**Electrospinning of fibers from polycaprolactone and polycaprolactone/collagen blends for neuronal cell guidance**

Kristina Klinkhammer[1], Eva Schnell[2], Paul Dalton[3], Jörg Mey[2], Gary Brook[4], Doris Klee[1], Martin Möller[1].
1) Institute of Technical and Macromolecular Chemistry, RWTH Aachen and DWI e.V., Aachen, Germany
2) Institute of Biology 2, RWTH Aachen, Aachen, Germany
3) School of Biological Sciences, University of Southampton, Southampton, United Kingdom
4) Department of Neurology, RWTH Aachen University, Aachen, Germany

**Statement of Purpose:** Peripheral nerve injuries cause several deficits including loss of motor function and neuropathic pain. Some injuries can be treated by using nerve autografts. Disadvantages of autografts include loss of function at the donor site, the need of extra surgery and limited material. An artificial nerve graft that can bridge such lesions is therefore an alternative to the autograft. The present research is directed towards the development of a 3D-construct where nanofibers are embedded in a loosely crosslinked hydrogel. This hydrogel matrix can be tailored to be cell adhesive or non cell adhesive and protein resistant while the fibers are the actual guides for the neurites. In a first attempt highly oriented nanofibers which are separated by a distance of approximately 10 µm have been laid on a protein resistant hydrogel layer. Therefore initially glial cells and neurites follow isolated tracks. Concurrent glial migration and neurite outgrowth from E10 chick dorsal root ganglia (DRG) has been monitored.

**Methods:** Polycaprolactone (PCL) (Mw = 67000 g/mol) and type I collagen from calf skin were mixed in different ratios and dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol. Pure PCL was dissolved in chloroform/methanol (3:1). Electrospinning was performed with variation of parameters (e.g. concentration, voltage and flow rate) to investigate their influence on nanofiber morphology. Nanofiber were either collected onto a single-plate target or between two parallel bars [1] and analysed by x-ray photoelectron spectroscopy (XPS) scanning electron microscopy and collagen I antibody staining. Prior to cell experiments, glass cover slips were coated with a star-poly(ethylene glycol) polymer (starPEG) [2] and passed through the suspended oriented collected nanofiber.
Explants and primary cultures of dissociated cells from DRGs were placed onto oriented nanofibers and cultivated at 37 °C, 5 % $CO_2$ for up to seven days *in vitro* (DIV). Antibodies against NF200 and S100 were used for immunocytochemistry and combined with DAPI nuclear staining. Cell migration, neurite orientation and outgrowth were measured.

**Results/Discussion:** Collagen I, a structural protein in the extracellular matrix, possesses specific ligands for cell adhesion and process outgrowth and has been used as cell adhesive substrate. Because electrospinning of pure collagen I resulted in poor fiber quality, we prepared blends with PCL which is biocompatible and degradable. In the electrospinning process, solution concentration, setup of the collection electrodes and collagen content had the most significant influence on nanofiber morphology. A solution concentration of 9 wt% resulted in good quality nanofibers with all blends but only when they were collected onto a single target. Nanofibers had diameters between 200 and 900 nm. The morphology of oriented fibers is dependant on collagen content. Blends with more than 50 % collagen resulted in poor orientation and wide range of fibre diameter distributions mainly due to adhesion and poor mechanical stability. XPS data and collagen I antibody staining indicated the existence of collagen at the nanofiber surface in the blends.
Oriented nanofibers of pure PCL or with 25 % collagen (CPCL) were chosen for *in vitro* cell experiments. They were collected onto starPEG surfaces prior to cell experiments (Figure 1A). StarPEG coated surfaces showed much lower cell adherence than the fibres. CPCL nanofibers were found to support 40 % greater Schwann cells migration (1.35 mm vs. 0.791 mm, 7 DIV) than PCL fibres. The orientation of migration was strongly influenced by nanofiber orientation. Outgrowing neurites also followed the orientation of the nanofibers and migrating glia (Figure 1B). Schwann cell process extension was also greater on CPCL (52.3 µm vs. 41.5 µm, 7 DIV). The stronger effects of CPCL nanofibers on cell migration and morphology indicate a biochemical interaction between the cells and the collagen at the nanofiber surface.

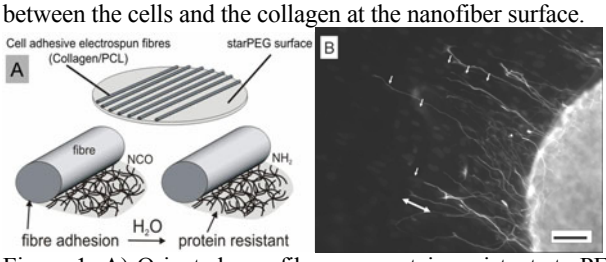


Figure 1: A) Oriented nanofibers on protein resistant starPEG surface. B) Oriented glial migration and neurite outgrowth from E 10 chick DRG on oriented electrospun nanofibers (doublearrow indicates nanofiber orientation, small arrows indicates Schwann cells, scale bar = 50 µm).

**Conclusions:** Electrospinning can be used to generate oriented PCL and CPCL fibers with diameters in the submicron range. While both materials are suitable substrates for glial migration and oriented neurite outgrowth, the inclusion of collagen clearly enhanced these interactions.

**Acknowledgements**: Simone Balzer and Nadine Seiler helped with cell culture experiments. This work was supported by the IZKF "BIOMAT." (TV B111) grant and by the DFG-Graduiertenkolleg 1035 "Biointerface".

**References:**

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