# Nanochemically oriented astrocytes direct adjacent nerve cell outgrowth

FanWei Meng, Vladimir Hlady and Patrick A. Tresco
The Keck Center for Tissue Engineering, Department of Bioengineering, University of Utah

**Statement of Purpose:** Following CNS injury, the disorganized extracellular milieu that ensues is one of the major impediments to axonal regeneration leading to restoration of function. To overcome this inhibitory environment, a variety of biomaterial strategies have been developed to bridge the gap and direct regrowing neurites to distant.Within this context we have proposed taking advantage of the gliotic reaction that encapsulates all CNS implants [1]. Previously, we have shown that is possible to use submillimeter topography to influence astrocyte orientation at the biotic-abiotic interface, and that such engineered cells can transfer their directionality to direct adjacent nerve outgrowth. However, in such studies it is unclear whether such guided neurite outgrowth is related to the physical topography of the bridging substrate or reflect an intrinsic property of astrocyte ansiotrophy. Toward this end, we asked whether the directional information contained in nanometer level surface patterned ligands can be used to engineer directed nerve cell outgrowth through an intervening encapsulating astrocyte layer.

**Methods:** Microcontact printing (μcP, Fig.1) using laminin (400μg/ml) was applied to silica substrates to generate oriented as well as random astrocytes monolayers. Primary P1 astrocytes were plated at a density of 17000 cells/cm$^2$ on both patterned and control surfaces in SATO- for the first 6 hrs and then switched to 10% FBS for four days until confluence. Fixed astrocytes monolayers were generated by exposing astrocytes to 4% PFA at day 4. The fixed culture was left in DMEM/F12 for 2 more days to deactivate residual aldehyde groups. Fresh P1 DRG neurons were seeded at a density of 580 DRGs/cm$^2$ for 2 days in 10% FBS with 10 ng/ml NGF. Cell nuclear morphology was used to determine astrocyte orientation. Image J was used to measure neurite orientation, as well as directed outgrowth length. Immunochemistry was performed using antisera against Glial Acidic Fibrillary Protein (GFAP), Laminin (LN), Cellular Fibronectin (CFN), Chondroitin Sulfate Proteoglycan (CSPG) and Neurofilament (NF-160) to reveal neuronal processes, secreted ECM and astrocytes.

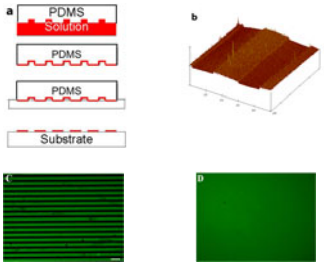
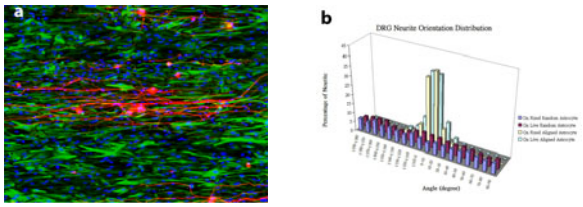



Fig.1 A) Overview of soft lithographic printing. B) AFM image of the LN pattern on coverslips C, D) Fluorescent image of stamped periodic LN lane and homogeneous stamped LN field. Scale bar = 100 μm.

**Results:** The printed LN pattern consisted of continuous lanes of laminin (15 microns wide) interspersed by continuous lanes of untreated glass (15 microns wide) with height on the order of 5 nm as measured by AFM. Astrocyte orientation was profounded affected by anisotropic LN lanes created by μcP. Approximately 80% of the astrocytes were aligned within 20 degrees of the direction of the lane orientation, whereas no biased orientation was observed on control uniformly stamped surfaces. The direction of overlying neurite outgrowth followed the orientation of the underlying astrocytes on both live and fixed astrocyte monolayers (Fig.2a). 89% of neurite growth was aligned within 20 degrees of the pattern long axis when grown on oriented monolayers whether alive or fixed, and no preference of neurite orientation was observed on randomly aligned monolayers (alive or fixed, Fig.2b). We observed a 370% and 280% increase of directed length on live and fixed oriented astrocyte monolayers compared to fixed random monolayers or a 250% and 200% increase when compared to live random astrocyte monolayers. CSPG and LN immunoreactivity was puntate and uniformly distributed over the monolayer and individual astrocyte surfaces and showed no directional bias on either surface. CFN, on the other hand, was observed to form linear arrays of fibrillar bundles on individual astrocytes irrespective of their orientation and were mostly aligned




on the patterned surfaces, suggesting a potential dominant role of CFN in astrocyte mediated neurite guidance.

Fig.2 A) DRG neurite outgrowth direction influenced by underlying astrocyte alignment, B) DRG neurite orientation distribution on four different conditions of engineered astrocytes cell layers. Red= NF160, green=GFAP

**Conclusions:** The current data indicate that nanometer level surface cues on a planar substrate are sufficient to affect the directional outgrowth of adjacent neural tissue through an encapsulating astrocyte monolayer. Studies in progress are focusing on understanding the mechanism

and translating it to a successful biomaterial bridging strategy for therapeutic applications

**References:**

[1] Biran R. Exp. Neurol. 2003; 184:141-152