PREPARATION OF END-GRAFTED POLYELECTROLYTE BRUSHES ON NANOSCALE PROBE TIPS USING AN ELECTRIC FIELD

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The atomic force microscope (AFM) and related high-resolution force spectroscopy (HRFS) instruments have become fundamental tools for studying molecular, colloidal, and surface forces in physiological fluid environments down to the pN range.1 A key AFM component is the soft microfabricated cantilever force transducer with a fine probe tip (with typical end radii, R_{tip}<5-60 nm) that deflects when interacting with a sample surface. Standard Si_{3}N_{4} probe tips have been used for studying the nanomechanical properties of macromolecules on surfaces2 and probe tips functionalized with proteins, ligands and receptors, cells, nanotubes, and self-assembling monolayers (SAMs) have enabled studies of biologically relevant intermolecular interactions.3 Tip functionalization has been achieved by covalent immobilization, nonpecific physiosorption, and conventional adhesives for larger particles (= 1 pm).4 However, attachment of macromolecules to the apex of a probe tip with a prespecified orientation, conformation and density is difficult due to the small surface areas involved and, for polyelectrolytes, the presence of fixed charge groups. Functionalization and subsequent characterization of parameters such as the chain grafting density (Γ, chains/nm²) in the vicinity of the tip apex are critical to the interpretation of HRFS data relating interaction force to tip-sample separation distance.

Our recent studies of the biological polyelectrolyte molecules in cartilage,5 chondroitin sulfate glycosaminoglycans (CS-GAGs), called for new methods of attachment to nanoscale probe tips in order to out carry HRFS experiments between two opposing, end-grafted polymer brushes which more closely mimic the physiological conformations found in native tissue. Hence, CS-GAG chains were chemically end-grafted to an Au-coated Si_{3}N_{4} probe tip by means of an electric field applied between the tip and a nearby Pt electrode. Previously, an electric field had been used to attract charged DNA oligonucleotides to an underlying monolayer of single-stranded DNA that had been immobilized to a Au coated sensor surface through a Au-thiol attachment and a 300 mV potential between the sensor and a Pt electrode was found to enhance hybridization of the DNA oligonucleotides to the DNA monolayer.5 We extended this to use an electric field to drive CS-GAGs to a nanosized probe tip, thus increasing the local polyelectrolyte concentration in the vicinity of the probe tip and chemisorption via an end-terminal functionality.

CS-GAG, a negatively charged, linear polysaccharide, is a major component of the extracellular matrix of cartilage, a tissue specialized to resist compressive loads in articulating joints. Each repeating disaccharide of CS-GAG has one carboxylic acid and one sulfate group spaced 0.64 nm apart, both ionized under physiological conditions (pH7.4, IS=0.15M). Intramolecular electrostatic repulsion between neighboring charge groups, as well as intermolecular repulsion between GAGs due to close packing (~4 nm separation distance), cause the GAGs to assume an extended, rod-like conformation (contour length, 35-45 nm) in vivo.7

![Figure 1. (a) End-grafting CS-GAG to a nanosized probe tip using an electric field, (b) 2-D map of E-field lines between tip and electrode; arrow length and direction correspond to E-field magnitude and direction, and (c) Current vs. voltage across tip in 0.1M NaCl, pH=5.6 aqueous solution](image)

We recently used HRFS to quantify long-range, repulsive nanoscale interaction forces between a CS-GAG end-grafted brush on a planar surface and probe tips functionalized with negatively charged (SO$_3^-$) or neutral (−OH) terminated monolayers in aqueous solutions of varying pH and ionic strength (IS).5 The measured stress was ~0.25 MPa, which compared well to macroscopic swelling pressures of cartilage.4 Comparison to electrostatic Poisson-Boltzmann (P-B) based theoretical models has shown that nonuniform charge distributions, and thus, lateral intermolecular electrostatic interactions are important to interpret nanomechanical behavior CS-GAGs.5 However, the interaction between two opposing CS-GAG brushes would more accurately mimic the molecular structure and deformation conditions responsible for the compressive stiffness of cartilage in vivo and shed new light on the nanoscale mechanisms of deformation, i.e. interdigitation versus compression of polymer chains, thus motivating the need to end-graft CS-GAGs to probe tips as well.

Methods described previously1 for chemically end-grafting mono(thiol)-terminated CS-GAGs to Au-coated Si planar surfaces were adapted for grafting CS-GAGs to Au-coated Si_{3}N_{4} probe tips (R_{tip} ~50 nm) at the end of a soft cantilever (Thermomicroscopes, Inc, V-shaped, spring constant = 0.01 N/m). “Passive functionalization” involved immersing Au-coated probe tips into 1 mg/ml phosphate buffer solution (PBS, pH = 7.4, IS = 0.17M) containing mono(thiol)-terminated CS-4-GAG for 9 hrs. After reaction, probe tips were immersed in 5 mM 11-mercaptopoundecanol, HS(CH$_2$)$_9$OH ethanol solutions (P. Laibinis, MIT) for 15 min to “backfill” the unreacted portions of the surface. “Active functionalization” involved applying an electric field for 9 hrs between the probe tip and a Pt electrode immersed...
in 1 mg/ml PBS solution containing 100 µg of CS-GAG in the closed liquid cell of an AFM (Multimode IIIA, Digital Instruments, Santa Barbara, CA). The probe tip was grounded and a negative voltage (-0.15 V) was applied to the Pt cathode via a cap on the piezoelectric scanner (Figure 1a). The separation distance between the probe tip and the Pt electrode was 100 µm. The sharp probe tip geometry resulted in a 10-fold higher E-field strength near the tip apex (~3000 V/cm) compared to 300 V/cm at the Pt surface below (Figure 1b, calculated using QuickField finite element solver, DK-5700 Svendborg, Denmark). Figure 1c is the current-voltage curve for the tip-Pt system which shows that at the low voltage used here (~0.15 V), the resulting small, non-Faradaic current (< 200 nA) minimizes chemical reactions at the probe tip that could lead to electrolysis or disrupt the CS-GAG end-grafting chemistry. The actively modified probe tip was backfilled using the same procedure as for the passive functionalization method. Planar OH-terminated SAM substrates were prepared using Si (100) wafers (Recticon Enterprises, Inc., Pottstown, PA) coated with a 2 nm-thick Cr layer followed by a 30 nm-thick Au layer, and then immersed in a 1 mM solution of 1-mercaptoundecanol for 24 hrs. HRFS measurements were performed using a Molecular Force Probe (MFP) (Asylum Research, Santa Barbara, CA) to measure force versus tip-substrate separation distance, D, at a constant z-piezo displacement rate of 1 µm/s at 23°C in NaCl aqueous solutions. A full description of this instrument, its limit of force and displacement detection in fluids (±5 pN using the present cantilever, ~3 Å respectively), and details of measurement errors, were given previously. HRFS data presented are averaged curves of ~10-15 individual experiments, on approach of the probe tip normal to the sample surface, at different sample locations and have a standard deviation of < 20 pN.

Figure 2a compares averaged Force/Radius vs. Distance profiles on approach for the actively and passively functionalized CS-GAG probe tips vs. a OH-terminated SAM planar substrate in 0.1 M NaCl, pH 5.6. Both probe tips showed a nonlinear, purely repulsive interaction force for D < 40 nm with no attractive jump-to-contact. The electrically functionalized probe tip produced a significantly higher force over a longer distance range, suggesting the presence of a higher density of charged GAGs. Since the electrical Debye length, \( \kappa^{-1} = 1 \text{ nm at } 0.1 \text{M IS, surface} \), surface anion adsorption to the probe tip, alone, could not account for the long distance ranges of the forces, which would begin at ~5\(\kappa^{-1}\). In order to estimate the grafting density obtained by electrical functionalization, the data of Figure 2a were compared to the predictions of a P-B based charged rod model (solid curves) for electrostatic double layer repulsion force between a neutral planar substrate and a hemisphere functionalized with a CS-GAG brush. The CS-GAGs were represented by cylindrical rods of finite height and uniform fixed volume charge density. The parameters that were fixed in the model included the polyelectrolyte brush height (\( h = 45 \text{ nm, the distance at which the force increased above the noise minus } 5\kappa^{-1} \)), the rod radius \( r = 2 \text{ nm, based on our previous study using a functionalized planar substrate} \), the known charge per CS-GAG chain (\( Q = -8.0 \times 10^{-18} \text{ C} \), and the hemisphere radius (\( R_{\text{HISP}} = 50 \text{ nm} \)). The only parameter left adjustable was the distance between neighboring CS-GAG chains (\( s \)) and was fit to the HRFS data between D=10-80 nm using the method of least squares. The resulting values, \( r=10 \text{ nm (}\Gamma=0.01 \text{ chains/} \text{nm}^2\text{) for passive and } s=6 \text{ nm (}\Gamma=0.028 \text{ chains/} \text{nm}^2\text{) for the electrical functionalization, showed that the E-field gave a ~3-fold increase in chain grafting density. The grafting density obtained by electrical functionalization also corresponded well with values obtained from previous measurements of metabolically radiolabeled CS-GAGs attached using the same chemical reaction conditions and measured via scintillation counting.

Figure 2b shows the average force/radius and stress vs. distance profiles for the actively functionalized CS-GAG probe tip vs. an OH-terminated SAM planar substrate on approach at various IS and pH=5.6. The repulsive force was observed to decrease in magnitude and range with increasing IS, consistent with the well-known effect of salt screening of the electrostatic double layer forces. In addition, the observed decrease in HRFS-measured force when the pH was decreased from 7 to 3 (Figure 2c, IS = 0.015M) is consistent with the fact that both SO\(_3^\text{-} \) and COO\(^{-}\) groups on the CS-GAG are charged at pH 7, whereas only the SO\(_3^\text{-} \) group remains charged at pH 3 due to the protonation of the COOH group, leading to a 50% reduction in the fixed charge.

To our knowledge, this is the first report of the use of an electric field to enable end-grafting of charged polyelectrolytes to a nanosized probe tip to study intermolecular interactions. The sharp tip geometry greatly enhanced the E-field strength near the tip apex which, in turn, greatly enhanced migration of charged CS-GAGs to the tip while simultaneously allowing a small total voltage drop and (non-Faradaic) total current. The presence of CS-GAG on the AFM tip was verified by HRFS force measurements at varying pH and IS in conjunction with a P-B based theoretical model to estimate \( \Gamma \).

Acknowledgement. The authors thank A. Plaa and S. Wong-Palms for providing CS-GAGs, and P. Laibinis for insightful advice. Supported by the Dupont-MIT Alliance, NIH Grant AR45779, and a Whitaker Foundation graduate fellowship (DD).

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