**Bioactive surface for neural electrodes: tethering transforming growth factor β1 to inhibit gliosis**

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**Statement of Purpose:** Implantation of deep-brain recording devices is a traumatic event which inevitably elicits reactive gliosis. The ensuing glial scar encapsulating the implanted device impedes the long-term functional recording capability of the microelectrode. In this work, a bioactive surface is prepared by conjugation of transforming growth factor-beta one (TGF-β1) and laminin to dextran which is in turn conjugated to a biomaterial substrate. Enzyme linked immunosorbent assay (ELISA) is used to determine whether TGF-β1 is covalently tethered to the biomaterial surfaces. Cell numbers are quantified after 48 hr culture on surfaces with and without tethered TGF-β1 to test the hypothesis that tethered TGF-β1 inhibits astrocyte proliferation.

**Methods:** Dextran coated tissue culture polystyrene surfaces are prepared following the method described by Massia et al.[1] Briefly, the wells of a 96-well plate coated with poly-L-lysine are incubated with oxidized dextran. Unreacted carboxyl groups are quenched with sodium borohydride followed by incubation with sodium metaperiodate to reactivate the dextran. Either laminin alone or laminin with 10 ng/ml TGF-β1 is incubated with this activated dextran surface overnight. Covalent conjugation of TGF-β1 is confirmed by ELISA.

A primary cell line of astrocytes is obtained from 0 - 2 d Sprague-Dawley rat pups as previously described with minor modifications.[2] Briefly, cortical tissue is dissociated in EDTA followed by DNAse. Cells are cultured until stratified, the flasks are shaken to free loosely attached microglia, neurons, and/or other cell types. The adherent cells are >90% astrocytes.

CellTiter 96® AQueous One Solution Cell Proliferation Assay is used to determine cell number. Primary rat astrocytes, counted using a hemocytometer, are added to wells conjugated with laminin or laminin and TGF-β1. After 48 hr culture, absorbance is quantified at 490nm. A calibration curve is created by plating known numbers of astrocytes in coated wells immediately before the assay.

**Results/Discussion:** Characterization of TGF-β1 conjugation to the biomaterial surface is performed by ELISA. Results, Figure 1 (Left), for the experimental surface, dextran chemically activated to form covalent bonds followed by incubation with 10 ng/ml TGF-β1, show statistically significant increases ($p < 0.006$, ANOVA with post-hoc Tukey test) from both the non-activated surface and the no-TGF-β1 surface. Absorbance due to non-specific adsorption of secondary antibody is subtracted from the values shown by preparing samples of the above surfaces to which primary antibody is not added. These results demonstrate that TGF-β1 is covalently bound to the biomaterial surface.

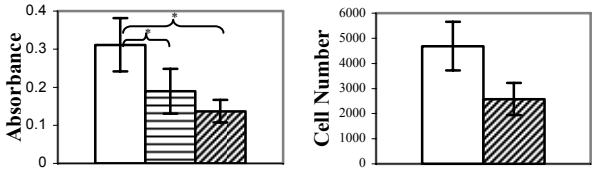


*Figure 1.* (**Left**) ELISA for TGF-β1 to assess tethering: TGF-β1 incubated with activated dextran surface (solid bar), TGF-β1 with unactivated surface (horizontal hash marks), and incubation with no TGF-β1 (diagonal hash marks). Asterisks indicate statistical significance (n=3). (**Right**) Surface conjugated TGF-β1 inhibits astrocyte proliferation. Negative control surfaces (diagonal hash marks) are reacted with molar equivalent concentrations of BSA; whereas, experimental samples are reacted with TGF-β1 (solid white bar) (n=6).

Soluble TGF-β1 is known to inhibit proliferation of primary astrocytes.[3] It is not obvious that a tethered form of TGF-β1 would elicit similar inhibition of astrocyte proliferation so we determined the rate of proliferation on surfaces to which TGF-β1 and laminin are conjugated and surfaces to which BSA and laminin are conjugated. Figure 1 (Right) shows that the surfaces to which TGF-β1 is conjugated elicit less astrocyte proliferation than the surface to which only laminin is conjugated ($p < 0.002$). Proliferation of the astrocytes is decreased approximately 57% by the surface conjugated TGF-β1 compared to the control group.

**Conclusions:** Covalent conjugation of the TGF-β1 is confirmed by enzyme-linked immunosorbent assay. Primary astrocytes incubated on surfaces conjugated with laminin and TGF-β1 exhibit 57% less proliferation ($p < 0.002$) than on the control surface (laminin with BSA). These data demonstrate that conjugated TGF-β1 retains its efficacy toward astrocyte proliferation and represents a potential strategy for reducing glial scar formation *in vivo*.

**References:**

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2. McCarthy, K.D. J Cell Biol, 1980. **85**(3): 890-902.
3. Lindholm, D. J Cell Biol, 1992. **117**(2): 395-400.

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