Cell-Specific ECM Down-Regulates the Inflammatory Response to Nervous System Implants

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Statement of Purpose: Microelectrodes have the potential for use as recording and stimulating interfaces for neuroprosthetic devices. However, current clinic application is limited due to their inconsistent performance over time. It is generally believed that the inconsistent performance is associated with the inflammatory foreign body reaction. Therefore, strategies which would reduce the inflammatory reaction surrounding these devices could lead to improvements in long-term functionality. Here we describe the ability of cell-specific extra-cellular matrix (ECM) derived from astrocytes and their progenitors to alter the phenotype of activated macrophages *in vitro*, as well as describe how such constructs may be applied to current electrode technologies for application *in vivo*. Additionally, we demonstrate the ability of these constructs to replace synthetic anchoring cuffs know to elicit an inflammatory reaction sufficient to alter morphometric parameters in peripheral nerves.

Methods: ECM-derived constructs were collected as previously described[1]. Briefly, open-cell polymeric foams were seeded with either primary rat astrocytes or rat dermal fibroblasts and kept in culture for 3 weeks. Following the incubation period, the polymer foams were removed and the ECM constructs were washed with DI water, frozen, and lyophilized. All constructs were ETO sterilized before use. For *in vitro* assays, astrocyte-derived constructs were placed in 24 well plates and seeded with primary P2 macrophages for 48 hours. Glass coverslips served as controls. Macrophages were then fixed with 4% paraformaldehyde and immunohistochemically stained for markers against IBA-1 and CD-68. For *in vivo* work, dermal fibroblast-derived constructs were implanted around the right sciatic nerve of male Sprague-Dawley rats for 60 days. Following the implantation period, implanted nerves were extracted and immunohistochemically stained at the implant for markers against CD68, vimentin, and NF-200. Additionally, sections immediately proximal and distal to the implant site were processed for morphometric parameters including total fiber counts, fiber density and packing, mean g-ratio values (ratio of axon diameter to fiber diameter), and fiber diameter distributions.

Results: Macrophages seeded onto control glass coverslips exhibited an amoeboid morphology indicative of activation (Figure 1A). Conversely, macrophages seeded on astrocyte-derived ECM constructs exhibited a ramified morphology, indicative of a resting phenotype (Figure 1B). For *in vivo* work, cross-sections through the implant site suggest incorporation or remodeling of the implanted ECM, with little associated inflammation as indicated by CD-68 staining (Figure 2). Morphometric data from sections proximal and distal to the implant did not exhibit any statistically significant differences from non-implanted control nerves (data not shown).

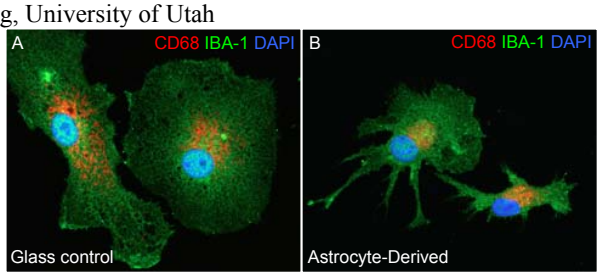

Figure 1: A) Macrophages seeded onto glass coverslips exhibited an amoeboid morphology indicative of an activated phenotype while those seeded on astrocyte-derived ECM constructs (B) exhibited a ramified morphology indicative of a resting phenotype.

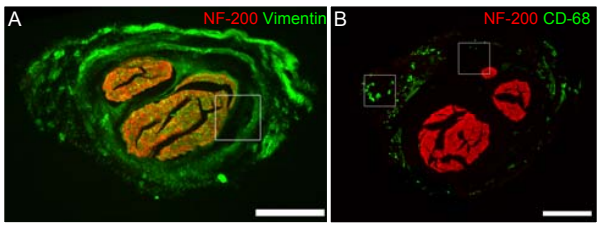

Figure 2: Cross-sections through the implant site of rat sciatic nerve after 60 days. A) Vimentin staining around the endoneurium suggest incorporation or remodeling of the originally implanted ECM. B) Small areas of inflammation are observed by CD-68 positive staining. Traditional silicone nerve cuffs have previously been demonstrated by our lab to exhibit a more pronounced reaction (data not shown).

Conclusions: Our studies demonstrate the ability of ECM-derived constructs to be used in place of synthetic anchoring devices, as well as their ability to reduce inflammation perhaps by down-regulating the inflammatory phenotype of activated macrophages. The incorporation of similar ECM as coatings for microelectrode devices (Figure 3) or other chronically implanted medical devices may help mitigate the inflammatory response around those devices, increasing their functional lifetimes.

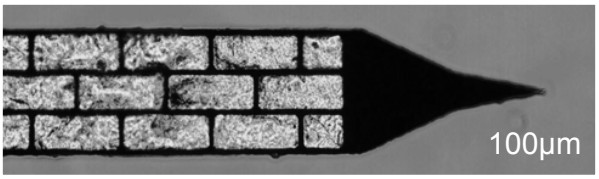

Figure 3: Michigan-style lattice microelectrode with astrocyte ECM proteins covalently bound to the surface.

References:

(Wolchock JC. Biomaterials. 2010;31:9595-9603)