A 3-dimensional, engineered astrocyte tissue construct for CNS repair

Fanwei Meng, Vladimir Hlady and Patrick Tresco
The Keck Center for Tissue Engineering, Department of Bioengineering, University of Utah, Salt Lake City USA

Statement of Purpose: Following CNS injuries, both the upregulation of repulsive ligands and the disorganized extracellular milieu inhibit axonal regeneration. While interventions focusing on ameliorating the inhibitory molecules have proven to be beneficial, strategies aiming at restoring the injured tissue architecture also have the potential to improve nerve regeneration especially after nerve cell grafting. Synthetic bridging materials are either implanted alone or in combination with cellular components in an attempt to mimic native tissue and direct axonal regeneration. However, the foreign body response associated with synthetic biomaterials is a major obstacle to this approach. Here we report on the development of 3-D, multi-layered astrocyte construct that is mechanically stable and possess anisotropic morphology that may be transplanted without an accompanying synthetic biomaterial to support neuronal outgrowth and potentially modulate the inflammatory response following implantation.

Methods: Microcontact printing was used to generate oriented ligand patterns on silica. Primary P1 astrocytes were plated at a density of 51,000 cells/cm^2 on both patterned laminin (LN) and control surfaces in SATO- for the first 4 hrs and then switched to 10% FBS. First cell layer reached confluence after two days. The seeding procedure was repeated every other day to achieve a dense astrocyte tissue construct that could be gently removed from coverslips by forceps. Both random and oriented multi-layered astrocyte constructs were fixed after 14 days in 4% paraformaldehyde for 15 mins. Some constructs were transferred into DMEM/F12 for 2 days to deactivate the residual aldehyde groups for subsequent DRG and microglia culture. Primary P1 DRG neurons or primary microglia were plated at a density of 2,500 neurons/50µl 10% FBS and 50,000 microglia /50µl 10% FBS onto each astrocyte construct and let it sit for 1 hr to attach. Then, 10 % FBS supplemented with or without NGF (10ng/ml) was added throughout culture period for DRG neurons and microglia, respectively. The astrocyte construct was wrapped against the 2.5% agar rod to engineer cylindrical astrocyte tube. Both astrocyte tube and multi-layered astrocyte construct was embedded in 0.8 % agar gel and left in 30% sucrose overnight before cryostat sectioning. Immunochemistry was performed using antisera against Glial Acidic Fibrillary Protein, Laminin, Fibronectin, Chondroitin Sulfate Proteoglycan, Neural cell adhesion molecule, ED-1, OX-42 and Neurofilament to visualize astrocyte derived ligands, neuronal processes and microglia.

Results: Oriented, multi-layered astrocyte tissue constructs were engineered after 14 days of culture (Fig.1A). The astrocyte constructs were mechanically stable and easily manipulated in the media with forceps. Following peeling off from the substrate, the oriented construct demonstrated anisotropy as it shrank more in the direction parallel to the long axis of patterned LN lane pattern. The preservation of anisotropy by the oriented construct was illustrated by fluorescent immuno-histochemical staining of various ECM proteins and cell surface ligands produced by astrocytes (Fig.1B). Both confocal microscopy and cryostat sectioning showed the preservation of oriented ECM proteins and surface ligands throughout the thickness of the astrocyte construct. We were able to manipulate multi-layered constructs into different shapes including stacks and tubes (Fig.1C-E), showing its potential as a transplantable pure cell derived biological material. Oriented astrocyte constructs supported directional outgrowth of neurons as compared to random constructs. Moreover, the astrocyte construct demonstrated the ability to modulate the inflammatory response of microglia cells in vitro as microglia became ramified when cultured on astrocyte construct, a phenomenon we did not see for those culturing on coverslips.

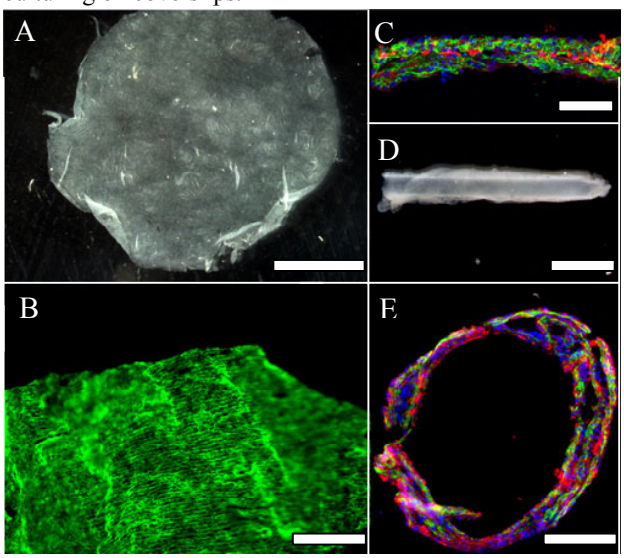

Figure1. (A) Centimeter scale 3D, multilayered astrocyte construct. (B) Preservation of FN alignment in the oriented construct. (C) Cross-section view of a multi-stack astrocyte construct. (D) Astrocyte tube. (E) Cross-section view of an astrocyte tube. Scale Bar=3mm in (A); 500µm in (B); 50µm in (C); 2mm in (D); 250µm in (E). Green:FN in (B,C,E), Red:GFAP in (C,E), Blue:DAPI in (C,E)

Conclusions: We engineered 3D, scaffold free astrocyte construct that possesses anisotropy and is mechanically stable and easily manipulated with simple hand held tools. Astrocyte constructs also support directed regenerating neurite outgrowth and may be capable of modulating inflammatory response of microglia. These results support the therapeutic potential of such an approach for CNS repair.

References:

[1] Matsusaki et al., Angew. Chem. Int. Ed. 2007