BULK TITANIUM MICROFLUIDIC NETWORKS FOR PROTEIN SELF-ASSEMBLY STUDIES

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ABSTRACT
We report the use of a newly developed micromachining technique to fabricate microfluidic networks in thin titanium foils. These devices are used to geometrically confine and align self-assembled protein systems for confocal microscopy and transmission x-ray scattering experiments. X-ray attenuation can be easily reduced or eliminated from the thin foil devices by backside etching or through-etching the substrate. A surface modification technique has been integrated into the fabrication process in order to minimize protein adsorption to the titanium. These devices have been used to study both actin filament bundles and microtubules under physiologically relevant conditions.

Keywords: bulk titanium, cytoskeleton proteins, microfluidics, surface modification

1. INTRODUCTION
The eukaryotic cell cytoskeleton is composed of a complex network of filamentous actin (F-actin), microtubules and intermediate filaments. These systems are formed by the repetitive assembly of smaller protein subunits in the presence of associated proteins, which help to determine overall filament structure and function. In order to study cytoskeletal filaments assembly in-vitro under physiologically relevant conditions, new strategies have been developed that utilize microfabricated devices to geometrically confine and align these systems both alone and in the presence of associated proteins. These microfluidic devices can be used for both small angle x-ray scattering (SAXS) [1] and confocal microscopy [2]. Each device consists of a network of microfluidic channels with widths and depths that approximate the persistence length of the protein system being studied. Confining biomolecules in channels of this size induces alignment passively without damaging the delicate protein assemblies. In turn, this controllable alignment allows for confocal imaging and x-ray scattering of highly-oriented samples. Traditionally, these microdevices have been fabricated using silicon substrates. We report the use of a newly developed technology, titanium bulk micromachining [3, 4], to fabricate microfluidic networks in titanium thin foils. These titanium devices were found to be more robust than their silicon counterparts and can be fabricated such that x-ray attenuation is reduced or eliminated during SAXS experiments. Both F-actin and microtubule self-assembly have been studied using this technique.

2. EXPERIMENTAL
This application utilized two different channel designs shown in Fig. 1. The first design consisted of two reservoirs interconnected via a network of channels. The second design used through-etched channels which acted to suspend the proteins in solution via capillary forces. The process flow used to fabricate the reservoir-based microfluidic devices is shown in Fig. 2. The through-etched channel devices were fabricated in a similar fashion. Several channel width variations were used, including 2, 5, 10, and 20 \( \mu \text{m} \). Prior to filling, the channels were coated with a thin layer of TiO\textsubscript{2} followed by a polycationic PEG graft copolymer poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) [5] known to reduce protein adsorption to the titanium surface. The cationic poly(L-lysine) backbone of PLL-g-PEG adsorbs to the anionic TiO\textsubscript{2} surface to form a comb-like structure, as shown in Fig. 2. This comb-like structure helped to eliminate protein-substrate interaction and contain the proteins in solution within the channels. After the surface was functionalized with the PEG...
coating, the channels were filled passively by capillary flow in order to minimize potentially damaging forces associated with more active filling mechanisms.

Two cytoskeleton protein systems, actin/α-actinin bundles and microtubules, have been studied using the bulk titanium microfluidic networks. The actin system consisted of G-actin (in monomeric form) polymerized to F-actin in KCl and bundled via the cross-linking protein α-actinin in the channels. The microtubule system consisted of tubulin polymerized in the channels at 37°C and stabilized with taxol.

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3. RESULTS AND DISCUSSION

Confocal microscopy results of a self-assembled filamentous actin system are shown in Fig. 3 for G-actin polymerized in the presence of α-actinin, an actin cross-linking protein, to form actin filament bundles. The dual reservoir design allowed for the addition of controlled protein concentrations simultaneously or at intervals. As shown in Fig. 3c, this made possible the study of real-time assembly dynamics for this system. Microtubules have also been studied using the titanium microfluidic networks. These experiments consisted of the in-situ polymerization of tubulin in the presence of GTP [2].

Small angle x-ray scattering measurements on aligned self-assembled protein systems can be used to provide nanometer length-scale structural information. The titanium microdevices work particularly well for these experiments because in a transmission geometry, incident beam attenuation can be significantly reduced through backside etching.
of the reservoir-based devices, or completely eliminated in the case of the through-etched channels. For this research, small angle x-ray scattering was performed at the Stanford Synchrotron Radiation Laboratory.

Figure 3. Confocal fluorescence microscopy images of actin filament bundles formed in 20 μm wide reservoir-based channels: (a) bundles are formed consistently in the majority of the channels; (b) the bundles are easily studied using fluorescence microscopy and show a complex branching network which is characteristic of the system; and (c) a bundle thickness gradient as a function of α-actinin concentration is highly visible.

4. CONCLUSIONS

Using recently developed micromachining techniques, microfluidic networks have been fabricated using thin titanium foil substrates. These devices have been used to geometrically confine and align filamentous cytoskeleton protein systems. These highly-oriented samples are then studied using a combination of confocal microscopy and SAXS. Surface modification techniques have been integrated into the titanium device fabrication in order to minimize protein adsorption to the substrate. Bulk titanium thin foils were found to be especially useful for this application because the devices can be easily backside-etched or through-etched to reduce or completely eliminate x-ray attenuation from the device. Bulk titanium thin foil technology offers a novel material platform for this and other microfluidic applications.

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