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Iterative Biomaterial Testing towards Applicability for Tissue Engineering

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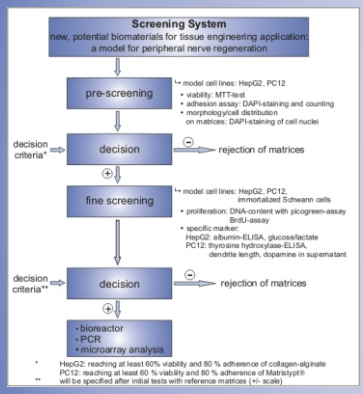
1 Introduction

One of the main objectives for tissue engineering is the choice of precise scaffold material in order to support and guide cell growth, cell differentiation and tissue formation. Therefore, numerous materials, fabrication techniques and modifications have been used and tested recently to fulfil different requirements depending on the target tissue. But so far, technology is far from optimized and there is great demand of new or modified biomaterials suitable for tissue engineering applications. The optimisation of biomaterial construct and its modifications requires a fast and efficient iterative testing of all accumulated materials in optimisation process. In this study a comparative screening of different biomaterials towards their applicability for tissue engineering was established. The setup for the screening of new materials in the field of peripheral nerve regeneration is presented. An example of several innovative biomaterials based on native polysaccharides and collagen is introduced. Model cell lines (HepG2, PC12) and immortalized Schwann cells (ISC) were seeded onto these matrices and cultured over a time period of two weeks. Within the presented pre-screening the cell morphology, viability and adhesion were analysed and compared with reference matrices. For selected experimental setups specific cell markers were determined. SEM micrographs demonstrate cell settling and morphology on the biomaterial constructs.



Itarative screeninmg of new biomaterials in tissue engineering

2 Materials and Methods

Cell culture: HepG2 cells were cultivated in DMEM containing 10 % NCS and antibiotics. PC12 cells were cultivated in DMEM supplemented with 10 % HOS, 5 % FCS, 1 % sodium-pyruvat, 1 % glutamine and antibiotics. ISC were cultured in DMEM containing 10 % FCS, 1 % Na-pyruvat, 1 % glutamine and antibiotics.

Cultivation on matrices: After desinfection with isopropanol (70 %) and over night incubation with cultivation medium the matrices were seeded with 5000 HepG2 and PC12 cells and 3000 ISC respectively.

Cell metabolism: Cell viability was assayed using **MTT-test**.

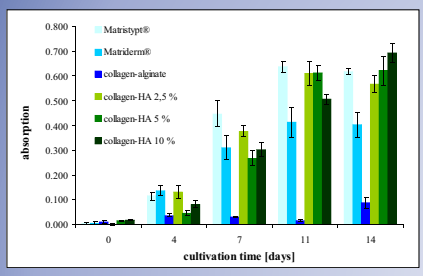
Cell marker: Gene expression of PC12 cells cultivated on the matrices in differentiation medium was observed via immunohistochemical staining of expressed thyrosine hydroxylase neuronal marker protein.

Matrices (kindly donated by Dr. Suwelack Skin & Health Care AG, Billerbeck, Germany)**:**

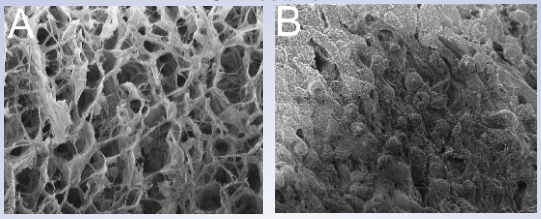
Matristypt®, Matriderm®, Collagen-alginate matrices
MATRISTYPT® is a pure collagen matrix, while MATRIDERM® and the collagen-alginate matrices additionally contain elastine and alginate respectively.

Collagen-hyaluronic acid 2,5-10 % (collagen-HA)
Collagen based biomaterials with different rates of incorporated hyaluronic acid as native polysaccharides.

3 Results

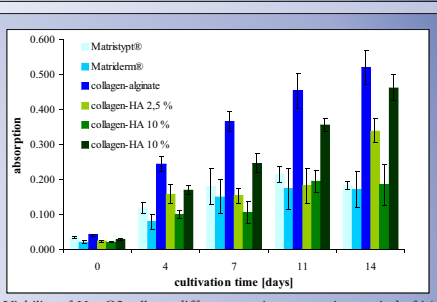


Viability of ISCs on different matrices over a time period of 14 days. Error bars reported as standard error of the mean (SEM) (n=6).

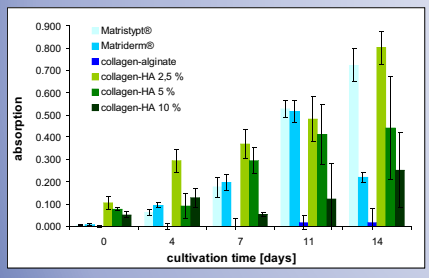


A: SEM micrograph of Matristypt® without cells. (magnification 400x) B: SEM micrographs of Matristypt® seeded with ISC and cultivation over 14 days. (magnification 1000x).

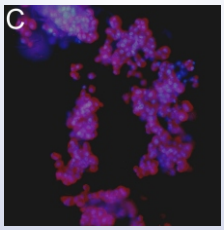
The matrix without cells shows a porous structure. After settling the matrix with cells and cultivation over 14 days the complete matrix is covered with cells.



Viability of HepG2 cells on different matrices over a time period of 14 days. Error bars reported as standard error of the mean (SEM) (n=6).



Viability of PC12 cells on different matrices over a time period of 14 days. Error bars reported as standard error of the mean (SEM) (n=6).



Immunocytochemical staining of PC12 cells on Matristypt®. (blue: cell nuclei stained with DAPI, red: staining of thyrosine hydroxylase in the cytosol) . Approximatly all PC12 cells express thyrosine hydroxylase on Matristypt®.

With regard to the respective references viability of cells on the new introduced collagen-HA matrices is comparable. Particularly, in a concentration of 2,5 % hyaluronic acid additive the ISCs and the PC12 cells proliferate on the collagen matrices. Especially the PC12 cells show higher viabilities on collagen-HA than on Matristypt® as reference. The porous structure of the collagen biomaterials seems to be very suitable since the ISCs grow in a dense cell layer on these materials.

4 Conclusion

In this study, we introduced a strategy for a parallel testing of several different materials and their modifications by means of a simple, fast and efficient screening system. The pre-screening of the compared materials revealed that the viability of cells on different matrices crucially depends on the applied cell type. Comparable reactions can be observed for cells with similar differentiation capabilities, thus PC12 cells can be neuronal differentiated whereas ISCs already are of neuronal origin. In conclusion, the introduction of different cell models in material screening is very important to differentiate information about the tested materials. After exemplary screening the collagen-HA matrices are of great interest for further testings in tissue engineering.

5 Acknowledgements

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