Nanofilament Directional Control within a Hybrid Microelectronic Actin-Myosin Motility Assay via Integrated Electric Field Addressing

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ABSTRACT

Control of biomolecular transport is essential to the advancement of nanokinematic systems whether for molecular cargo delivery in sensing or assembly processes, or as a means to interface micro-electro-mechanical systems with the nanoscale regime. Actin-myosin and nanotubule-kinesin systems represent two protein-based systems being explored as basic building blocks for realization of linear and rotary biomolecular motors based on biological nanoscale transport phenomena. While macroscopic electro-motility assays have been documented in the literature, harnessing this biological nanokinematic system to achieve spatially addressable transport of cargo on the micrometer scales of an integrated chip surface is of significant interest. In this work, we undertake a fundamental exploration of the interaction of microscale localized electric fields with the nanoscale actin-myosin motility assay. Electric fields established with arrays of integrated electrode structures under the assayed surface are used to experimentally characterize the effect of these local fields on nanoscale linear biomolecular motor filament alignment, direction of motion, and assay ambient. Fluorescence techniques are used to optically observe actin motion in assay and determine field effects on the actin-myosin system. This paper will describe our current results in this research. These results will contribute to the understanding of the governing electro-mechanics of the actin-myosin molecular transport system that can serve as a framework for the control of nanoscale biomolecular motors from within a microelectronic environment.

Keywords: biomolecular motors, myosin, motility assay, electric field

1. LINEAR BIOMOLECULAR MOTOR SYSTEMS

A major thrust in nanotechnology research has been in the area of nanoscale biosystems, the application of biological materials and processes to effect the advancement of nanoscience. Due to the variety of disciplines involved, this field is beginning to see a merging of medicine and biology with engineering and physics research. There are two main protein-based linear biomolecular motor systems being examined for use in nanoscale biosystems, the microtubule-kinesin system and the actin-myosin system. Both systems operate on the same principle; myosin or kinesin motor heads drive specific polymer filaments made up of protein monomers (actin or tubulin) while utilizing ATP as a chemical energy source (Figure 1 [1]).

Actin monomers have an average length of 5.5 nm and join together to form twisted-rope chains 10 nm in diameter with lengths of 50-200 nm. The filaments associated with kinesin, hollow microtubules, are significantly larger at 25 nm in diameter and up to 100 μm long, usually composed of 13 parallel strands of 8 nm long tubulin monomers. While they both perform the same basic function, there are many variations among kinesin and myosin molecules, including head size, hinge length, and mode of motion.

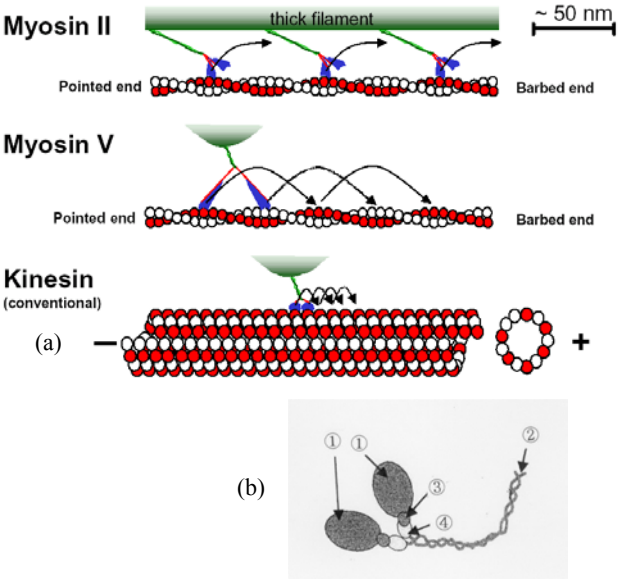

Figure 1: (a) Biomolecular protein motor systems [1]. The black arrows indicate the mode of motion of each type of motor protein. (b) A detailed diagram of one myosin protein: 1-heavy chain peptide motor domain (heads), 2-heavy chain peptide tail, 3-essential light peptide chain, 4-regulatory light peptide chain.

The elements of both types of linear biomolecular motor systems can be isolated, and operation can be examined *in vitro* through the use of a motility assay. The motion of both motor and filament proteins can be observed optically through the use of phosphorescent dyes under 60-100x magnification, with motion lasting until the ATP in the buffer solution is exhausted or the buffer conditions deteriorate due to waste chemical buildup.

Harnessing the motion of biomolecular motors for nanosystems requires actuation rate control, directional control, cargo attachment to filament or motor molecules, and the viability of the proteins in a non-cellular environment.

The direction of filament travel is dictated by filament alignment because motor proteins bind to filaments in a specific orientation. The tail of a motor protein is rotationally flexible, so, even when attached to a substrate, the motor head can rotate to match the alignment of the filament protein and bind to it, defining the direction of motion.

The application of electric fields to biological nanosystems has been explored as a method of controlling the direction and alignment of nanoelements. The alignment of DNA and separation of different cells has been achieved using dielectrophoresis. Traveling wave dielectrophoresis has also been used to transport nanometer-scale latex beads in fluid environments [2]. Large-area electric fields have been used to apply alignment force to inherently negative charged actin filaments in macroscale motility assays, as well as achieve some degree of velocity control [3]. Electric field strengths on the order of 10^3 V/m lead to an electromotive force on filament elements of 2 pN/μm, and micron-scale field strengths of 1 mV/μm. Fields of this strength are sufficient for actin alignment, but are generally too small to affect the direction of travel of larger microtubules.

As these biomolecular transport systems evolve and mature, it is critical that an interface be established between their nanoscale motion and the chip-level microelectronic environment that will enable reconfigurable control of their nanoscale motion via electronic signaling. Building on prior work which has established the macroscale electromotility characteristics of the myosin-actin system [3], the scope of this research encompasses the first fundamental exploration of the interaction of electric fields localized on the micron scale with the nanoscale actin-myosin motility assay. Electric fields established with integrated electrode structures under the assayed surface will be used in order to understand their effect on nanoscale linear biomolecular motor filament alignment, direction of motion, and assay ambient. This research seeks to lay the groundwork for subsequent research of electronically controllable nanoscale cargo delivery systems able to arbitrarily address chip surface locations.

The development of electric field controllable biomolecular motor systems has the potential to be utilized in a wide range of applications. Commercial devices are currently available that are able to electrophoretically move DNA and other biological elements on a self contained chip consisting of an array of micron-scale electrodes by taking advantage of the inherent polarity of the species to be moved [4]. The use of linear biomolecular motor proteins in these systems would play a major role in significantly increasing their capabilities. A long-term application of electric field actin motility is the development of addressable biomotor substrates with which to perform nanoscale manufacturing and assembly.

In anticipation of the inevitable merger of linear biomolecular motor systems and microelectronics, a study of the application of micron-scale electrodes in motility experiments for use as a singular or complementary method of direction control is warranted. The focus of this work is an exploration of the electrokinetics of biological nanosystems that show promise for utilization in nanoscale manipulation and manufacturing processes. The main area of emphasis of this research is a fundamental study of nanoscale motility dynamics of a conventional actin-myosin system under the effect of electric fields localized on the micrometer scale.

The actin-myosin system has been chosen for study due to the small size of the actin filaments (requiring lower electric field strengths to effect alignment and direction) and their inherent flexibility [5]. Previous studies that achieved success in the application of e-fields to align actin filaments utilized large area fields and were limited due to the high temperatures developed during field application, leading to shortened myosin lifetimes [3]. The key goals of this study are twofold: 1.) Development of a combined *in vitro* microelectronics/motility assay testbed with which to observe the effects of micron-scale localized electric fields on actin alignment and direction of motion, and 2.) Examination of myosin before, during, and after e-field application to observe and understand the effects of the localized fields on the motor proteins as well as the chemical cell ambient which fuels and carries away the byproducts of protein motor action.

2. THE ACTIN-MYOSIN SYSTEM AND MOTILITY ASSAY PROCEDURE

Skeletal muscle myosin was extracted from back muscle of a rabbit. Heavy meromyosin (HMM) was prepared by digestion of myosin with chymotrypsin. Actin was purified from actin-acetone powder and F-actin was labeled with rhodamine-phalloidin.

The motility assay aspect of the testbed mirrors standardized procedures developed during more than a decade of protein motility research by the medical and pharmacological community [5]. The hardware used to perform motility assays includes several pieces of standard laboratory equipment. A flow cell consisted of a glass coverslip ($18 \times 18 \times 0.15$ mm^3) coated with three different adsorption layer of 0.2% collodion (nitrocellulose) only, PMMA only or 0.2% collodion on silicon dioxide (SiO_2) as

well as a microscopic glass slide (76 × 26 × 1 mm^3) and tapes at 0.09 mm in thickness. It was filled with 70 µg/ml HMM in assay buffer (KCl 25, $MgCl_2$ 2.0, $CaCl_2$ 0.20 and imidazole 25 in mM at pH 7.0) for 5 min followed by 1 mg/ml BSA for 5 min. After washing with the buffer, the flow cell was exchanged for 0.25 µg/ml labeled F-actin-containing buffer together with 14 mM 2-mercaptoethanol, 4.5 mg/ml glucose, 0.22 mg/ml glucose oxidase and 0.036 mg/ml catalase in the presence of 1.5 mM ATP. A microscope with a camera mount with 1.4 NA, 100x objective is used to observe fluorescing actin motion. A CCD camera with 25-50 µm field diameter is employed in conjunction with a VCR video capture to record actin motility. Labeled F-actin was observed under a fluorescence microscope in room temperature (23-25ºC).

2. Electrode Design

In order to perform this fundamental examination of the effects of localized electric fields on an actin-myosin system, a hybrid microelectronic motility assay is developed. Electric field simulation of the microelectronic motility assay was undertaken to determine the proper electrode shape, size, spacing, and voltage needed to affect actin alignment without causing degradation of motor proteins in high e-fields. After the optimum geometry is established, the assay, consisting of PMMA channels fabricated on gold electrodes, separated from the high-magnitude electric fields at the electrode surfaces by a SiO_2 passivation layer, was fabricated in microfabrication lab. The pattern of the electrode chip, shown in Figure 2, has three sets of parallel electrode patterns and four circular electrode structures of varying dimensions and angles respectively. The parallel electrode patterns have varying sizes of the electrodes and the widths between them. Each parallel electrode pattern has varying size of electrodes keeping the width between the electrodes constant. i.e. the first pattern has electrode widths varying from 5-10-15-20µm keeping the width between the electrodes as 10µm. The second pattern has the same electrode width variation keeping the space width constant at 20µm. Similarly the third pattern has a space width of 40µm. The circular electrodes have widths of 20µm. The circular patterns have an angular variation of electrodes. The angles of variation are 11.25^0, 22.5^0, 30^0, 45^0.

The electrode structures on the motility chip are made of Chrome and Gold. The chip is made on a glass substrate, on which 200nm Chrome is deposited first and then followed by a 400nm deposition of Gold. The process is done in an e-beam evaporator. The electrode structure is then patterned on the slide by Photolithography and the subsequent development process. The patterned glass slide is then subjected to a gold etch followed by a chrome etch. An insulating layer of SiO_2 is then deposited on the surface of the chip; the thickness of SiO_2 is approx 1µm. Silver epoxy paste is used to bond wires on to the chip for the electrical connections.

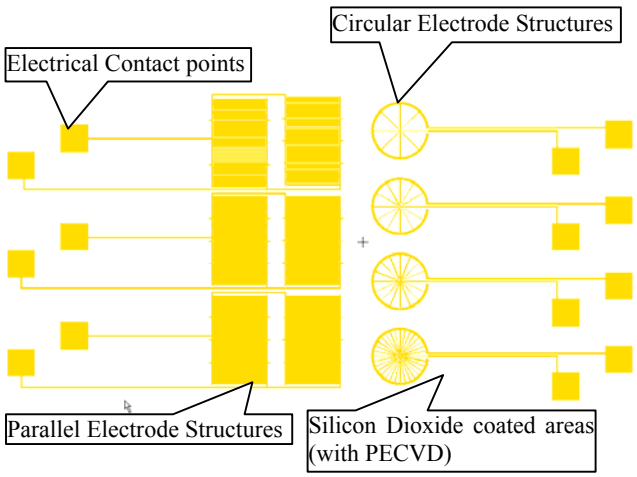

Figure 2: The pattern of the electrode chip

3. Experimental Results

Figure 3 shows example of F-actin motions on HMM bound to 0.2% collodion surface without silicon dioxide every 4 sec in a total of 12 sec. F-actin moved randomly and fast for 0.2% collodion surface. Figure 4 shows the velocity contribution of the moving F-actin on HMM-bound surface of 0.2% collodion only, PMMA only at 49.5 and 95.0 kDa, or 0.2% collodion on silicon dioxide as two layers. Two molecular weights of PMMA produced much lower velocity and narrower distribution (0.4 ± 0.1 µm/sec) in F-actin than those (2.2 ± 0.4 µm/sec) for collodion used generally as the standard adsorption layer. The number of adhesive F-actins for PMMA also was less than that for collodion. When the silicon dioxide surface was coated with 0.2% collodion as two layers, the velocity (1.4 ± 0.3 µm/sec) little decreased as compared with collodion only.

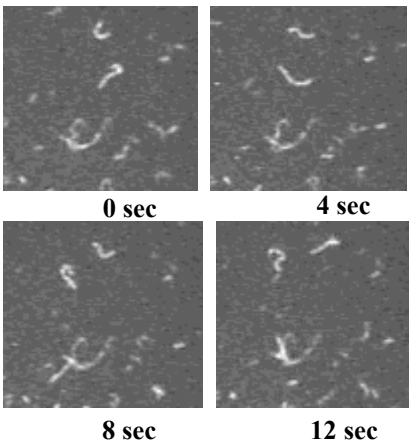

Figure 3: Micrographs of motions of fluorescent F-actin on heavy meromyosin (HMM) every 4 sec for 12 sec for 0.2% collodion (nitrocellulose) surface of a glass coverslip without silicon dioxide (SiO_2). Scale bar is 10 µm

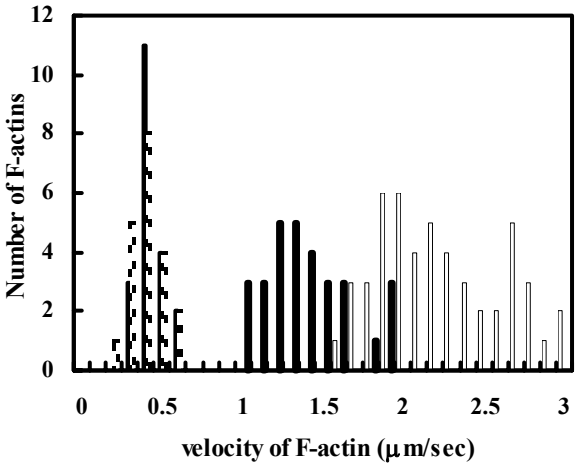

Figure 4: Velocity distribution of the moving F-actin on heavy meromyosin (HMM) for four different surfaces: 0.2% collodion (open bars) and PMMA at 49.5kDa (line) and at 95.0 kDa (dashed line) without silicon dioxide treatment of coverslip as well as 0.2% collodion with silicon dioxide treatment (closed bar)

4. Conclusions

This paper describes the research efforts to fundamentally understand the governing mechanics of biological molecular transport mechanisms that can serve as a foundation for their direct use in integrated biomolecular systems or the development of nanoengineered systems that mimic biological processes. Actin-myosin system represents a protein-based system being explored as basic building blocks for realization of linear and rotary biomolecular motors based on biological nanoscale transport phenomena.

The motility assay experiments are conducted and results were presented. Fluorescence techniques was used to optically observe actin motion in assay. Moreover, electrode chip was designed to explore the interaction of electric fields localized on the micron scale with the nanoscale actin-myosin motility assay. The next step is to integrate the electrode chip with established electric fields under the assayed surface experimentally and characterize their effect on nanoscale linear biomolecular motor filament alignment, direction of motion, and assay ambient.

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