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A study on different biomaterials for tissue engineering and peripheral nerve regeneration

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Introduction

The first and foremost function of scaffolds for tissue engineering and likewise peripheral nerve regeneration is its biocompatibility and biodegradability. Additionally, the material has to utilize the role as substrate for cell attachment, cell growth and differentiation to the desired phenotype. Although peripheral nerves show capacity for regeneration after injury, in case of severe defects, axons often extend randomly and nerve regeneration competes with the formation of scar tissue, resulting in a permanent loss of peripheral nerve function. Thus, there have been raising attempts on the development of biosynthetic nerve guidance channels that could provide an optimized environment for enhanced and guided peripheral nerve regeneration and would mimic autografts.

In this study, we investigated spider silk with ist biocompatible properties and high mechanical capacity in neuronal cell culture. Moreover, we used a biopolymer that disclaims to be involved in nerve repair *in vivo* with regard to peripheral nerve repair potential. Polysialic acid (PSA) is a dynamically regulated posttranslational modification of the neural cell adhesion molecule (NCAM). NCAM-PSA acts as important regulator in the development of brain structures and in processes accompanying learning and memory.

Here the material- cell interactions were investigated using the model cell lines Hep-G2, PC-12 and immortalized Schwann cells (ISC). The investigations include the cytotoxicity, viability and proliferation of the cells (MTT assay). Moreover, testing of different cell markers by RT-PCR and the differentiation status of the PC-12 cells were performed.

Materials and methods

Cell culture:
PC-12 cells were cultured in DMEM, 10 % HOS, 5 % FCS, L-glutamine, Na-pyruvate and antibiotics.
Hep-G2 cells were cultured in DMEM containing 10 % NCS and antibiotics.
ISC were cultured in DMEM, 10 % FCS, L-glutamine, Na-pyruvate and antibiotics.

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

Coating procedure and cell seeding:
The wells of 96-well plates were covered with 40 µl of each solution respectivly, and incubated for 1 h at room temperature and 30 min at 6°C. Plates were washed twice with PBS and seeded with 4000 cells (Hep-G2 and PC-12) per well.

Cell culture experiments
Uncoated tissue culture plastic and PLL (0.5 mg/ml) were appointed as control.

Cytotoxicity tests: 0.1-5 mg/ml of colominic acid (CA)(purchased) was dissolved in medium. Silk strand was added to plated cells.

Coating experiments: 5 mg/ml of PSA-K1 (isolated from *Escheria coli K1* by our group) and 5 mg/ml CA (purchased) were dissolved in ddH_2O respectivly.

Cytotoxicity of hydrogels: CA was differently crosslinked with diepoxyoctan and purified by dialyses.

Spider silk

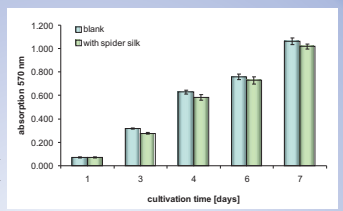


Figure 5:
Cell cytotoxicity of ISC.
Values represent the mean of 6 experiments in parallel +/-SEM.

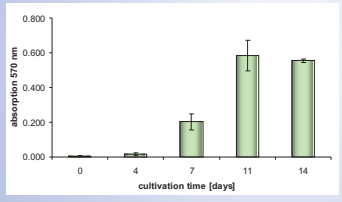


Figure 6: Cell viability of ISC on spider silk. Values represent the mean of 6 experiments in parallel +/-SEM.

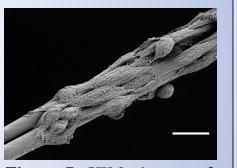
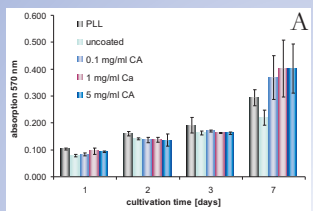


Figure 7: SEM pictures of ISC growing on native spider silk (1000x), Rohde/Dittmar, HZI Braunschweig, Germany

The native spider silk has no cytotoxic effect. Neuronal ISC grow well and almost enwrap the whole strand. Therefore, with regard to peripheral nerve regeneration this native material may be an ideal for innovative engineered biomaterials.

Polysialic acid



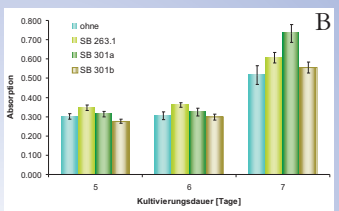


Figure 1: Cell cytotoxicity of Hep-G2. Values represent the mean of 6 experiments in parallel +/-SEM.
Figure A: After addition of different CA concentration.
Figure B: After addition of different crosslinked hydrogels.

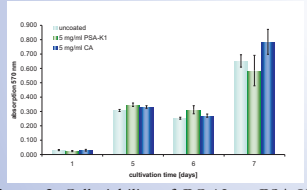


Figure 3: Cell viability of PC-12 on PSA-K1, CA and uncoated surfaces. Values represent the mean of 6 experiments in parallel +/-SEM.

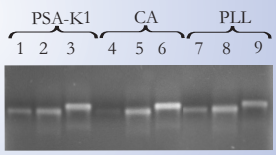


Figure 4: RT-PCR of PC-12 cells:
1, 4, 7: Tyrosin Hydroxylase, 596 bp
2, 5, 8: beta-Actin, 536 bp
3, 6, 9: GAPDH, 452 bp

The viability results show increasing viability in connection to all used materials. The colominic acid (CA) and the PSA-K1 indicate to have no cytotoxic effect. The RT-PCR results confirm the expression of the specific cell marker *tyrosine hydroxylase* and the house keeping genes *GAPDH* and *beta-Actin*.

Conclusion and Outlook

Spider silk indicated no cytotoxic effect and SEM revealed that the neuronal ISC spread and grow well on the native spider strands. In conclusion, with regard to the immunogenic, mechanical and biological properties of spider silk this native biomaterial displays a applicable biomaterial itself as well an ideal for new biomaterials. Other biopolymers that disclaim to be involved in nerve repair *in vivo*, as the polysialic acid, have to be tested with such regard to cell culture applicability. The results of the cell viability assays show that the cells are viable in presence of PSA-K1, CA and modified CA. The CA was tested in its soluble form and also as modified hydrogels. Both used cell lines reached high viabilities after addition of different CA concentrations. The comparison of the CA and the PSA-K1 results in a similar course of the cell viability. The crosslinked CA materials do not show cytotoxic effects. In this study the application of polysaccharide polysialic acid as putative biomaterial for cell cultivation was demonstrated.

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