



Development of a fast and efficient screening system for new biomaterials in tissue engineering

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1 Introduction

The use of three-dimensional biodegradable matrices is one major issue in tissue engineering. Therefore, numerous materials, fabrication techniques and modifications have been used and tested in different areas of tissue engineering recently. But nevertheless, technology is far from optimized and optimal constructs with bioidentical and mechanical properties have not been described in the literature so far. Hence, there is great demand of new suitable biomaterials for tissue engineering applications. In this study a fast and efficient screening system for initial testing of biomaterials for cell culture application was developed. For this purpose HepG2, PC12 and immortalized Schwann cells (ISC) were seeded onto different matrices and cultured over a time period of two weeks. Within the pre- and fine-screening the cell morphology, viability and some specific cell markers were analysed and compared. SEM micrographs demonstrate cell settling and morphology on the biomaterial constructs.

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 % N-pyruvat, 1 % glutamine and antibiotics.

Cultivation on matrices: After disinfection 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 tyrosine hydroxylase neuronal marker protein.

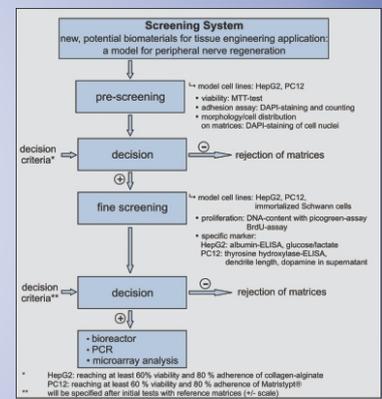
Matrices (kindly donated by Dr. Suwelack Skin & Health Care AG, Billerbeck, Germany):

Matristypt®, Matriderm®, Collagen-alginate matrices

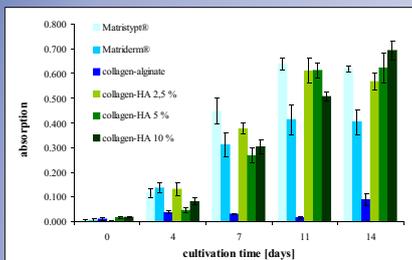
MATRISTYPT® is a pure collagen matrix, while MATRIDERM® and the collagen-alginate matrices additionally contain elastine and alginate respectively.

Collagen-hyaluronic acid 2,5-10 % (collagen-HA)

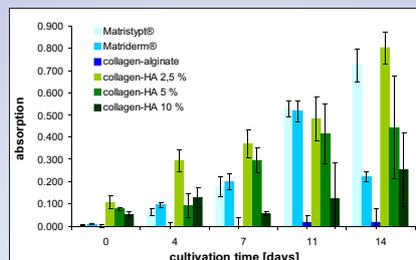
Collagen based biomaterials with different rates of incorporated hyaluronic acid as native polysaccharides.



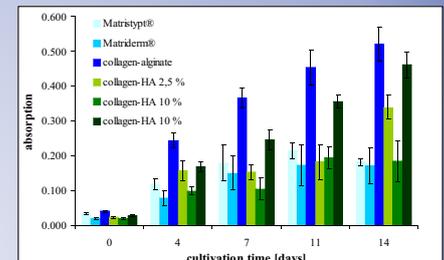
3 Results



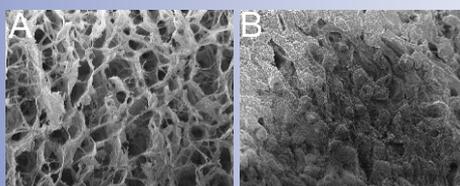
Viability of ISCs on different matrices over a time period of 14 days. Error bars reported as standard error of the mean (SEM) (n=6).



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).

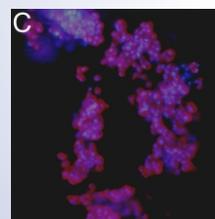


Viability of HepG2 cells on different matrices over a time period of 14 days. Error bars reported as standard error of the mean (SEM) (n=6).



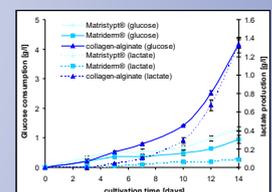
A: SEM micrograph of Matristypt® without cells, (magnification 400x) B: SEM micrographs of Matristypt® seeded with ISC and cultivation over 14 days, (magnification 1000x).

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.



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



Glucose consumption and lactate production of HepG2 cells cultivated on selected materials over a time period of 14 days. Error bars reported as standard error of the mean (SEM) (n=6).

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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