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Effects of Ridges on Spinal Cord Neuron Outgrowth

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INTRODUCTION: One fundamental question in neurobiology is how neurites and neuronal connections can be localised with high precision. Control of neurite outgrowth and guidance of growth cones on material surfaces have become important topics in biomedically oriented material science. They have implications for the rational design of neuroimplants such as artificial nerve conduits or bioelectronic interfaces.

The aim of the present study was to investigate in how far ridges are able to affect neurite growth properties. For this, reaggregates of ventral spinal cord neurons of chicken embryos were cultured close to an array of ridges with different dimensions and surface chemistry

METHODS: Polyimid spin coated oxidized silicon wafers were patterned by photolithography. The resulting polyimide structure consists of parallel ridges with ridge width and interridge distances of 5µm to 100µm, and ridge height of 1.3 µm or 3.0 µm.

To obtain fluorescence, spinal cord neurons of chicken embryos were transfected *in ovo* with a modified RFP-plasmid vector pRFP-N1 of Clonetech (USA) after breeding the eggs for 70 hours at 37 ºC. Motoneurons were isolated 74 hours after transfection. Single cell suspensions of spinal cord sections were obtained by trypsinisation. Reaggregates were prepared by gyratory shaking of cell suspensions for 1 day. Reaggregates were placed near the structures and pictures were taken every 5 min. for the following 16 hours. Cells were kept under cell culture conditions during the whole process (37°C and 5 % CO_2). Dislocation of marked growth cones was analysed using Visiometrics software as previously described [1].

RESULTS: Polyimide is very neurocompatible taking the neurite outgrowth into account. Furthermore, nerve cells behaved similarly on SiO_2, polyimide and on a-C:H coated surfaces.

Neurites changed their direction in parallel orientation to the ridges being trapped between 2 ridges forming a channel-like structure. The ridge-like structures also reduced neurite branching. The tendency of crossing the ridges was not only increased by reducing the ridge height from 3.0 to 1.3 µm but also by decreasing the interridge distance from 100 to 5µm (Fig. 1). The velocities found for outgrowth (v+) were around 18 % higher than for retraction (v-). By increasing the period between the measurements the retraction of neurites increasingly affects the measured velocity. By measuring *net* velocity instead *mean* velocity (v_{mean}) measured every 5 minutes SiO_2-PI surfaces with 10µm width -10µm ridges were able to significantly promote the outgrowth by a factor of two. However, no differences were found for v_{mean}.

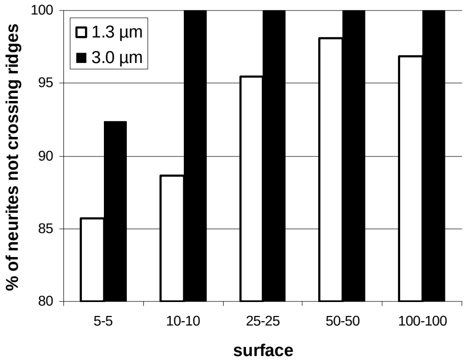

Fig. 1. Percentage of neurites crossing the ridges (data of SiO_2-PI and a-C:H coated surfaces pooled). Values are based on behaviour of 28-85 neurites.

DISCUSSION & CONCLUSIONS: We conclude that a-C:H and polyimide are neurocompatible coating materials. Furthermore, topographic structures in the low micrometer range have a strong influence on outgrowth behaviour of spinal cord neurons, and may therefore be used to optimise surfaces of neuroprostheses. The present study shows that topographical cues with dimension of around 3 µm in height and 5-10µm interspacings are enough to result in a precise, stereotypical pathfinding and that these cues do not alter nerve functionality taking outgrowth and retraction velocity as indices.

REFERENCES: [1] J.P. Kaiser, A. Reinmann, A. Bruinink (2006) *Biomaterial* **27**:5230-41.

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