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A Preliminary Study on Spider Silk as Biomaterial for Peripheral Nerve Regeneration

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Introduction

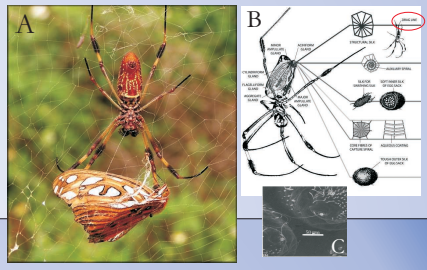
Although peripheral nerves show capacity for regeneration after injury, there are limitations in the recovery of peripheral nerve function depending on the type of injury and particularly on the length of the gap. Thus, since the 1980s there have been raising attempts on the development of biosynthetic nerve guidance channels that could provide an optimized environment for enhanced peripheral nerve regeneration. Our approach was a preliminary study on spider silk as innovative biomaterial.
Silk from the silkworm, *Bombyx mori*, contains two proteins, the fibroin core fibres encased in a sericin coat, which seems to be the major cause of adverse problems with biocompatibility and hypersensitivity to silk. In contrast, the dragline silk from *Nephila clavipes* has no allergic effect. Moreover, spider silk provides a remarkable combination of strength and toughness. The distinguishing features of the spider silk are the very high strength in combination with excellent elasticity in comparison with all other biomaterials.
In this study, two model cell lines (immortalized Schwann cells, PC-12) were seeded onto defined silk constructs and cultured statically over a time period of at least two weeks. Cytotoxicity, viability and proliferation of the cells were observed. SEM pictures indicate the preferred attachment of cells on and a directed growth along the silk fibres. Moreover, mechanical properties in regard to ultimate tensile strength and elasticity were investigated.

Materials and methods

Cell culture:
PC-12 cells were cultured in DMEM, 10 % HOS, 5 % FCS, L-glutamine, Na-pyruvate and antibiotics.
Immortalized Schwann cells (ISC) were cultured in DMEM, 10 % FCS, L-glutamine, Na-pyruvate and antibiotics.

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

Mechanical properties: Mechanical properties were investigated by means of *zwicki*® material test control unit (Zwick/Roell).



Results

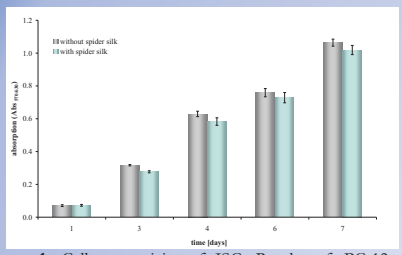


Figure 1: Cell cytotoxicity of ISC. Results of PC-12 cells comparable (not shown). Values represent the mean of 6 experiments in parallel +/-SEM.

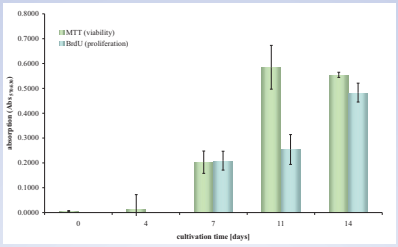


Figure 2: Cell viability and proliferation of ISC on spider silk. Results of PC-12 cells comparable (not shown). Values represent the mean of 6 experiments in parallel +/-SEM.

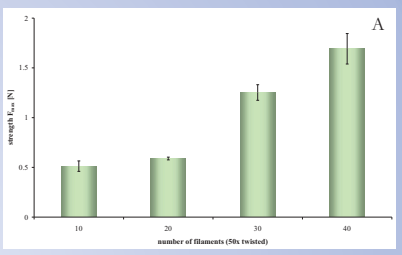


Figure 3: Mechanical properties of spider silk strand. **A:** maximal strength **B:** elongation at break

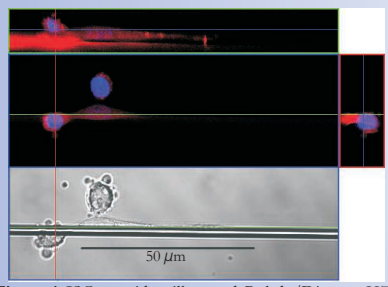


Figure 4: ISC on spider silk strand, Rohde/Dittmar, HZI Braunschweig, Germany, blue: cell nucleus, red: cytosol

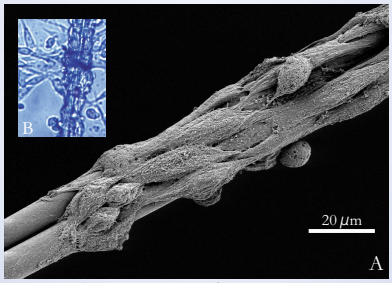
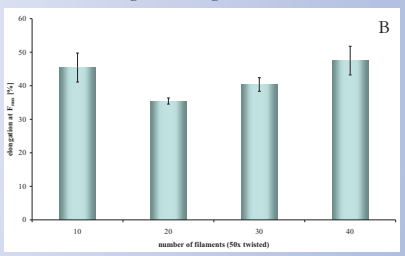


Figure 5: A: SEM pictures of ISC growing on native spider silk (1000x), Rohde/Dittmar, HZI Braunschweig, Germany **B:** confocal microscopy



The native spider silk has no cytotoxic effect. Neuronal ISC and PC-12 cells show an increasing viabiliy and proliferation rate on the spider silk strands over the time period of 14 days. Confocal and SEM micrographs indicate that the ISC almost enwrap the whole strand and contemporarily begin to produce extracellular matrix (not shown). Figure 4 demonstrates how the ISC spread along the spider silk strand. With regard to the mechanical properties of the spider silk described in the literature so far, very high strength in combination with excellent elasticity could be approved. In the tested range, the maximal strength is continuously increasing with increasing numbers of filaments. Although, elongation capacities of the filaments stay constantly high indepent of the increasing cross section. Thus, an increasing toughness does not implicate a loss of elongation capacity. Filaments in wet surroundings could be elonagted twice compared to their started length (data not shown).

Conclusion and Outlook

In this study we investigated spider silk as biomaterial in cell culture with regard to peripheral nerve regeneration. Moreover, we characterized the spider silk filaments by means of their mechanical properties. Beyond the excellent observed viability and proliferation rate, the presented morphology of the cells on the spider silk strands are striking. The ISC enwrap the silk strand in widespread areas nearly thoroughly. Thus, these structures were ideal constructs for directed nerve guidance. The high strength and elasticity promote the application of spider silk in this field, as such mechanical properties are favoured for uncomplicated use in medicine and for sufficient integration in the desired tissue.
In a next step, dynamic cultivation in a suitable bioreactor may lead to constructs which meet the demands of scaffolds for tissue engineering of peripheral nerves.

Acknowledgement

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