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Screening of innovative biomaterials in comparison to well established towards tissue engineering applicability in peripheral nerve repair

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1 Introduction

Tissue engineering aims at finding alternatives and ideally advancements for transplantations of organ, tissue and functional substitutes in case of severe injuries or defects. In contrast to the central nervous system, the peripheral nervous system shows minor capacity for regeneration after injury. Depending on the type of injury and particularly on the length of the gap limitations in the recovery of peripheral nerve function exists. Conventional neurosurgical strategies include the end-to-end coaptation, which is impossible after severe loss of substance, and nerve grafting, which is limited on one hand by the availability of autologous nerve grafts and on the other hand by immunological reactions against allogenic grafts. Hence, as in other fields of tissue engineering research numerous materials, fabrication techniques and modifications of three-dimensional biodegradable matrices need to be developed and tested to enhance technology and finally find optimal constructs with bioidentical and suitable mechanical properties.

In this study, several innovative biomaterials were screened in comparison to well established materials towards their applicability in peripheral nerve repair. Model cell lines (PC12, HepG2) and immortalized Schwann cells were seeded onto different materials and cultured over a time period of at least two weeks. Viability and proliferation of the cells on all materials were observed. For selected experimental setups specific cell markers were determined. SEM micrographs demonstrate cell settling on the biomaterial constructs. Gene expression analysis will further on be performed by RT-PCR and microarray technique. Results are exemplarily shown with collagen (Matristypt®) variations, silk and hydroxyapatite (Sponceram®HA).

2 Materials and Methods

matrix	compound	details	source
Matristypt®	collagen	predefined reference matrix for PC12/ISC cells	Dr. Suwelack Skin & Health Care AG, Billerbeck, Germany
collagen-alginate	collagen + alginate	predefined reference matrix for HepG2 cells	
collagen-HA 2,5/5/10 %	Collagen + hyaluronic acid 2,5-10 %	hyaluronic acid as native polysaccharide with promising use in cell culture	
collagen-silk	collagen + fibroin	defined proportion of silk fibroin from bombyx mori	
spider silk	fibres of nephila clavipes	ball of spider silk fibres	Klinik für Plastische- Hand und Wiederherstellende Chirurgie, Medizinische Hochschule Hannover
Sponceram®HA	hydroxyapatite coated Sponceram®	pore size of 600-900 µm and a surface area of 0,8 m²/g	Zellwerk GmbH, Oberkrämer, Germany

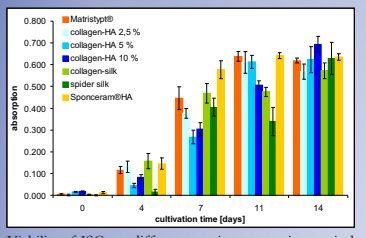
Cell culture: HepG2 cells were cultivated in DMEM containing 10 % NCS and antibiotics. PC12 cells were cultivated in DMEM supplemented with 10 % HOS, 5 % FCS, 1 % sodium-pyruvat, 1 % glutamine and antibiotics. ISC were cultured in DMEM containing 10 % FCS, 1 % Na-pyruvat, 1 % glutamine and antibiotics.

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

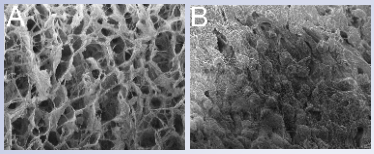
Cell metabolism: Cell viability was assayed using *MTT-test*

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

3 Results

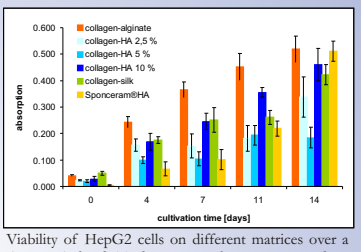


Viability of ISCs on different matrices over a time period of 14 days. Error bars represented as standard error of the mean (SEM) (n=6).

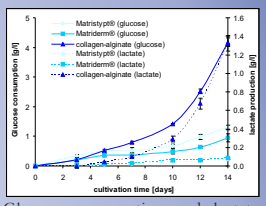


A: SEM micrograph of Matristypt® without cells. (magnification 400x) B: SEM micrographs of Matristypt® seeded with ISC and cultivation over 14 days. (magnification 1000x).

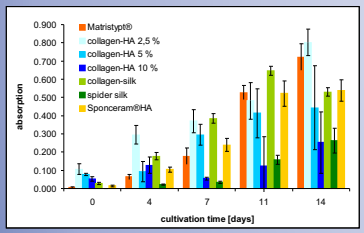
The matrix without cells shows a porous structure. After settling the matrix with cells and cultivation over 14 days the complete matrix is covered with cells.



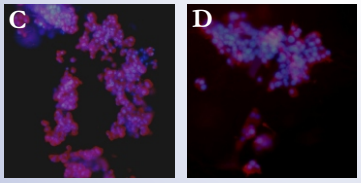
Viability of HepG2 cells on different matrices over a time period of 14 days. Error bars represented as standard error of the mean (SEM) (n=6).



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



Immunocytochemical staining of PC12 cells on Matristypt®. C: Undifferentiated PC12 cell D: Neuronal differentiated PC12 cells (blue: cell nuclei stained with DAPI, red: staining of tyrosine hydroxylase in the cytosol). Independent of differentiation approximately 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. Silk enhances cell growth and indicates to be attractive especially for the ISC. The in bone regeneration well established Sponceram® HA shows also capacities in regard to nerve tissue engineering.

4 Conclusion

The strategy of parallel testing of innovative in comparison to well established biomaterials by means of a simple, fast and efficient screening system indicates to be convenient. The viability results revealed that cell growth on the 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. At actual state the collagen-HA matrices as well as silk variations are of great interest for further testings in peripheral nerve repair. This further screening may lead to an enhancement towards optimal and suitable biomaterials in this research field.

5 Acknowledgements

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