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Quantitative Investigation of FGF-2-Modified Nanofibrillar Prosthetic for Neural Cell System Re-establishment

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Recent advances in regenerative medicine have improved understanding of the materials properties of nanofibrillar scaffolds used to aid cell system re-establishment. A picture is emerging that a successful scaffold has a defined set of physical properties including mechanical properties, topographical properties, and growth factor inclusion, and that the weighting of each may vary depending on the cell system and its functionality. In the present work, results from investigations of a slowly biodegradable nanofiber prosthetic for neural cell system re-establishment are presented. Preliminary data from in-vivo investigations (rat model) indicate that a nanofiber prosthetic device of FGF-2-modified nanofibers contributes to system re-establishment including aligned guidance for regenerating axons across an injury gap, and angiogenesis. Research by Meiners' group also demonstrated that FGF-2 retains biological activity significantly longer when immobilized on nanofibers than when presented as a soluble molecule [1]. The present investigations use atomic force microscopy operated in a new mode, Scanning Probe Recognition Microscopy (SPRM) [2], developed by Ayres' group, to quantitatively investigate properties of FGF-2-modified nanofibers. The SPRM system is given the ability to auto-track on regions of interest through incorporation of recognition-based tip control realized using algorithms and techniques from computer vision, pattern recognition and signal processing fields. Statistically meaningful numbers of reliable data points are extracted using an automatic procedure that maintains uniformity of experimental conditions. Properties under quantitative SPRM investigation include nanofiber stiffness and surface roughness, nanofiber curvature, nanofiber mesh density and porosity, and growth factor presentation and distribution. Each of these factors has been demonstrated to have global effects on cell morphology, function, proliferation, morphogenesis, migration, and differentiation.

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Multi-detection Device For Studying Neuronal Cell Networks

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We are developing new analytical devices in order to analyse molecules involved in neuronal cell signalling in individual cells and networks of cells. The unique chip design will allow us to characterize the pattern of synaptic connections between neurons by stimulation with directed microflows to specific individual cells and subsequent detection of cell activity by fluorescence imaging or detection of release (exocytosis) by electrochemical imaging with electrode arrays. We will present the fabrication scheme for this device and preliminary results obtained by culturing pheochromocytoma (PC12) cells onto the chip. The location of the cells will be determined by microcontact printing of surface adhesion proteins (e.g. laminin or poly-L lysine) on the surface of the device. Upon stimulation, intracellular calcium levels in PC12 cells increase and they release dopamine by exocytosis. The former can be monitored with ratiometric fluorescence imaging and the latter can be quantified by electrochemistry and both are non-invasive.

These combined measurements represent an important technical development and will help bridge the gap between single cell measurements and those at neuronal systems in organisms. Once characterized, they will be applied to understand patterns of cell signalling and to develop an *in vitro* model of degenerative diseases like Parkinson's disease.

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Observations of Sensory Neuron Behaviors on Substrates with Various Stiffnesses through Living Cell Imaging

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With the development of materials science and fabrication techniques, we can fabricate an elastic contexture similar to the physiological condition of living organisms to address how cultured cells behave when grown on materials of physiologically realistic elastic moduli. In our previous studies, we found that neurons cultured on a substrate with a low stiffness coated with fibronectin exhibited a higher excitability responding to a stretching force compared with those cultured on the same substrate coated with poly-L-lysine. Neurons cultured on fibronectin-coated soft substrate also altered the morphology of microtubules. Herein, we further attempt to address how culture substrates with various stiffnesses influence the glia cell-neuron interaction and neurite outgrowth of cultured sensory neurons. To examine these effects on dorsal root ganglion (DRG) neurons, we first prepared substrates for neuron culture by using polydimethylsiloxane (PDMS) that is composed of a base and a curing agent with a ratio of 35:1. The elastic modulus of this soft PDMS is around 88 kPa. CD1 mice at 8-12 weeks old were used for DRG primary culture. Through living cell imaging, we found that the neurite outgrowth velocity of DRG neurons cultured on a coverslip was faster than cultured on PDMS matrix. In addition, the glial cells did not attach and spread well on a soft matrix compared with cells on a coverslip. When cycloheximide was applied to inhibit the synthesis and secretion of extracellular matrix molecules from glial cells, DRG neurons hardly survived after culturing for 24 hours. In conclusion, extracellular matrix signalling and substrate stiffness have profound effect on the velocity of neurite outgrowth and the survival of both neurons and glia cells.

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Natural-born Pacemakers

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The pre-Botzinger complex is a group of about 100 neurons in the brain stem that produce perdiodic bursting. The complex regulates breathing rythms. Individually, these neurons do not produce bursts, while the bursting is not regulated by pacemaker neurons. We report on a numerical and analytical study of periodic bursting by an array of coupled identical neurons showing the spontaneous emergence of clusters of neurons that act like pacemakers. The bursting is periodically interrupted by ''dephasing events'' that are encountered in in-vivo and in-vitro studies, which are compared with our results.

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Morphological Amplification of Action Potentials in Axonal Varicosities

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Action potentials, the regenerative electrical waves traveling along axons, are the electrical trigger of synaptic neurotransmitter release. Aside from propagation failures at branch points, axon geometry is presumed to be immaterial to action potential propagation. Our numerical simulations, in contrast, indicate that in-line varicosities can significantly modulate action potentials, and over large distances. A single in-line varicosity can severely depress action potential amplitudes upstream from it while, within the varicosity, depolarization is amplified. Amplification within varicosities varies in a non-trivial manner with varicosity size, and is most pronounced for varicosities close in dimensions to Herring bodies, the secretory specializations of neurohypophysial axons. Enhancement in secretory terminals is equally significant. Amplification is dominated by geometrical factors, but does vary with the kinetics of voltage-gated ion channels.

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Morphology of Neurofilament Protrusions: Sequence-Based Modeling of Neurofilament Brush

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Neurofilaments are essential cytoskeletal filaments that impart mechanical integrity to nerve cells and regulate the cross-sectional area of axons. They are assembled from three distinct molecular weight proteins (NF-L, NF-M, NF-H) bound to each other laterally forming 10 nm diameter filamentous rods along with side-arm extensions. These protrusions contain an abundance of charged amino acid residues. The charged side-arms are considered to mediate the interactions between neighboring filaments, regulating interfilament spacing, and hence axonal caliber. The precise mechanism by which neurofilament protrusions regulate axonal diameter remains unsettled. In particular, the role of individual proteins has remained to be a matter of debate. Computer modeling is instrumental in demonstrating the details about the structural arrangement of individual protrusions. The present study employs Monte Carlo Simulations of neurofilament to reveal the role played by individual side-arms. The simulations are conducted under different phosphorylation state by making use of physically motivated 3D model of neurofilament brush. The model consists of a neurofilament backbone along with side-arm extensions that are distributed according to the stoichiometry of the three subunits. The side-arms are modeled at amino acid resolution with each amino acid represented by a hard sphere