5-4-2010

Application of Poly(amidoamine) Dendrimers for Use in Bionanomotor Systems

Madhukar Babu Kolli
Marshall University, kolli@marshall.edu

B. Scott Day
Marshall University, day17@marshall.edu

Hideyo Takatsuki
Marshall University

Siva Krishna Nalabotu
Marshall University, nalabotu@marshall.edu

Kevin M. Rice
Marshall University, rice9@marshall.edu

See next page for additional authors

Recommended Citation
Kolli, M. B., Day, B. S., Takatsuki, H., Nalabotu, S. K., Rice, K. M., Kohama, K., ... & Blough, E. R. (2010). Application of poly(amidoamine) dendrimers for use in bionanomotor systems. Langmuir, 26(9), 6079-6082.

Authors
Madhukar Babu Kolli, B. Scott Day, Hideyo Takatsuki, Siva Krishna Nalabotu, Kevin M. Rice, Kazuhiro Kohama, Murali K. Gadde, Anjaiah Katta, and Eric R. Blough

Langmuir
Letter

pubs.acs.org/Langmuir

Application of Poly(amidoamine) Dendrimers for Use in Bionanomotor Systems

Madhukar B. Kolli,[†,‡] B. Scott Day,[§] Hideyo Takatsuki,[†,∥] Siva K. Nalabotu,[†,‡] Kevin M. Rice,[†,∥,⊥] Kazuhiro Kohama,[†,#] Murali K. Gadde,[∥] Sunil K. Kakarla,[†,‡] Anjaiah Katta,[†,‡] and Eric R. Blough*[,†,‡,∥,⊥]

[†]*Cell Differentiation and Development Center,* [‡]*Department of Pharmacology, Physiology and Toxicology, Joan C. Edwards School of Medicine,* [§]*Department of Chemistry,* [∥]*Department of Biological Sciences, and* [⊥]*Department of Exercise Science, Sport and Recreation, College of Education and Human Services, Marshall University, Huntington, West Virginia, and* [#]*Department of Molecular and Cellular Pharmacology, Gunma University, Gunma, Japan*

Received July 2, 2009. Revised Manuscript Received March 18, 2010

The study and utilization of bionanomotors represents a rapid and progressing field of nanobiotechnology. Here, we demonstrate that poly(amidoamine) (PAMAM) dendrimers are capable of supporting heavy meromyosin dependent actin motility of similar quality to that observed using nitrocellulose, and that microcontact printing of PAMAM dendrimers can be exploited to produce tracks of active myosin motors leading to the restricted motion of actin filaments across a patterned surface. These data suggest that the use of dendrimer surfaces will increase the applicability of using protein biomolecular motors for nanotechnological applications.

Introduction

Bionanomotors such as myosin and kinesin efficiently convert the energy stored in adenosine triphosphate (ATP) into mechanical work, leading to suggestions that these molecules could be used in the development of novel nanoscale transport or sensor devices.[1,2] Although recent research efforts have significantly increased our understanding of bionanomotor function, one limitation precluding the advancement of bionanomotor applications is the ability to effectively pattern active bionanomotors in a controlled fashion. Ideally, the bionanomotors should be immobilized onto a substrate at high density with uniform distribution while retaining their enzymatic activity. Thus far, several different approaches have been tried including the construction of physical barriers,[3−6] chemically defined surface patterning,[7−9] and streptavidin−biotin chemistry.[10] Herein, we report a simple, efficient, and versatile method for patterning active myosin bionanomotors onto solid substrates by constructing supramolecular structures composed of dendrimers.

Dendrimers are a unique class of monodisperse, synthetic macromolecules that are of a defined molecular structure and topology.[11] These polymers are synthesized by an iterative sequence of reaction steps that allows tight control over size (i.e., G2 dendrimers are ∼30 Å; G4 dendrimers are ∼45 Å) and composition.[12] These molecules exhibit a three-dimensional hyperbranched structure composed of numerous chain termini that increase exponentially as the generation size increases. Of particular interest is the fact that dendrimers can be easily functionalized with a number of different surface groups.[13] In recent years, these molecules have attracted increasing interest as potential building blocks for use in bottom-up nanotechnology.[11] Indeed, dendrimers have been explored as chemical sensors and cross-linking agents, and have been investigated in the biomedical field for drug delivery,[14] gene therapy,[15] and imaging contrast agent delivery.[16] Here we examine the potential utility of poly(amidoamine)

*To whom correspondence should be addressed. Mailing address: Laboratory of Molecular Physiology, Room 241N, Robert C. Byrd Biotechnology Science Center, 1700 Third Ave., Marshall University, Huntington, WV 25755-1090. E-mail: blough@marshall.edu.

(1) Schliwa, M.; Woehlke, G. Molecular motors. *Nature* **2003**, *422* (6933), 759−765.
(2) van den Heuvel, M. G.; Dekker, C. Motor proteins at work for nanotechnology. *Science* **2007**, *317* (5836), 333−336.
(3) Suzuki, H.; Yamada, A.; Oiwa, K.; Nakayama, H.; Mashiko, S. Control of actin moving trajectory by patterned poly(methylmethacrylate) tracks. *Biophys. J.* **1997**, *72* (5), 1997−2001.
(4) Nicolau, D. V.; Suzuki, H.; Mashiko, S.; Taguchi, T.; Yoshikawa, S. Actin motion on microlithographically functionalized myosin surfaces and tracks. *Biophys. J.* **1999**, *77* (2), 1126−1134.
(5) Byun, K. E.; Kim, M. G.; Chase, P. B.; Hong, S. Selective assembly and guiding of actomyosin using carbon nanotube network monolayer patterns. *Langmuir* **2007**, *23* (19), 9535−9539.
(6) Bunk, R.; Klinth, J.; Montelius, L.; Nicholls, I. A.; Omling, P.; Tagerud, S.; Mansson, A. Actomyosin motility on nanostructured surfaces. *Biochem. Biophys. Res. Commun.* **2003**, *301* (3), 783−788.
(7) Sundberg, M.; Rosengren, J. P.; Bunk, R.; Lindahl, J.; Nicholls, I. A.; Tagerud, S.; Omling, P.; Montelius, L.; Mansson, A. Silanized surfaces for in vitro studies of actomyosin function and nanotechnology applications. *Anal. Biochem.* **2003**, *323* (1), 127−138.
(8) Sundberg, M.; Bunk, R.; Albet-Torres, N.; Kvennefors, A.; Persson, F.; Montelius, L.; Nicholls, I. A.; Ghatnekar-Nilsson, S.; Omling, P.; Tagerud, S.; Mansson, A. Actin filament guidance on a chip: toward high-throughput assays and lab-on-a-chip applications. *Langmuir* **2006**, *22* (17), 7286−7295.
(9) Sundberg, M.; Balaz, M.; Bunk, R.; Rosengren-Holmberg, J. P.; Montelius, L.; Nicholls, I. A.; Omling, P.; Tagerud, S.; Mansson, A. Selective spatial localization of actomyosin motor function by chemical surface patterning. *Langmuir* **2006**, *22* (17), 7302−7312.
(10) Manandhar, P.; Huang, L.; Grubich, J. R.; Hutchinson, J. W.; Chase, P. B.; Hong, S. Highly selective directed assembly of functional actomyosin on Au surfaces. *Langmuir* **2005**, *21* (8), 3213−3216.
(11) Yang, H.; Kao, W.J. Dendrimers for pharmaceutical and biomedical applications. *J. Biomater. Sci., Polym. Ed.* **2006**, *17* (1−2), 3−19.
(12) Newkome, G. R.; Childs, B. J.; Rourk, M. J.; Baker, G. R.; Moorefield, C. N. Dendrimer construction and macromolecular property modification via combinatorial methods. *Biotechnol. Bioeng.* **1998**, *61* (4), 243−253.
(13) Frechet, J. M. Functional polymers and dendrimers: reactivity, molecular architecture, and interfacial energy. *Science* **1994**, *263* (5154), 1710−1715.
(14) Majoros, I. J.; Myc, A.; Thomas, T.; Mehta, C. B.; Baker, J. R., Jr. PAMAM dendrimer-based multifunctional conjugate for cancer therapy: synthesis, characterization, and functionality. *Biomacromolecules* **2006**, *7* (2), 572−579.
(15) Eichman, J. D.; Bielinska, A. U.; Kukowska-Latallo, J. F.; Baker, J. R., Jr. The use of PAMAM dendrimers in the efficient transfer of genetic material into cells. *Pharm. Sci. Technol. Today* **2000**, *3* (7), 232−245.
(16) Kobayashi, H.; Kawamoto, S.; Saga, T.; Sato, N.; Ishimori, T.; Konishi, J.; Ono, K.; Togashi, K.; Brechbiel, M. W. Avidin-dendrimer-(1B4M-Gd)(254): a tumor-targeting therapeutic agent for gadolinium neutron capture therapy of intraperitoneal disseminated tumor which can be monitored by MRI. *Bioconjugate Chem.* **2001**, *12* (4), 587−593.

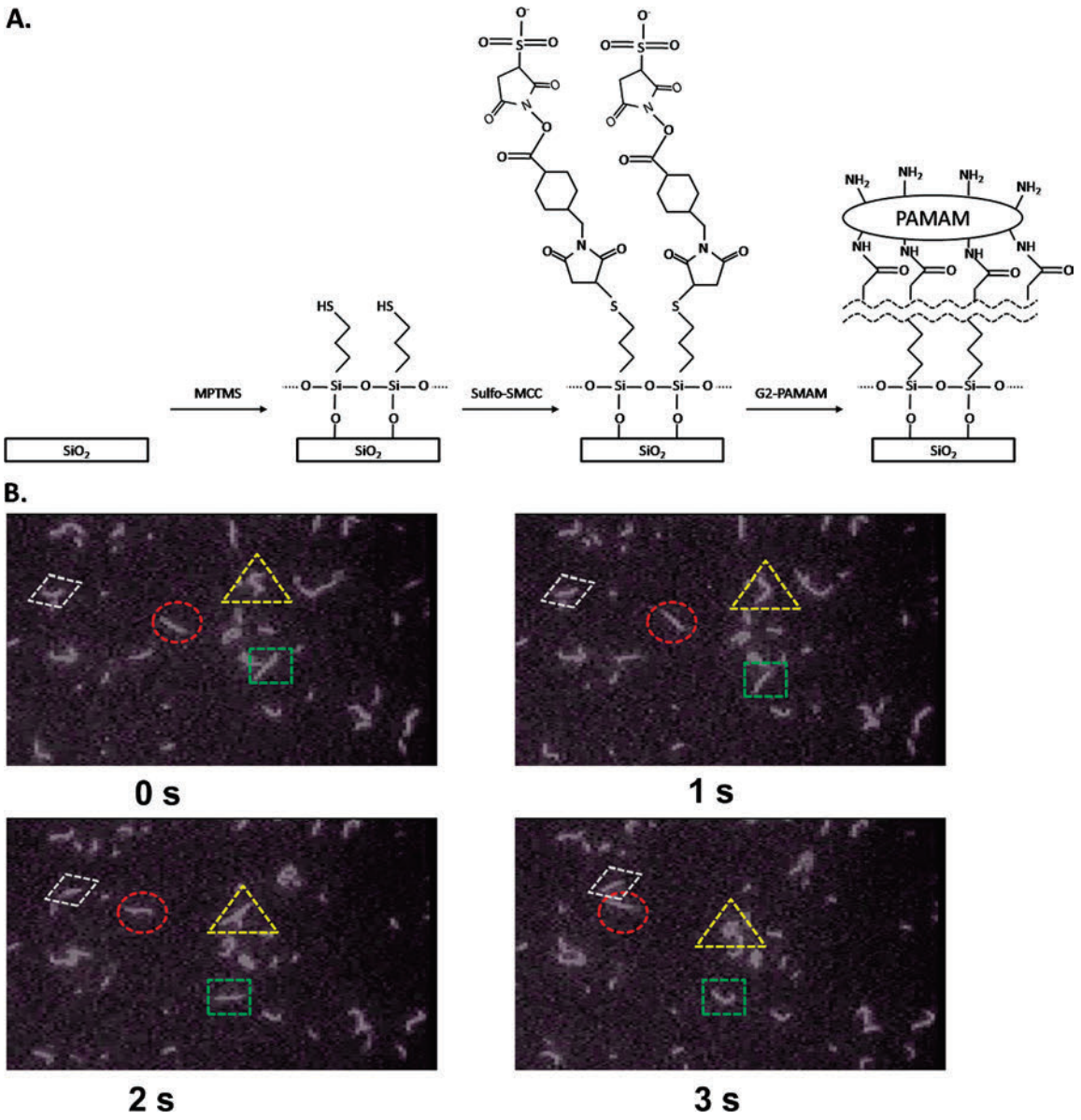

Figure 1. Synthesis and preparation of surfaces coated with PAMAM dendrimers (A). Actin filament movement on nitrocellulose (B), at different time points.

(PAMAM) starburst dendrimers to support myosin activity. Our data suggest that actomyosin motility is of similar quality on dendrimer coated surfaces as that observed using a standard nitrocellulose substrate. In addition, our findings demonstrate that dendrimers can be used to support motility on conductive surfaces such as indium tin oxide (ITO). This fact suggests that these molecules may allow the interface of bionanomotors with electrical devices. Finally, our data reveal that microcontact printing may be used to restrict myosin motor activity to defined locations on the motility surface.

Results and Discussion

PAMAM starburst dendrimers are spherical hyperbranched starlike polymers composed of a core of tertiary amines and primary amines at their surface. Here we demonstrate that PAMAM surfaces are capable of supporting high quality actin−myosin motility and provide evidence that active bionanomotors can be patterned on a PAMAM surface. Taken together, these findings suggest that PAMAM dendrimers may be useful for the future development of novel bionanomotor applications.

Silanization of glass surfaces was achieved by chemical vapor deposition of 3-mercaptopropyl trimethoxysilane (MPTMS) as outlined previously[17] (Figure 1A). Once silanized, the heterobifunctional cross-linker, sulfosuccinimidyl 4-[*N*-maleimidomethyl] cyclohexane-1-carboxylate (sulfo-SMCC), was then used to covalently conjugate the amine terminated dendrimers to the surface. To confirm our ability to functionalize the surface, we next examined the surface hydrophobicity of the preparation by measuring the contact angle of the surface at each step of the functionalization process. Contact angle studies showed that the MPTMS surface was more hydrophobic than bare glass (glass = 17.9° ± 0.2° versus MPTMS = 67.7° ± 0.2°, $P < 0.05$; Figure S1, Supporting Information). Compared to the MPTMS surface, the addition of the sulfo-SMCC decreased surface hydrophobicity slightly (52.2° ± 0.9°, $P < 0.05$). The contact angle of PAMAM coated surfaces was less than that of the MPTMS and nitrocellulose

(17) Le Berre, V.; Trevisiol, E.; Dagkessamanskaia, A.; Sokol, S.; Caminade, A. M.; Majoral, J. P.; Meunier, B.; Francois, J. Dendrimeric coating of glass slides for sensitive DNA microarrays analysis. *Nucleic Acids Res.* **2003**, *31* (16), e88.

(86.2° ± 0.4°; $P < 0.05$) but did not change with PAMAM generation (G-2 = 46.7° ± 1.4° and G-4 = 46.5° ± 2.0°; Figure S1, Supporting Information). To further characterize the surface homogeneity and supramolecular structure, we next examined the surface topography using AFM measurements. As expected, analysis of surface roughness indicated that the PAMAM-macromolecule coated glass was smoother (bare glass = 0.412 ± 0.061; nitrocellulose = 2.138 ± 0.340 nm versus G-2 = 1.170 ± 0.201 nm, and G-4 = 1.015 ± 0.140 nm; $P < 0.05$; Figure S2, Supporting Information) compared to the surface roughness observed for the nitrocellulose coated surfaces. Taken together, these experimental analyses suggest that PAMAM dendrimers form a uniform nanostructured layer on the glass surface.[18]

In an effort to further confirm PAMAM binding to the surface through the sulfo-SMCC linkage, the sulfo-SMCC surface was treated with potassium hydroxide (KOH) prior to PAMAM addition. Potassium hydroxide hydrolyzes the *N*-hydroxysuccinimide (NHS) esters, thereby preventing the covalent conjugation between the amine groups of the PAMAM macromolecules and the substrate.[19] For these experiments, surfaces were treated with KOH and then incubated with dendrimers according to standard procedure. After exposure to dendrimers, the surfaces were incubated with NHS-fluorescein. The absence of functional NHS ester groups following KOH hydrolysis should eliminate PAMAM binding to the surface. The NHS-fluorescein cannot react on the substrate without the primary amines from the dendrimers, and therefore, fluorescence should be limited. As expected, treating the surface with KOH prior to the addition of PAMAM dendrimers greatly diminished the fluorescent signal emanating from the surfaces relative to the signal observed from parallel experiments where the KOH treatment was omitted. The mean pixel intensity for the PAMAM dendrimer coated glass was 49.9 ± 1.2 arbitrary units (AU) compared to 23.4 ± 1.4 AU for the PAMAM dendrimer coated glass that had been treated with KOH. These intensity values are much higher than those for bare glass surfaces (4.8 ± 0.2 AU) and hydrolyzed surfaces not exposed to the dendrimers (4.4 ± 0.3 AU). This confirms that the covalent binding between the cross-linker sulfo-SMCC and the primary amines is necessary for effectively coating the surface with the dendrimers.

To evaluate the role of PAMAM dendrimers on protein adsorption, we exposed bovine serum albumin/fluorescein isothiocyanate (BSA-FITC) to similar surfaces as those in the NHS-fluorescein study. The mean pixel intenisity for PAMAM dendrimer coated glass was 54.9 ± 1.3 AU compared to 21.6 ± 2.0 AU for PAMAM dendrimer coated glass that had been treated with KOH. Surfaces treated with BSA-FITC that had not been exposed to PAMAM dendrimers showed substantially lower fluorescence, 5.3 ± 0.3 AU for bare glass and 4.5 ± 0.1 AU for bare glass hydrolyzed with KOH (Figure S3, Supporting Information). This suggests that protein binding to the surface is mediated, at least in part, by the presence of dendrimers.

Bionanomotor powered filament movement is dependent on both the successful binding of the motor proteins to the surface and the enzyme activity of the bionanomotor. To determine if PAMAM coated substrates are capable of supporting myosin ATPase activity, we measured the relative ATPase activity of heavy meromyosin (HMM) coated PAMAM surfaces to that of a known concentration of HMM on standard nitrocellulose. We observed that the relative ATPase activity of HMM on nitrocellulose and PAMAM dendrimers was similar and that ATPase activity did not appear to change with increasing dendrimer generation (Table 1). These results are consistent with the notion that the amount of functional HMM bound to PAMAM substrates and nitrocellulose coated glass is of similar quantity. To confirm this finding, we next examined the ability of MPTMS, sulfo-SMCC, and PAMAM coated surfaces to support actin−myosin motility. To provide a functional comparison, motility assays on both PAMAM coated surfaces and nitrocellulose were performed under the same conditions and using the same aliquot of HMM. At least three independent motility experiments were performed for each surface preparation. As expected, actin filaments became motile upon the addition of ATP on nitrocellulose coated surfaces (Figure 1B; Table 1). Neither the MPTMS nor the sulfo-SMCC coated surfaces supported actin filament movement; however, PAMAM coated glass surfaces demonstrated a similar average velocity and percentage of motile filaments to that observed for the nitrocellulose surface (Figure S4a, Supporting Information; Table 1). Previous reports have demonstrated a positive correlation between filament sliding velocity and contact angle of the surface.[20,21] Why similar findings were not observed in the current study is unclear but may be related to differences in the methodology of contact angle measurement,[9] HMM preparation, concentration of HMM used, ionic strength of the buffer, method for the quantification of velocity, and differences in the preparation of the surface.[20] In addition to surface hydrophobicity, it is also possible that the degree of negative surface charge could also be correlated to HMM motor function.[21] Future experiments using PAMAM coated surfaces and experimental conditions mimicking previous work will be useful in determining how contact angle, surface charge, and surface preparation affect bionanomtor function.

Table 1. Effect of Motility Surface on Myosin Function

substrate	velocity (μm/s)	percent of motile filaments[a]	HMM ATPase activity[a]
nitrocellulose	2.44 ± 0.06	1.00 ± 0.02	1.00 ± 0.11
G-2 PAMAM	2.41 ± 0.11	0.86 ± 0.05	1.11 ± 0.11
G-4 PAMAM	2.39 ± 0.12	0.86 ± 0.09	0.93 ± 0.04

[a] Data expressed relative to that observed for nitrocellulose coated surface.

To expand upon these findings, we next examined if dendrimers could be used to support myosin powered actin motility on ITO coated substrates. ITO was chosen for its optical transparency and electrical conductivity. These two properties are thought to have wide applicability for use in several types of biomedical and nanotechnological applications. As expected, dendrimer coated ITO surfaces supported actin filament motility of similar speed (1.75 ± 0.05 μm/s) to that observed using a dendrimer coated glass surface (Figure S4b, Supporting Information). The translation of actin filaments on the PAMAM dendrimer surfaces was virtually indistinguishable to that observed on nitrocellulose (Figure 1B). These findings indicate that myosin powered actin filament movement on dendrimer coated surfaces is similar to that observed using standard nitrocellulose surfaces.

In an effort to determine if the unique properties of the PAMAM macromolecules can be exploited in patterning bionanomotor

(18) Degenhart, G. H.; Dordi, B.; Schonherr, H.; Vancso, G. J. Micro- and nanofabrication of robust reactive arrays based on the covalent coupling of dendrimers to activated monolayers. *Langmuir* **2004**, *20* (15), 6216−6224.
(19) Jang, L. S.; Keng, H. K. Modified fabrication process of protein chips using a short-chain self-assembled monolayer. *Biomed. Microdevices* **2008**, *10* (2), 203−211.
(20) Nicolau, D. V.; Solana, G.; Kekic, M.; Fulga, F.; Mahanivong, C.; Wright, J.; Ivanova, E. P.; dos Remedios, C. G. Surface hydrophobicity modulates the operation of actomyosin-based dynamic nanodevices. *Langmuir* **2007**, *23* (21), 10846−10854.
(21) Albet-Torres, N.; O'Mahony, J.; Charlton, C.; Balaz, M.; Lisboa, P.; Aastrup, T.; Mansson, A.; Nicholls, I. A. Mode of heavy meromyosin adsorption and motor function correlated with surface hydrophobicity and charge. *Langmuir* **2007**, *23* (22), 11147−11156.

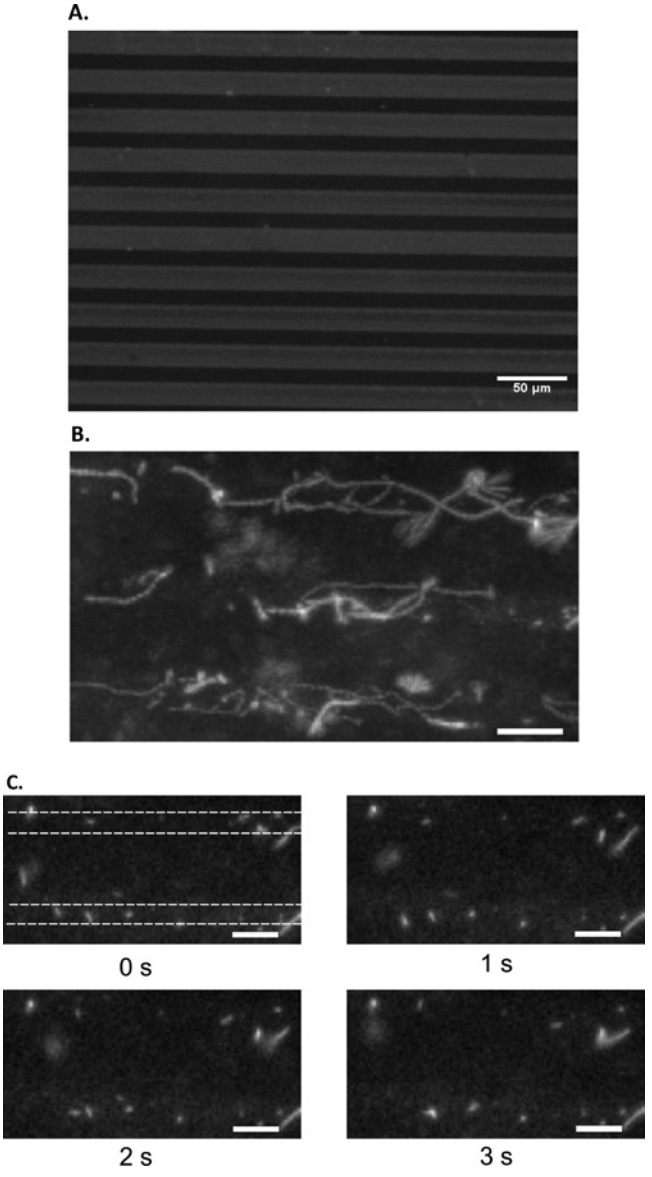

Figure 2. Patterning of PAMAM dendrimers and OEG visualized under fluorescence following incubation with BSA FITC and extensive washing (A). In vitro motility assay using micropatterned PAMAM dendrimers. Sum of 30 flourescence images of rhodamine labeled actin filaments showing filament trajectories (B). Position of actin filaments taken at 1 s intervals as the filaments move across patterned PAMAM lines (C). Scale bar = 5 μm.

motors, we next investigated microcontact printing to form defined patterns of PAMAM molecules on the glass substrate. This method was chosen because of its simplicity and low cost, and because it can be used to generate patterns over a relatively large surface.[22] For these experiments, PAMAM macromolecules were patterned by first transferring them onto sulfo-SMCC coated surfaces using a poly(dimethylsiloxane) (PDMS) stamp, followed by backfilling the nonprinted area with *O*,*O*′-bis(2-aminoethyl)octadecaethylene glycol (OEG). To confirm pattern fabrication, the printed surface was incubated with BSA-FITC. When surfaces of this type were observed under a fluorescence microscope, we found a clearly defined pattern that corresponded to the stamped features (Figure 2A). Similar to previous studies, these observations suggest that PAMAM macromolecules can be effectively patterned by microcontact printing[18] and OEG can be used to prevent the nonspecific adsorption of proteins.[23] To examine if this technique could also be used to pattern myosin, PAMAM molecules were transferred to the substrate, backfilled with OEG, and exposed to a solution of fluorescently labeled HMM (Figure S5, Supporting Information). The observance of a pattern using fluorescently labeled HMM indicates that BSA-FITC is a good analogue for predicting adsorption of HMM on these surfaces. A PAMAM patterned surface was then placed in a flow cell and used as in the standard motility assay. Consistent with our ability to pattern fluorescently labeled HMM on the PAMAM surface, our motility experiments revealed that the microcontact printing of PAMAM macromolecules could also be used to restrict myosin powered actin filament translation to specific spatial regions (Figure 2B and C, Movie S1 in the Supporting Information). Sliding actin filaments were observed only within the channels with no moving actin filaments on the OEG surface, suggesting that functional HMM bound only to the dendrimers or at a low enough density not to support motility on the OEG areas. As expected, moving filaments remained inside the tracks. Filament movement was efficiently redirected (97.4%, $N = 39$) after coming in contact with the OEG area, demonstrating that filament translation across the surface could be mediated by the alternate patterning of dendrimers and OEG. During an observation period of 30 s, a small proportion of filaments were observed to diffuse into solution (5.2%, $N = 39$). These data suggest that PAMAM macromolecules could be of use in developing new and novel uses for bionanomotors.

Acknowledgment. The authors are grateful to Dr. Shinji Yoshiyama, Gunma University for his help in performing the ATPase assay experiments and Dr. Michael Norton and the Molecular Biology Imaging Core facility at Marshall University for the use of their AFM. Funding for this was provided in part by NSF - EPSCOR to Marshall University.

Supporting Information Available: Details regarding the materials and methods, and additional data pertaining to the characterization of the surface and myosin motility. This material is available free of charge via the Internet at http://pubs.acs.org.

(22) Whitesides, G. M.; Ostuni, E.; Takayama, S.; Jiang, X.; Ingber, D. E. Soft lithography in biology and biochemistry. *Annu. Rev. Biomed. Eng.* **2001**, *3*, 335−373.

(23) Rusmini, F.; Zhong, Z.; Feijen, J. Protein immobilization strategies for protein biochips. *Biomacromolecules* **2007**, *8* (6), 1775−1789.

(24) Fujita, K.; Ye, L. H.; Sato, M.; Okagaki, T.; Nagamachi, Y.; Kohama, K. Myosin light chain kinase from skeletal muscle regulates an ATP-dependent interaction between actin and myosin by binding to actin. *Mol. Cell. Biochem.* **1999**, *190* (1−2), 85−90.

(25) Kodama, T.; Fukui, K.; Kometani, K. The initial phosphate burst in ATP hydrolysis by myosin and subfragment-1 as studied by a modified malachite green method for determination of inorganic phosphate. *J. Biochem.* (*Tokyo, Jpn.*) **1986**, *99* (5), 1465−1472.

(26) Samizo, K.; Ishikawa, R.; Nakamura, A.; Kohama, K. A highly sensitive method for measurement of myosin ATPase activity by reversed-phase high-performance liquid chromatography. *Anal. Biochem.* **2001**, *293* (2), 212−215.

(27) Kohama, K. Heterogeneity of amino acid incorporation rate in adult skeletal muscle actin. *J. Biochem.* (*Tokyo, Jpn.*) **1980**, *87* (3), 997−999.

(28) Giang, U. B.; Lee, D.; King, M. R.; DeLouise, L. A. Microfabrication of cavities in polydimethylsiloxane using DRIE silicon molds. *Lab Chip* **2007**, *7* (12), 1660−1662.