Facile use of cationic hydrogel particles for surface modification to improve neuronal cell adhesion and differentiation: an *in vitro* investigation

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Statement of Purpose: The low regenerative capacity of the central nervous system (CNS) has spurred intense research of interventions to restore lost neural functions. Regardless of what format biomaterials are introduced into the CNS such as neural electrodes or three-dimensional tissue regenerative scaffold, their successful integration with the host tissue is hinged largely on the extent of neural interactions with these abiotic entities. Since neuron adhesion bears a critical effect on all other aspects of neural functions, it is crucial to ensure that biomaterials are conducive for neuron adhesion. Many substrate factors such as topography, charge, and stiffness can influence neural cell adhesion.[1-3] While one can improve neural adhesiveness of biomaterials by modifying any of the above properties, it has been suggested that a combination of multiple factors will provide synergistic benefits promoting neuron adhesion.[4] In order to achieve this goal, in the study we investigate a cationic nanogel system as a versatile adhesion "liaison" between neurons and planar nonadhesive abiotic biomaterials.

Methods: A bilayer precursor film was used as a model surface on glass for nanogel investigation. Polyethylenimine (PEI) and polystyrenesulfonate (PSS) were incubated on piranha treated glass to construct $(PEI/PSS)_2$ LbL precursor films. Nanogels (NG) (1 mg/mL)[5] were then incubated on this precursor substrate for 10 min to prepare NG/LbL. Polyacrylic acid (PAA) was also used to prepare PAA/NG/LbL samples to study the charge effects of the nanogel. Poly-L-lysine (PLL) was used as a control. Samples were UV sterilized for 30 min. AFM and SEM were employed for substrate morphology characterization. For adhesion assay, PC12 cells, a rat adrenal pheochromocytoma cell line, were seeded on various surfaces at 15,000 cells/cm^2 and cultured at 37 °C in a 5% CO_2 atmosphere for 12 hr. The number of adhered cells on various surfaces was then quantified. Differentiation study was carried out by supplementing the culture medium with nerve growth factor (50 ng/mL).

Results: Average size and zeta potential of the nanogels used were 320 nm and 60 mV respectively (Fig. 1). The AFM image (Fig. 1) showed that cationic nanogels can adsorb readily onto the LbL precursor surface. The adhesion study showed that the number of cells attached was significantly higher on the nanogel modified surface as compared to the plain glass, LbL precursor, and PAA capped NG layer (Fig. 2). It is worth noting that PLL control group had the highest number of cell attachment among all the conditions, implying the prominent effect of surface charge on neuronal cell adhesion. SEM imaging from on our initial differentiation study revealed close interactions between nanogels and extending neurites (Fig. 2). Quantitative analysis of neurite morphology on various surfaces is currently underway.

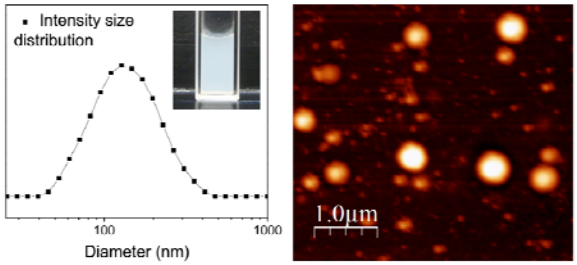

Fig. 1: Left: DLS depicting average nanogel size, inset shows concentrated nanogel solution at 3.5 mg/mL. Right: AFM image of nanogels distributed on the LbL precursor surface.

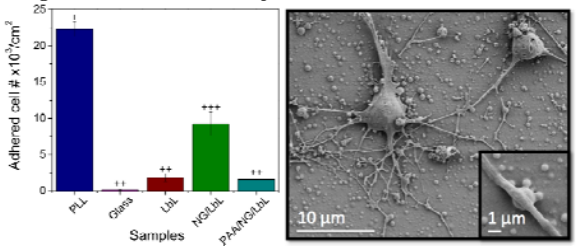

Fig. 2: Left: Neuronal adhesion after 12 hr (indicator signs in common denote a lack of significant difference, p<0.01). Right: SEM images showing neuron attachment and neurite extension on a NG/LbL modified planar substrate. Magnified view illustrates close interaction between nanogels and the neurite.

Conclusions: Neuronal attachment to nonadhesive substrates can be improved using nanogel as an interfacing layer. Balanced tuning of the nanogel properties such as size, charge, and surface density could further influence its effects on cells. Additionally, the use of nanogels for surface modification adds the benefit of localized delivery of bioactive molecules to modulate cell behaviors.

Acknowledgements: The authors thank support from the NSF (Award No. DMR-1055208). EAM is grateful to the fellowship provided by the NIH-funded PEER program at UTK. KLR is supported by the NSF-funded RISER program at UTK.

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