In vitro Study of Astrocyte and Macrophage Response to Silicon Surface Modified with Cationic Microgels

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Statement of Purpose: Neural implants have the potential to improve the lives of patient suffering from conditions like paralysis, Parkinson's, and epilepsy. Because neuron adhesion plays a critical role in neural functions, successful integration and short-term functionality of these devices with the patient's tissue depends largely on the extent of neural adhesion and integration. Unfortunately, typically used implant materials, such as silicon, do not promote these crucial neural interactions. Previous research has shown that cationic microgels adsorbed on a layer-by-layer (LbL) surface have facilitated primary chick cortical neuron and PC-12 cell, a neuronal-like cell line, adhesion and development onto these typically non-adhesive silicon substrates.[1] Contrary to short-term device functionality, long-term functionality is hindered by the bodies' chronic tissue reaction, in particular chronic inflammation resulting in non-neural glial cells like microglia and astrocytes, encapsulating the implant and inhibiting essential neuron-implant interactions.[2] Following the encouraging preliminary outcome of cationic microgel and neuron integration, we investigate glial cell responses towards the microgels.

Methods: The details of sample preparation have been described elsewhere.[2] Briefly, polyethylenimine (PEI) and polystyrenesulfonate (PSS) were incubated on piranha treated silicon substrates to construct $(PEI/PSS)_2$ LbL precursor films with either microgel particle (MP,1 mg/mL) or PEI adsorbed as the final layer (control). SEM and fluorescent imaging were employed for substrate morphology characterization, cell counting, and cell morphology. WST-1 assay was employed for proliferation studies. A-172 astrocytes were seeded on various surfaces at 10,000 cells/cm^2 and cultured at 37 °C in a 5% CO_2 atmosphere for 1-3 days. RAW 264.7 cells, a mouse leukaemic monocyte macrophage cell line (identical to activated microglia), were seeded on various surfaces at 10,000 cells/cm^2 and cell adhesion was examined after 2 h of culture.

Results: Average size and zeta potential of the cationic microgels used were 375 nm and 50 mV, respectively. Early point adhesion of macrophages showed cells were able to attach to all sample types, while a significant difference was found between LbL/MP and the piranha treated silicon groups (Fig. 1). Astrocytes were also able to attach to MP modified silicon.

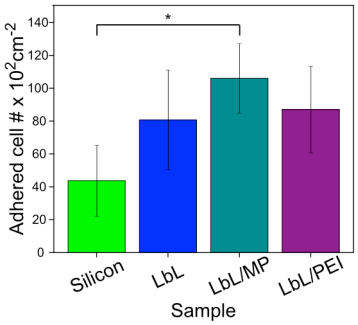

Figure 1: Macrophage adhesion after 2 h culture (n = 3, * $p < 0.05$, Student's t-test).

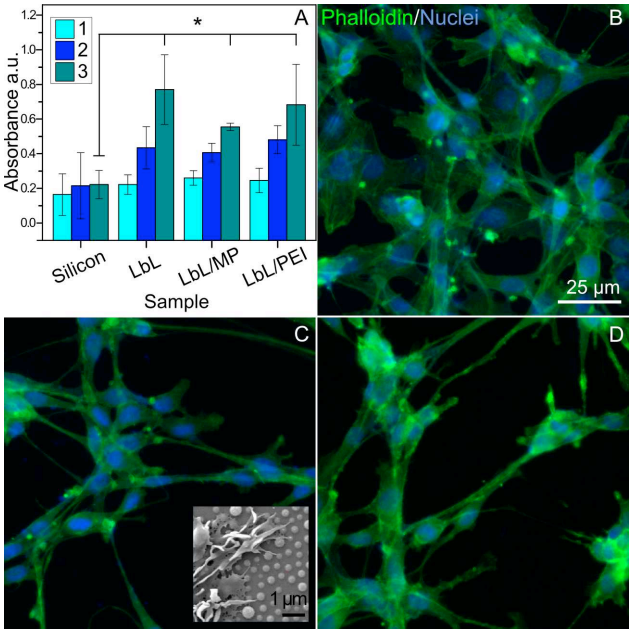

Figure 2. (A) WST-1 assay after 1-3 days of A-172 astrocyte cell culture (n = 3, * $p < 0.05$, Student's t-test). Fluorescent staining of cells on LbL (B), LbL/MP (C), and LbL/PEI (D) on day 3. SEM inset (C) shows cell interaction with MP.

Results from the 3-day proliferation study showed that cell growth was supported by all samples except the piranha treated silicon control (Fig. 2A). Fluorescent staining showed that cells on the LbL/MP sample (Fig. 2C) had small round bodies and thin processes when compared to those on the LbL group (Fig. 2B), an indicator of a more differentiated and less proliferative[4] astrocyte cell form. Close interaction of astrocyte cell extension with MPs was observed with SEM (Fig. 2C inset). The LbL/PEI group (Fig. 2D) had similar cell morphology to LbL/MP, suggesting the prominent effect of surface charge affecting astrocyte response. Quantitative analysis of cytokine release from macrophages and glial fibrillary acid protein (GFAP) expression in astrocyte is currently underway.

Conclusions: Cationic microgels adsorbed on silicon surfaces were non-toxic to both macrophage and astrocyte. The observed astrocyte proliferation on the modified surface warrants in-depth molecular investigation of the astrocyte activity to determine whether the presence of microgels induce reactive astroglial response. To overcome potential adverse cellular activation, the reservoir function of microgels can be explored for localized delivery of bioactive molecules or drugs.

Acknowledgements: The authors thank support from the NSF (Award No. DMR-1055208). EAM is supported by the Center for Materials Processing at UTK. KLR is supported by the NSF-funded RISER program at UTK **References: (1)** Morin et al. Society For Biomaterials **2014** April 16-19, Denver CO, p.35 abstract no. 53. **(2)** Polikov, V. et al. Neurosci. Meth. **2005,** *148*, 1. **(3)** Tang, S et al. J. Mater. Chem. B **2013**, *1*, 1628. **(4)** Chen T. et al. Lab. Invest. **1998**, *78*, 165.