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Polysialic Acid:
A New Bioidentical Scaffold Material For Peripheral Nerve Regeneration

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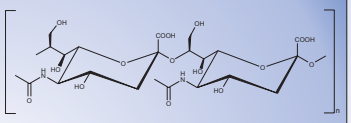
1 Introduction

The aim of this interdisciplinary study is the testing of polysialic acid and its derivatives towards putative biocompatible scaffold material. First of all coating of cell culture surfaces with the soluble material was established and cytotoxicity was studied. The cell-matrix interaction was investigated using different well established model cell lines as Hep G2 and PC 12 with regard to proliferation and viability (MTT-test).

Long-term objective is an alternative approach in the treatment of non-regenerative peripheral nerve lesions by use of tubes as interponats between the proximal and the distal nerve stumps. For the bridging with autologous Schwann cells preferential bioresorbable scaffolds will be applied.

2 Materials and Methods

Polysialic acid: Polysialic acid (PSA) occurs as a cell surface structure which provides a post-translational modification of the vertebrate neural cell adhesion molecule. For these first material-testings the physiological, soluble PSA and a crosslinked derivative were used.



Cell culture: Hep G2 cells were cultivated with DMEM containing 10 % NCS and antibiotics (pen/strep).
PC 12 cells were cultured in DMEM containing 10 % HOS, 5 % FCS, 1 % Na-pyruvat, 1 % glutamine and antibiotics (pen/strep).

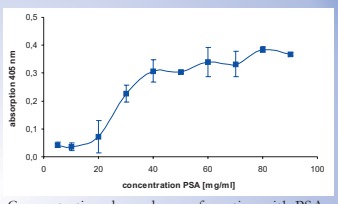
Coating: Wells of 96-well-plates were covered with 40 µl of 0,1/1/5/10 mg/ml PSA- and 0,5 mg/ml poly-L-lysine (PLL) in dH_2O respectively, incubated for one hour at room temperature or for 30 minutes at 37 °C and subsequently washed three times with PBS.
PSA-coating was detected by immunocytochemistry with mAk 735 and visualised by CY3-labeled secondary antibody. Concentration dependance was tested by direct ELISA with HRPO-labeled secondary antibody and ABTS as substrate.

Cell seeding: 4000 cells were seeded into each coated well of a 96-well-plate.

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

3 Coating

Detection of PSA-coating with CY3-labeled secondary antibody after coating with 5 mg/ml PSA-solution (original magnification 400x).

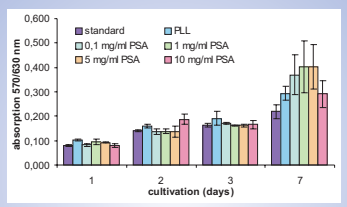


Concentration-dependance of coating with PSA by direct ELISA.

The immunocytochemistry with CY3 evidences that coating of the whole surface was reached with PSA after the mentioned treatment.

Up to a concentration of 40 mg/ml PSA the coating extent directly depents on the applied concentration of PSA-solution. From 40 mg/ml a plateau level of bound PSA-amount is reached (monolayer formation).

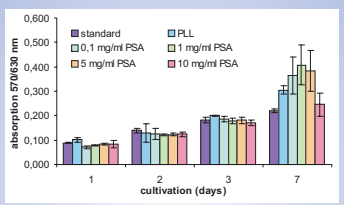
4 Cytotoxicity



Viability of Hep G2 cells after addition of solutions of different PSA-concentrations to the medium compared to PLL and standard medium over a time period of 7 days. Values represent the mean of 4 samples +/-SD.

PSA shows no cytotoxic effect on the cells. After adaption over 7 days viability of the cells can be increased with PSA in the medium in contrast to standard medium. Best effect over the whole period results by addition of 5 mg/ml PSA to the medium.

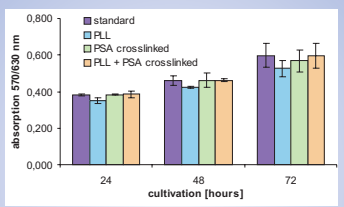
5 MTT - PSA standard coatings



Viability of Hep G2 cells on different PSA-coatings compared to coating with PLL and standard well over a time period of 7 days. Values represent the mean of 4 samples +/-SD.

The cells proliferate on all used surfaces. The cells cultivated on 1 and 5 mg/ml PSA show the highest proliferation rate. Similar effects can be observed for PC 12 cells.

6 MTT - Coatings with crosslinked PSA



Viability of Hep G2 cells on crosslinked PSA compared to standard, coating with PLL and double coating with PLL and PSA over a time period of 7 days. Values represent the mean of 4 samples +/-SD.

Cells proliferate on crosslinked PSA coatings as well as on standard material. The double coating of PLL and crosslinked PSA shows an cumulative effect. The viability of PC 12 cells on crosslinked PSA shows the same results.

7 Conclusion

The first material-testings with soluble PSA and a crosslinked derivative prove the applicability of PSA as a putative biocompatible scaffold material. The addition of PSA solution to the medium shows no cytotoxic effect and the proliferation on PSA coated surfaces is comparable to well established cell culture coatings. Viability of the cells on crosslinked PSA gel like coatings reveal the suitability of this new, bioidentical scaffold material for cell culture application.
In further experiments well established scaffold materials will be compared to promising PSA based matrices with regard to proliferation (BrdU-incorporation) and viability (DNA-content). Furthermore cell differentiation on these matrices will be monitored by immunocytochemistry, ELISA and specific microarrays.

Acknowledgement

The authors thank Prof. Claudia Grothe (MHH) for providing PC12 cells and the CY3 antibody,
Prof. Rita Gerardy-Schahn (MHH) for providing mAk 735 and
Dipl. Chem. Silke Berski as well as Dr. Gerald Dräger for providing the crosslinked PSA.