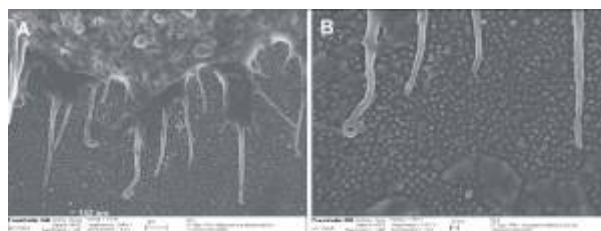


Fig. 1: **A)** With SEM filopodia-like structures of primary keratinocytes can be illustrated. **B)** Direct interaction of these filopodia-like structures with nanoparticles.

DISCUSSION & CONCLUSIONS: Long term objectives of these studies are to cope with crucial points of Tissue Engineering: effective isolation of primary cells, long term cultivation of stem cells, selective differentiation and functional cultivation of primary cells.

Insight into the cells' preferences concerning structure and chemical functionality of the substrate helps to built up improved cell-culture systems, optimized biomaterials and tailored surfaces for implants without the use of cost-intensive biological components.

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