Nanomolecular Interactions of Novel Biological and Synthetic Polyelectrolyte Brushes

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The overall goal of this research program is to use powerful new nanoscale experimental and theoretical methodologies to develop a fundamental understanding of novel, important polyelectrolyte (PE) brush and brush-like systems. Our interdisciplinary research team includes 4 PI's representing 5 different academic departments at 3 universities, including **Grodzinsky** (MIT - tissue and cell structure, biomechanics, electrical, mechanical, and biologicalengineering, electrostatic double layer continuum theory), **Ortiz** (MIT - nanomechanics, surface science, polymer mechanics, materials science and engineering), **Genzer** (NCState - surface science, polymer physics and chemistry, directed self-assembly, chemical engineering), and **Szleifer** (Purdue - theoretical physical chemistry including statistical mechanics of complex fluids, computer simulations, and polymer structure and dynamics).

AIM I: Nanomechanics and Nanostructures of Connective Tissue Polyelectrolytes: In Dean et al. [2], micro-contact printing was used to produce patterned surfaces of densely packed, chemically end-grafted cartilage aggrecan proteoglycans (composed of a core protein to which are attached highly negatively charged glycosamino-glycan (GAG) chains) surrounded by a neutral hydroxyl-terminated self-assembled monolayer. Under fully hydrated conditions, aggrecan height and compressibility were measured in NaCl electrolyte solutions 0.001-1M (pH = 3-7), as well as normal compressive force (0-40 nN) using contact mode AFM.

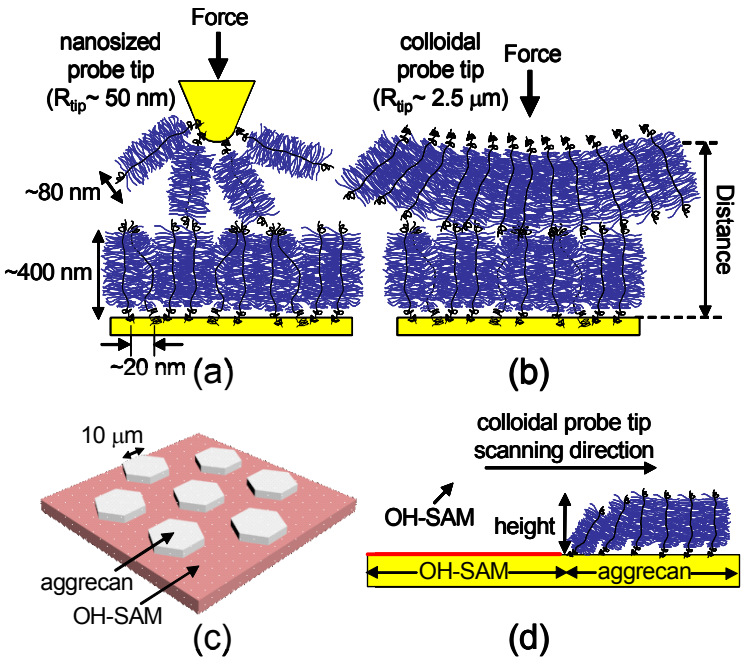

Fig. 1. (a) Nanosized and (b) micron-sized probe tips with chemically end-grafted aggrecan brushes (core protein spaced ~ 20 nm apart, CS-GAG side chains ~2-4 nm apart) interacting normally with aggrecan on planar substrates. (c) μ-contact printed sample with aggrecan inside a hexagonal pattern. (d) Colloidal probe tip functionalized with OH-terminated SAM imaging the height of a chemically end-grafted aggrecan layer on a planar substrate during contact mode AFM.

In Dean et al. [3], aggrecan molecules from fetal bovine epiphyseal cartilage were chemically end-grafted at physiologically relevant densities (~ 45 mg/ml) to planar substrates, standard nanosized atomic force microscopy (AFM) probe tips (end radius R_{tip} ~50 nm), and larger colloidal probe tips (R_{tip} ~ 2.5 μm). To assess normal nanomechanical interaction forces between opposing aggrecan layers, substrates with micro-contact printed aggrecan were imaged using contact mode AFM, and aggrecan layer height was measured as a function of solution ionic strength and applied normal load (Fig. 1).

Then, using force spectroscopy, nanoscale compressive forces between opposing aggrecan on tip and substrate were measured versus tip-substrate separation in 0.001M-1M NaCl. Nanosized tips enabled measurement of molecular stiffness of 2-4 aggrecan while colloidal tips probed the stiffness of larger assemblies (~10^4 molecules).

In Han, et al. [4], lateral force microscopy in conjunction with micro-contact printing was employed to measure the lateral deformation mechanisms and proportionality constants between normal and lateral force of chemically end-grafted aggrecan layers as a function of scan rate, solution ionic strength, and loading history. Normal adhesion force measurements of functionalized aggrecan surfaces were also conducted. We recently examined the temporal evolution of the nanostructural and mechanical properties of individual cartilage chondrocytes and their newly synthesized pericellular matrix (PCM) with time in culture (Fig. 2) [5]. Our objectives were (1) to visualize the synthesis of extracellular matrix (ECM) molecules from individual chondrocytes using atomic force microscopy, and (2) to compare the kinetics of the production of the pericellular (PCM) matrix with the temporal evolution of the nanomechanical properties of cell and PCM with increasing time in culture via the technique of nanoindentation. A novel *in vitro* experimental design incorporated the development of etched silicon wells to immobilize the cells, enable visualization of *de novo* synthesis of PCM, and measure nanomechanical properties of resident cells (Fig. 2). Chondrocytes freshly extracted from bovine calf knee cartilage were cultured in alginate gel beads. Cell with their newly synthsized PCM were removed from alginate culture at selected times from 1-4 weeks; a portion of the cells was used for quantitative analysis of the total content in the PCM of sulfated GAGs and collagen. The results showed that cells cultured in the combination of insulin-like growth factor-1 (IGF-1) and osteogenic protein -1 (OP-1) synthesized a PCM that became stuffer than the freshly extracted cells by 28 days in culture, and significantly stiffer than the PCM of cells cultured in standard serum supplemented (10% FBS) medium.

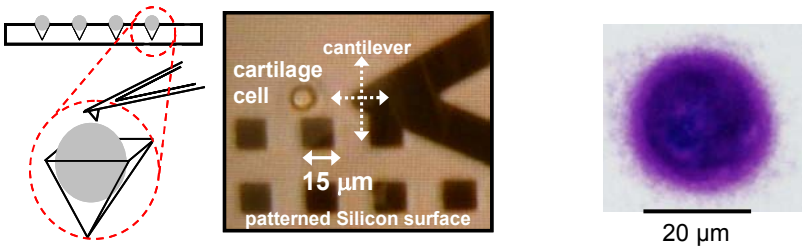

Fig. 2. (*Left*) Schematic of individual cell in silicon etched pyramidal well; (center) optical microscope of an individual cell on top of patterned silicon surface and AFM cantilever; (right) cell removed from alginate culture on day 21 and stained with toluidine blue for sulfated GAG in the PCM.

AIM II: Synthesis and Nanoscale Interactions of Synthetic PE Brush Gradients: About 4 years ago, the Genzer research group initiated a program geared towards developing multivariate grafted polymer substrates with two overarching goals: (1) creation of novel assemblies with a continuous and systematic variation of the above-mentioned parameters of grafted polymers, and (2) utilization of these combinatorial substrates to understand the behavior of grafted polymers and their interaction with foreign materials. To this end, we created substrates having gradients in MW and composition of grafted polymers using surface-initiated controlled radical polymerization. Successful application of one-property gradient in providing insight into the complex behavior of anchored polymers prompted us to construct the second generation of combinatorial grafted polymer substrate wherein effect of two independent system parameters can be simultaneously studied. For example, one can vary MW along one direction of the substrate and σ along the other direction, thus generating a (MW,σ) matrix on the substrate.

Such substrates are called orthogonal gradients wherein two material properties vary continuously along two mutually perpendicular substrate directions. Since these two properties are changing continuously, every possible combination of the two properties can be probed systematically by using such a set-up. Working with orthogonal gradient substrates not only saves time and resources but it also minimizes the systematic errors associated with carrying out a large set of individual experiments. Additionally, concurrent variation of the two properties facilitates investigation of their cooperative effect on a given phenomenon. During the last year, new methodologies were developed leading to the formation of two different orthogonal gradient motifs, involving: (1) molecular weight and grafting density (MW/σ) gradients of a given polymer, and (2) molecular weight gradients (MW1/MW2) of two different polymers. In addition, it was demonstrated how these orthogonal structures can be utilized to map out systematically: (1) protein adsorption and cell proliferation, and (2) chain conformations of diblock copolymers exposed to selective solvents.

AIM III: Multi-Scale Polyelectrolyte Theory: The Szleifer group has been concentrating on the development and application of molecular theoretical approaches that enable the systematic study of the properties of tethered polymer layers. For the purpose of the current project the aim is to study the behavior of weak and strong polyelectrolytes tethered to surfaces of varying geometries. The study of aggrecans requires the understanding of weak polyelectrolytes (polysaccharides, GAG) end tethered to a linear and flexible backbone. On the other hand, the development of synthetic polyelectrolytes with controlled properties, such as the ones studied in the Genzer group, require fundamental understanding of end-grafted polyelectrolytes on planar surfaces. To fulfill both types of systems, theoretical molecular theories have been developed that enable: (1) studies of the behavior and interactions between polymers end-tethered into linear (cylinder with nanoscopic radius) backbones and (2) studies of the behavior of weak and strong polyelectrolytes grafted on planar surfaces. Recent resuls have focused on models of aggrecan-aggrecan interactions layered end grafted to a cylinder (Fig. 3). Of particular interest are the effects of polymer charge and surface geometry on the interactions.

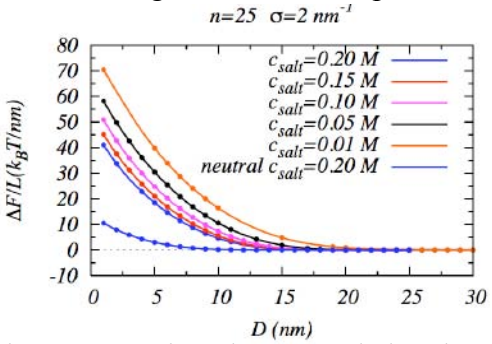

Fig. 3. Interactions between polyelectrolytes layers end-grafted to a cylinder, as a model for aggrecan-aggrecan interactions: effect of polymer surface density and salt concentration

[1] For further information about this project link to **http://stellar.mit.edu/S/project/nsf-nirt/materials.html** or email **<alg@mit.edu, cortiz@mit.edu, Jan_Genzer@ncsu.edu, igal@purdue.edu >**

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