Astrocyte and Glial Restricted Precursor Derived Biomaterials as Coatings for Chronically Implanted CNS Devices

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Introduction: Long-term recording performance of implanted microelectrode arrays is believed to be adversely affected by the tissue response. Irrespective of the type of implanted electrode, researchers have observed persistent inflammation, reactive gliosis and neuronal loss in the tissue surrounding the implanted array. In contrast, cell derived biomaterials show a level of biocompatibility and integration not found with synthetics currently used for microelectrode fabrication technology. To create more biocompatible microelectrodes that better integrate into surrounding CNS tissue, we have developed a novel approach to harvest and then couple extracellular matrix produced by astrocytes and glial restricted precursors (GRPs) grown on and then extracted from sacrificial, open celled polymer foams[1,2] to lattice microelectrode arrays.

Materials and Methods:

Substrate Preparation: Porous, rectangular, polyurethane foams (Tecoflex SG-80, Thermedics) were custom fabricated using a phase inversion method. Each foam substrate was incubated in a fibronectin solution (20ug/ml) overnight, and seeded (2 million cells/cm^3) with either primary astrocytes or glial restricted precursors (GRPs) harvested from Sprague Dawley rats. Seeded substrates were cultured for 3wks in DMEM F12 supplemented with 10% FBS.

Cell-derived Material Extraction: Following culture, samples were rinsed in DI and frozen to -80°C. Samples were weighed and then soaked in the solvent DMAC for 72hrs at 37°C to remove the PU foam. Extracted material was rinsed three times in DI H_2O and lyophilized.

Characterization of Extracted Material: Yield of cell-derived material was determined by measuring the mass of extracted material (n=4 foams/cell type). The effectiveness of the DMAC dissolution process was assessed using Fourier transform infrared spectroscopy (FTIR). Immunohistochemical analysis and tandem mass spectroscopy (MS/MS) was performed to identify ECM and proteomic components found in harvested material.

Cytotoxicity Study: To assay cytotoxicity, cell derived material was reseeded with P4 rat DRG cells at a density of 50K cells per sample (approximate sample size 2x2x2 mm) in Sato$^-$. At the end of the 48h cultivation period cell viability was assessed with calcein AM (2 mM in DMEM/F12) and neuronal outgrowth with NeuroFilament 160 (NF 160).

Cell-Derived Material Coating: To improve the integration of microelectrodes into tissue and create a more biological interface we covalently tethered cell-derived material to Si/SiO^2 electrode surface using an epoxy silane (GPTMS).

Results:

We collected bulk cell-derived material from both astrocytes and GRPs. Yield ranged from approx. 1.8mg to 4.4mg of material per seeded foam. We did not observe strong characteristic polyurethane linkage peaks within the FTIR spectra of cell-derived biomaterials. However, we did observe increased absorption corresponding to amine chemistries characteristic of ECM. Immunohistochemical analysis and tandem mass spectroscopy indicated that the lacey material consisted of a porous network of ECM components containing fibronectin, laminin and glycosaminoglycans (Fig 1).

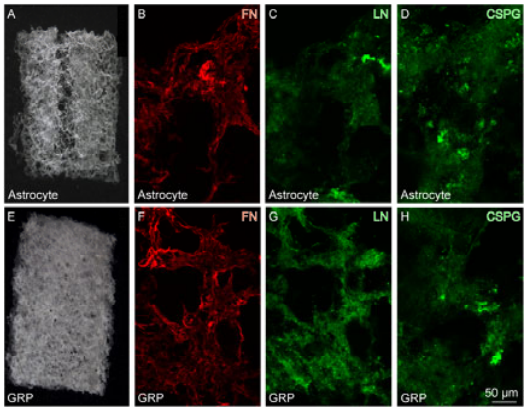

Figure 1: (A and E) Representative light micrographs of astrocyte and GRP-derived biomaterials after dissolution of the culture substrate. Isolated material from both cell types was white, lacy and porous. Panels B-D and F-H show confocal images of astrocyte and GRP derived material stained for FN, LN and CSPG respectively.

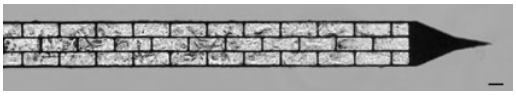

Figure 2: GRP derived biomaterial coated within and on the lattice structure of a silicon microelectrode array used in neural prosthetic application for better tissue integration. Scale bar = 100µm.

Conclusions: In this study we describe a method to couple accumulated cell-derived ECM material from astrocyte and glial restricted precursor cells cultured within sacrificial substrates to microelectrodes. Following culture, the cell-derived material was harvested through treatment with a polar, aprotic, organic solvent. This approach represents a new method to create cell-derived biomaterials that is not limited to the cells and substrates described in this study. A wide range of phase separated polymers whose chain entanglements can be overcome with such solvents can be used. Additionally, this method could be used with other cell types to create a new class of materials with potentially distinct properties and applications. Ultimately we suspect the complex and all-natural composition of this harvested material to show a level of biocompatibility and therapeutic effectiveness that is not found with synthetic materials currently used to fabricate microelectrode arrays and other devices implanted into the nervous system. Investigations in progress are examining the tissue response to microelectrodes coated with cell-derived materials.

1. Wolchock JC and Tresco PA, Biomaterials 2010
2.Wolchock JC et al, Biomaterials 2009