Evaluating Adhesion Strength of Biological Molecules to Nanofabricated Substrates

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ABSTRACT

Our research efforts are aimed at developing a new class of nanoscale mechanical devices powered by biomolecular motors. Currently, advanced techniques are being employed for creating nanofabricated substrates that provide precise positioning and orientation of the biomolecular motors. High-velocity laminar flow tests were used to evaluate the chemical bonding strength of His-tagged microspheres to gold, copper, and nickel substrates. All three substrates served as efficient media for binding His-tagged microspheres. Chemical bonding strength, however, increased from gold, to unoxidized copper, to nickel. Because of its superior bonding strength and routine application in nanofabrication, nickel is the best suited for further development of biomolecular motor-powered, nanomechanical systems.

Keywords: NEMS, F_1-ATPase, nanotechnology

INTRODUCTION

A motor can be defined as a device that harnesses input energy to perform work. In the realm of biology, motors such as kinesin and F_1-ATPase serve a myriad of functions in living organisms, e.g., synthesizing/hydrolyzing ATP, powering bacterial flagella, or contracting muscle fibers [1, 2]. While the general properties of such biomolecular motors have been extensively studied, further examinations of their potential use in hybrid organic/inorganic devices can offer insight on new methods of manipulating biological systems. The advent of advanced nanotechnology, as well as the latest nanofabrication techniques, offers the ability to achieve the integration of biomotors with nanometer scale devices.

The creation of hybrid organic/inorganic devices permits the utilization of the optimal attributes of both the living and non-living world. However, platforms for the hybridization of biomolecular motors and nanomechanical devices must first be established. These prerequisite technologies include (i) the production of a recombinant biomolecular motor that offers genetic and biochemical flexibility, (ii) precise positioning and orientation of biomolecular motors within hybrid devices, (iii) attachment of inorganic devices for the generation of useful work, and (iv) methods for evaluating the physical and engineering characteristics of biomolecular motors.

Current research efforts in our laboratory are focused on the creation and evaluation of hybrid nano-electro-mechanical devices (NEMS) powered by the biomolecular motor, F_1-ATPase [2]. Objectives that have been achieved to date include (i) the large-scale production of a thermostable F_1-ATPase from *Bacillus* PS3, (ii) evaluation of the recombinant F_1-ATPase performance as a biomolecular motor, and (iii) the chemical attachment of inorganic molecules to the γ subunit (the work end) of the F_1-ATPase [2]. The objectives of the following study were to nanofabricate various metallic substrates for attachment of biological molecules, and evaluate the chemical bonding strength of His-tagged microspheres on each substrate. The use of nanofabricated metallic substrate will provide a means for the specific attachment of biomolecular motors on a surface with precise positioning, spacing, and orientation.

MATERIALS AND METHODS

Substrate Preparation

Evaporative deposition was used to coat glass coverslips (24 x 60 mm) with 200 Å of gold (Au), copper (Cu), or nickel (Ni) for the bonding substrates. In order to prevent rapid oxidation of the copper substrate, coated coverslips were stored under vacuum prior to experimentation. Several glass coverslips coated with 500-µl of 1% nitrocellulose in amyl acetate (Sigma Chemical Co., St. Louis, MO) were used to establish a baseline for comparing the bonding strengths among the metallic substrates.

Microsphere Attachment

To evaluate the bonding strength of the different substrates, a synthetic peptide containing a 6x His-tag (NH_3-Gly-Gly-Lys-Gly-Gly-Lys-Gly-Gly-His-His-His-His-His-His-CO_2H) was covalently coupled to carboxylate-modified 2- and 10-µm fluorescent microspheres (Molecular Probes, Eugene; or Polyscience, Inc., Warrington, PA) using a water-soluble carbodiimide. A 50-µl aliquot of His-tagged microspheres was placed at the center of the slide, and incubated for 5 minutes at room

temperature. Unattached microspheres were removed using a series of washes.

Adhesion Strength Assessment

A custom fabricated flow-cell consisting of fluid input/output and vacuum ports was used to mount the substrates, and apply a variable fluidic flow across the surface. Vacuum was applied to secure the substrate onto the flow-cell. Microspheres were observed at 50X magnification using a stereomicroscope; images were captured with an attached Photometrics CCD camera.

A direct-drive infusion pump provided laminar fluid flow, and microsphere removal was observed at each flow rate. The flow rate was controlled using the infusion pump equipped with a 60-ml syringe filled with deionized water. Time of application and the volume of water leaving the flow cell were recorded in order to determine flow rates. This provided the necessary range of flow rates to generate a broad distribution of data points, and determine the point of adhesion force disruption for different substrates. Experiments were repeated for each substrate type with slight variations in flow rates between trials.

To calcukatw the force required to disrupt microsphere adhesion, the number of microspheres within the field of view was determined for each timed interval (1 to 5 minutes) and flow rate (0 to 52 ml/min). Navier-Stokes equations representing three dimensional flow through rectangular ducts was utilized to estimate the velocity of flow at 5-μm above the substrate [3]:

$$u_z = (u_c + u_p)_z \qquad (1)$$

NOTE: u_c and u_p are the respective complementary and particular parts of the velocity equation in the z (vertical)-direction.

Because laminar flow of water was applied to the flow cell, the calculated velocity was subsequently transformed to a shear stress using Stoke's Law:

$$F = 6\pi\mu_T (u_c + u_p)_z r \qquad (2)$$

where:

F represents the force in nano-Newtons (nN)
r is the radius of microsphere (5-μm)
μ_T is a linear function relating temperature to kinematic viscosity (μ 1.51-0.025T)

The magnitude of force (shear stress), F_{r66} required to remove approximately 66% of the original number of microspheres appearing in the field of view was determined, and used for relative comparisons among substrates.

RESULTS & DISCUSSION

Gold, unoxidized copper, and nickel served as suitable substrates for attaching His-tagged microspheres. Oxidized copper, did not serve as a suitable substrate (data not shown). Chemical adhesion strength varied according to the individual substrates (Figure 1). At low flow rates (<5-ml/min), microspheres were removed from only the glass, nitrocellulose-, and gold-coated coverslips. Adhesion strength to the metallic substrates increased from gold, to unoxidized copper, to nickel (Figure 2). All three of these metals are suitable media for the specific adhesion of biological molecules, and are compatible with current nanofabrication techniques.

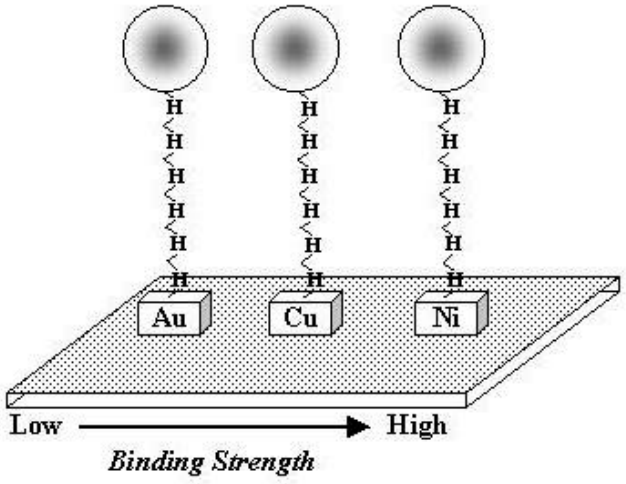

Figure 1: Attachment of His-tagged microspheres to gold (Au), copper (Cu), and nickel (Ni) substrates.

The force required to remove 66% of the microspheres (F_{r66}) from a substrate further demonstrated the differences among the various media (Table 1). The F_{r66} for each substrate was determined by locating the intersection point at 66% removal using Figure 2. Glass and gold possessed relatively similar F_{r66} values (Table 1). The F_{r66} increased from nitrocellulose, to unoxidized copper, to nickel (Table 1). The exact F_{r66} of nickel could not be determined because only a small percentage (<1.32%) were removed at the maximum flow rate attainable with this system. Further tests utilizing greater flow rates are required in order to accurately evaluate the bonding strength of nickel.

Together, these experiments demonstrate a chemical mechanism for attaching biological molecules to nanofabricated substrates. The metals utilized in this study are widely used in nanofabrication, and possess a range of affinity for biological molecules. Using this knowledge in conjunction with standard and novel electron beam lithographic methods [4,5,6,7], proteins can be attached to metallic substrates with precise orientation, positioning, and spacing.

Substrate Type	Calculated F_{r66} (nN)
Glass	53
Nitrocellulose	75
Gold	41
Copper	150
Nickel	>160

Table 1: Shear force (F_{r66}) required to remove 66% of the His-tagged microspheres attached to the various substrates.

Compositional and structural changes of biomolecular motors are often essential for their integration with NEMS devices. Current molecular biological techniques permit the insertion or modification of genetic sequences with relative ease. In our laboratory, we have engineered a biomolecular motor, an F_1-ATPase from a thermophilic bacterium, with specific modifications essential for its incorporation into a hybrid nanomechanical device. His-tags were attached to the coding sequence for the α and β subunits in order to specifically attach the motor protein with precise orientation and spacing [2]. Utilizing electron beam lithography, a patterned array of gold was used to demonstrate the ability of the motor to bind with specific spacing and orientation (Figure 3). These technologies have opened the door for the creation of novel classes of nano-electro-mechanical devices (NEMS) powered by biomolecular motors.

The confluence of molecular biology and nanofabrication provide, for the first time, the ability to create hybrid organic/inorganic NEMS. These devices will provide a means for the seamless integration of NEMS with living systems. Imagine a NEMS device powered by a cell's own energy which is capable of pumping fluids across cell membranes. Further, imagine the biomolecular motors of the same NEMS device being continually replaced by the cell as their function ceases. Only human innovation and imagination limit the application and evolution of such devices.

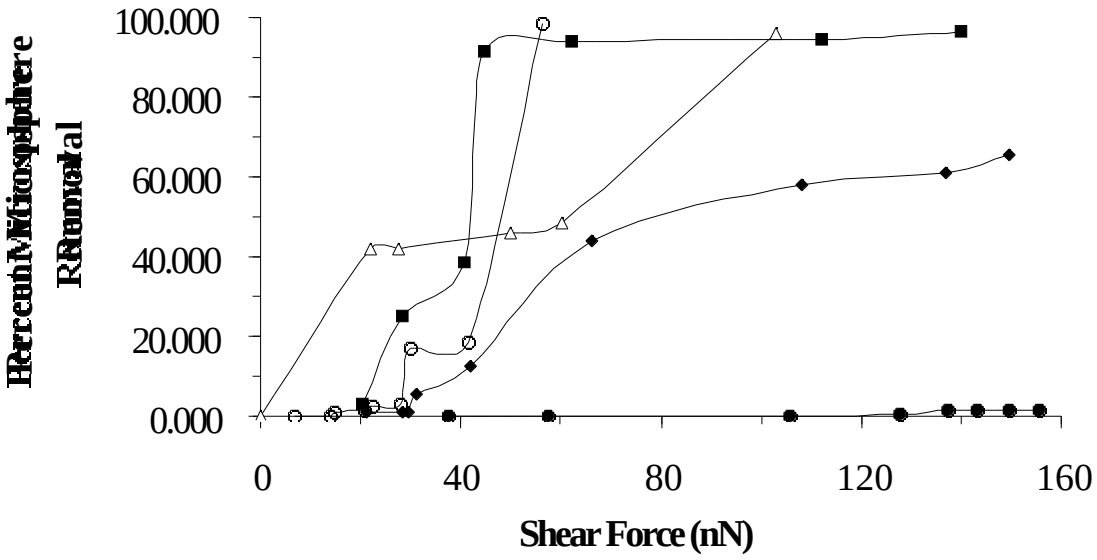

Figure 2: Percent removal of His-tagged microspheres attached to gold (-■-), copper (-◆-), nickel (-ஜ-), nitrocellulose (-❒-), and glass (-ᘺ-) coverslips at various shear forces applied using high velocity laminar flow.

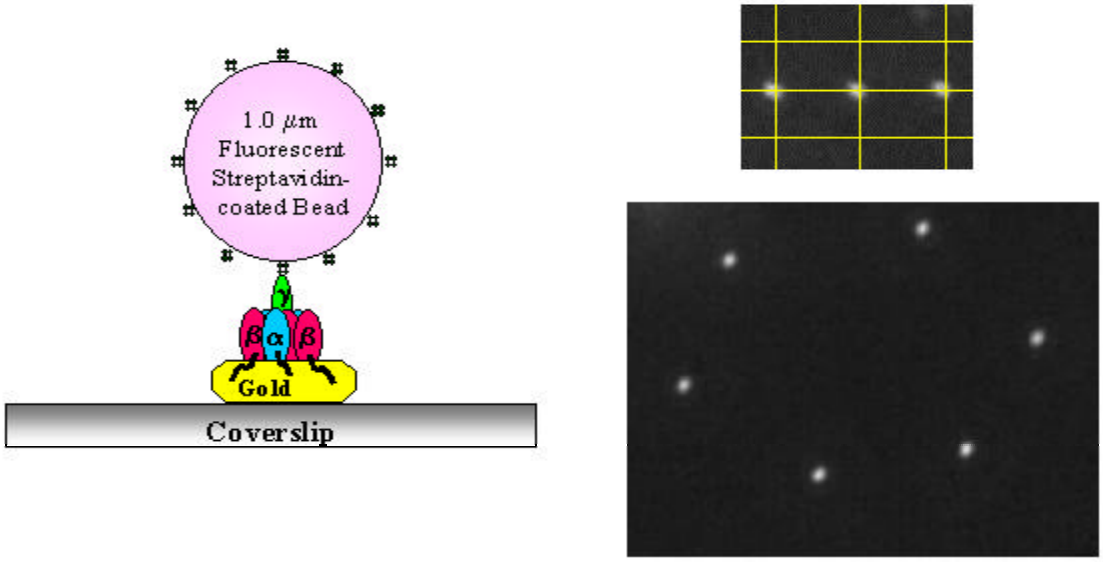

Figure 3: Specific attachment and precise orientation of F_1-ATPase molecules on a patterned array of 30-50 nm gold dots created using electron beam lithography.

CONCLUSION

This study has demonstrated the efficacy of nanofabricated substrates for attachment of biological molecules. All three substrates (i.e., gold, unoxidized copper, and nickel) were suitable for attachment of biomolecules, and are compatible with current nanofabrication techniques. These substrates vary in their adhesion strength of His-tagged microspheres (up to and greater than 160 nN), and offer a choice of media depending upon the specific requirements.

These experiments provide crucial information for the selection of the appropriate substrates for further use in NEMS development. Future areas of research include using nanofabrication technologies to generate patterned arrays of various metals as previously reported [2]. In addition, more complex arrangements, including positioning in the z dimension, also will be explored. Nanofabrication will be used to create a magnetic rotor bar to evaluate the load-dependent performance of the biomolecular motor, F_1-ATPase. Ultimately, such technologies will be used to seamlessly integrate NEMS devices with biomolecular motors to pump fluid, open and close valves on microfluidic devices, and provide mechanical drives for a new class of nanomechanical devices.

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