Nanoscale Cues and Astrocyte Responses in Neural System Regenerative Medicine

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Abstract- The goal of regenerative medicine is to create a scaffold which is biomimetic for the native attachment environment of a given cell group, which, prompted by nanoscale cues, re-colonizes the scaffold. In the present investigation, astrocyte responses are correlated with the mechanical, topographical and biochemical cues of a prosthetic environment.

Recent efforts in the field of regenerative medicine have yielded impressive successes [1]. The basis of regenerative medicine is to create a scaffold which is biomimetic for the native extracellular matrix (ECM) of a given tissue and to then expose it to a desired cell population, which may contain adult, embryonic or pluripotent stem cells. If the scaffold has the correct nanoscale cues, the cells will naturally re-colonize the prosthetic ECM matrix and resume normal functions. Fundamental understanding is still needed to design scaffolds with appropriate mechanical, topographical and biochemical cues for particular cell classes. Understanding the nature of the physical interactions of cells within these biomimetic structures and deriving information that would correlate mechanical, topographical and biochemical properties of the scaffolds with the promotion of specific cellular responses would have a major impact on their design and utility.

This study is focused on astrocyte response to different types of scaffolds. Astrocytes are members of the neural cell system that provide nutrients to and remove wastes from neurons [2]. They also provide mechanical support for neurons both directly and through the expression of interstitial extracellular matrix molecules. Furthermore, astrocytes express directive growth factor proteins that promote neuron axon growth and alignment, resulting in healthy synaptic transmission. Astrocytes are themselves in contact with a dense ECM termed the basement membrane, which surrounds each capillary at the blood-brain barrier. We will report our recent research that indicates that astrocytes respond strongly to the mechanical, topographical and biochemical cues provided by the native, or by a prosthetic, ECM.

The prosthetic scaffold currently under investigation is a synthetic electrospun polyamide dense nanofibrillar matrix that has demonstrated promise for the repair of the injured spinal cord and is architecturally mimetic for the capillary basement membrane at the blood brain barrier [3]. The mechanical, topographical and biochemical cues of the prosthetic nanofibrillar matrix are investigated at the nanoscale using atomic force microscopy techniques, including a new technique developed by this group, scanning probe recognition microscopy [4], which enables property measurements along individual nanofibres within a scaffold, followed by compilation of results into statistical representation for the nanofibrillar matrix as a whole .

The astrocyte responses to the nanoscale cues presented by three different substrates are investigated. The substrates are: an unmodified nanofibrillar matrix (NANS), a surface activated nanofibrillar matrix (SANS) which been treated with a polyaniline coating, and a nanofibrillar matrix which has been covalently modified with the directive growth factor fibroblast growth factor-2 (FGF-2). A fourth 2d plastic substrate is used a control.

Atomic force microscopy images (tapping mode) that show details of the topographical differences amongst the nanofibrillar substrates are shown in Figure 1. The topological differences translate into several physical differences. As further shown in Figure 1, the frictional environment is changed by the addition of the growth factor. We have recently reported additional nanoscale physical changes in surface roughness and elasticity caused by the FGF-2 growth factor modification [5]. Astrocyte responses to nanofibrillar versus 2D substrates include both differences in cell morphology and, significantly, up-regulation of FGF-2 expression by astrocytes. The latter induces longer and more branched axon development by neurons in co-culture [6].

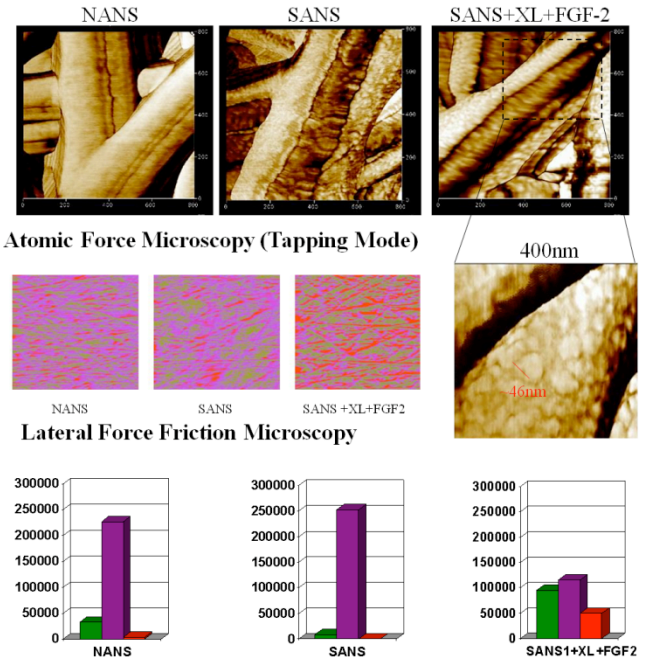

Figure 1. AFM investigation of unmodified, coated, and FGF-2 growth factor modified nanofibrillar matrices shows topographical differences. Friction, assessed by lateral force microscopy, is one of several changed nanoscale physical properties.

Friction, surface roughness and elasticity have all been recently shown to influence cell morphogenesis and differentiation Therefore, when performing growth factor modification of prosthetic tissue scaffolds to achieve a desired biochemical directive goal, it is necessary to consider the changed nanoscale physical environment presented to re-colonizing cell groups.

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