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AFM Bond-Rupture Forces on Neuron Receptors and Protein-Patterned Surfaces: Biomaterials for Neuron Pathfinding

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Injuries and diseases of the central nervous system (CNS) are the most catastrophic and costly human ailments. A lot of studies have been focused on developing biomaterial bridges bearing extracellular matrix (ECM) components to promote and control nerve outgrowth in the damaged CNS. Although previous studies have described and determined a causal link between ECM and neuron integrin regulation, more research needs to be done to develop a fundamental understanding of the molecular-level details that govern neurite outgrowth.

In order to investigate the interaction of neuron integrin receptor and ligands, specific and careful preparation and characterization of the biomaterial substrates to which the neurons bind is an essential first step. The surfaces were covalently attached with ECM proteins (e.g., fibronectin (FN), laminin (LN)) and protein derived peptides (e.g., RGD-derived peptides) via a heterobifunctional crosslinker (GMBS) method. Various surface analytical techniques such as contact angle measurement, X-ray photoelectron spectroscopy (XPS), and time-of-flight secondary ion mass spectrometry (TOF-SIMS) were used for the characterization of the surfaces at each step of the chemistry. After the confirmation of successful ligand attachment, the modified surfaces were then plated with postnatal day 1 (P1) Sprague-Dawley rat dorsal root ganglia (DRG) neurons and incubated in serum-free medium. Both the protein- and peptide-modified substrates supported significantly greater neurite outgrowth than control. Mean neurite outgrowth tests showed that P1 neuron outgrowth was significantly greater (~25%) on the whole-protein FN surfaces than on the RGD surfaces under the same conditions (Fig.1).

In order to spatially control cell adhesion and to direct outgrowth on the surface, single-protein patterned surfaces were created using the protein of interest and a comb polymer because of the latter's incorporation of short poly ethylene glycol (PEG) side chains for excellent protein and cell resistance. Surface-sensitive analytical techniques (atomic force microscope (AFM) and TOF-SIMS) confirmed the successful micron-scale extracellular matrix protein (FN, LN)-patterned areas prepared by micro-contact printing of comb polymer. P1 DRG neurons only attached and extended along protein (FN or LN)-immobilized stripes, avoiding the comb polymer regions. Results also showed that FN surface regions similar to or wider than the size of the cell body ~ 30 - 40 μm were necessary for successful neuron attachment and outgrowth (Fig. 2).

In order to investigate the role of ligand-receptor affinity on neuron outgrowth, ligand-receptor bond-rupture forces were studied by atomic force microscopy (AFM) on the molecular level. The first-ever direct AFM force measurements were conducted on the membranes of living neurons under physiological conditions, with the protein fibronectin (FN) or linear GRGDSY peptide covalently attached to the AFM tips. A statistical analysis method, making use of the properties of the Poisson distribution, was successfully applied to the neuronal model systems (Fig. 3). The magnitude of the interactions between the protein FN and integrin receptors on the neuron membrane was found to be 106 ± 8 pN (Fig.4) and this value is in good agreement with that reported by Li et al [1] in analyzing the K562 cell line that expresses only the $\alpha_5\beta_1$ receptor. Similar measurement of the interactions between linear GRGDSY and

receptors resulted in a magnitude of 170 ± 10 pN. The result that a significantly higher affinity was observed for attached linear GRGDSY peptide and its integrin receptor relative to the protein FN indicates that the RGD binding site may be presented in a more conformationally favorable context in the surface-attached GRGDSY peptide than in the attached protein FN. Neuron migration and outgrowth on biomaterial surfaces involves the formation of new attachments at the growth cone front and the breakage of attachments at the rear. The higher binding affinity between the neurite's receptors and the GRGDSY makes the formation/breakage process harder for this system when compared to the FN system.

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References

[1] F. Li et al, *Biophys. J.* 84 (2003) 1252.
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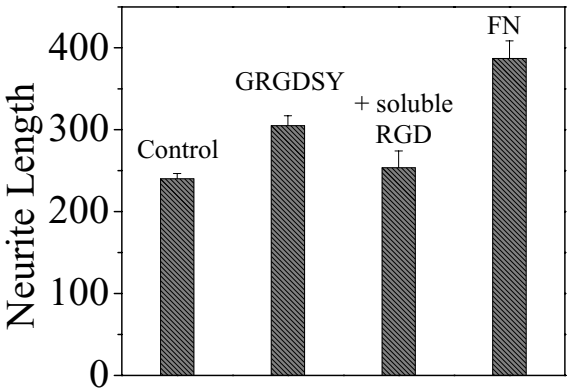

Fig. 1. P1 DRG neuron outgrowth results after 24 h in serum-free medium on various surfaces: GMBS control surface, GRGDSY-modified surface, GRGDSY-modified surfaces with competing soluble GRGDSY peptide present.

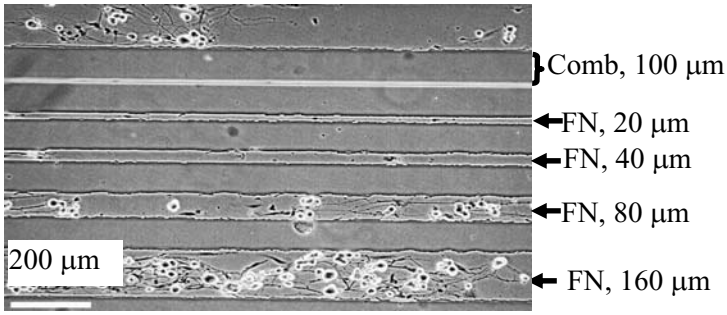

Fig. 2. Neurite outgrowth on a FN/comb polymer patterned substrate as observed by phase-contrast optical microscopy. The pattern was designed with a 5-line repeating master. FN stripes of different widths (10 μm, 20 μm, 40 μm, 80 μm, 160 μm) were separated by a 100-μm comb polymer stripe.

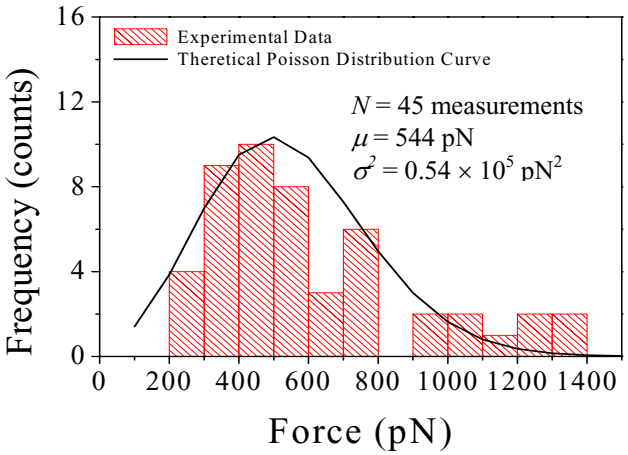

Fig. 3. Histogram of AFM bond-rupture forces from one set of 45 measurements between a FN-modified AFM tip and the growth cone of a living neuron. The data for this system fits a Poisson distribution curve $P_p(x) = e^{-\mu}\mu^x / x!$ reasonably well, where $P_p(x)$ is the probability of observing x events, μ is the mean of the expected number of events.

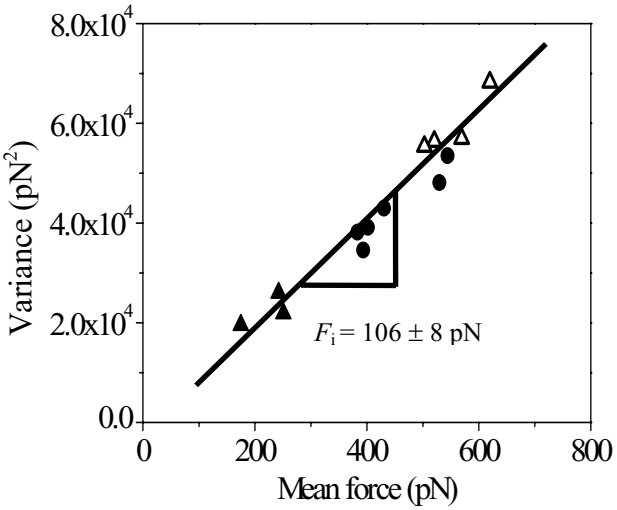

Fig. 4. Plots of force variance σ_m^2 vs. mean μ_m for the FN-integrin receptor system acquired under physiological conditions for growing neurons. Each data point represents a data set obtained from a different neuron growth cone. Different symbols depict a set of measurements obtained by a different AFM tip.

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