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Metallization of surface- attached actin networks

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Abstract – **Several biopolymers have been used as templates for nanowire construction, however the metallization procedures typically occur in solution and do not address the issue of wire placement on a surface. Among the biopolymers, actin has a large array of regulatory handles and the potential for point-to-point self-assembly of nanocircuitry. As a step toward the use of actin in more sophisticated constructs, we report here on methods for the successful metallization of surface-attached actin networks.**

I. INTRODUCTION

To improve the cost and performance of future electronics, tremendous efforts are dedicated to creating technologies that fabricate sub-micron-sized conductive wires and interconnected structures. As part of these efforts, investigators have demonstrated how self-assembling biological polymers can be used as templates to construct wires 10-200 nanometers in size [1] - [5]. Because biopolymers themselves are typically not conductive, the techniques involve coating biopolymers with conductive metals. Naturally occurring binding proteins and antibodies provide tremendous specificity for these laminated constructs, and along with the controllable self-assembly of the polymer, make the prospect of mass assembled nanostructures realistic.

It has been previously shown that amyloid fibers [5] , [6], DNA molecules [1], [2], and actin microfilaments [3] can serve as templates for building metal nanowires. Among these, actin has immense potential to assemble into structures covering a wide range of geometries [7] , [8]. Indeed such potential is essential to life as actin creates diverse structural elements in both muscle and non-muscle cells. Most exciting and unique is the capacity to spatially control the growth of actin filaments by patterning with molecules that naturally bind or nucleate actin filaments. With this ability one can imagine self-assembling circuitry with wires that grow between pre-specified locations in a circuit, or dynamic circuits that re-wire in response to immediate energy or computational requirements.

The use of actin filaments in dynamic circuitry will require two core abilities: 1) the controlled assembly of actin networks between pre-specified locations on surfaces, and 2) the conversion of assembled actin networks into conducting replicas. Previous transformations of actin and other filamentous proteins to metal wires have been done in solution. Here we present methods for the conversion of surface-adhered actin networks into metal replicas.

II. METHODS

To metallize individual actin filaments we follow procedures typical for the metallization of biological template materials. We begin by reacting actin filaments (F-actin) with 1.4 nm gold nanoparticles (Nanoprobes; Yaphank, NY) to serve as initiators of electoless metallization steps (below). The nanoparticles were purchased functionalized with mono-sulfo-NHS to cause reaction with primary amines on the surface of the filaments. The gold-labeled F-actin was dialyzed overnight against buffer B (0.15M KCL, 5 mM MgCl, 1 mM EGTA, 5 mM ATP, 0.1 M Tris, 5 mM BME, pH 7.4) in 50kD MWCO dialysis tubing to remove unreacted nanogold. Filaments were then disassembled into monomers by dialyzing over several days against a buffer A (2 mM Tris, 0.2 mM CaCl, 0.5 mM BME, 0.5 ATP, pH 7.5). Gold labeling of filament surfaces rather than unpolymerized actin ensures that the stored monomers are not labeled at sites essential for filament assembly.

To determine the degree of gold labeling we combined absorbance assays on gold actin with the standard Bradford protein assay. We estimated the concentration of bound gold at 6uM by measuring absorbance at 420nm

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assuming only gold contributed to the absorbance at this wavelength. We estimated the concentration of actin at 12uM by mixing with Bradford reagent and measuring absorbance at 595nm. Thus roughly 50% of actin monomer was gold labeled. As a check, we confirmed that these estimates account for the total absorbance of the solution at 280nm where both actin and gold have significant extinction coefficients. Optical absorbances were taken from the nanogold optical spectrum (provided by Nanoprobes), Biorad's Bradford kit, and for actin at 280nm we used the customary value of 0.63 $(mg/ml)^{-1}$.

We polymerized gold-labeled actin (GA) monomers (12 uM) in polymerization buffer supplemented with 2 uM rhodamine phalloidin. The phalloidin enhances the rate of filament assembly and helps stabilize the filaments for subsequent processing. We used rhodamine-labeled phalloidin in order to visualize surface-attached filaments by fluorescence microscopy (see Results below).
We created surface-attached actin networks by incubating

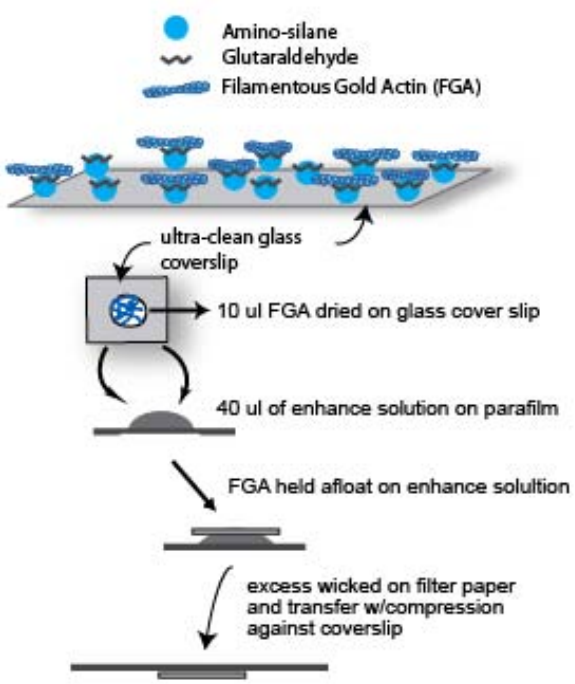

Figure 1. Method of actin network enhancement. Nanogold-labeled actin filaments are coupled to ultraclean glass coverslips via an amino-silane – glutaraldehyde bridge. The solution is allowed to air dry and then inverted atop enhancement solution for a brief exposure. Coverslips are then dabbed with filter paper and compressed against a glass slide for observation.

filamentous gold actin (FGA) with amine-reactive glass coverslips. We created amine-reactive coverslips by treating stringently cleaned coverslips [9] with 2% aminopropyl triethoxysilane following standard protocols, and then soaking treated coverslips in 2.5% glutaraldehyde for 30 minutes. We then incubated these coverslips with a 10 uL solution of FGA and allowed the solution to air dry. We determined that the resulting adhered networks were immobile under fluid shear by pipetting buffer through custom-made flow cells that incorporated coverslips for microscope observation. By contrast networks that were only physically adsorbed to coverslips could be disrupted by shear in the flow cells. The creation of strong adhesion between filaments and glass was required to minimize aggregation and protein loss when surfaces were later introduced to electroless plating solutions.

We metallized FGA networks by floating coverslips on a 40 uL drop of complete Li silver enhance solution for 1 second. Coverslips were blotted with a filter paper to reduce the fluid volume and immediately compressed against a slide for inspection by light microscopy. Even on functionalized coverslips, longer incubation times caused protein loss and aggregation (not shown). Inversion of the coverslips atop the enhancement solution minimized the amount of desorbed materials and background nucleation that settled back onto coverslips. For electron microscopy the same enhancement procedures were used on silicon wafers that were then imaged on a Zeiss-Leo DSM982 Field Emission source Scanning Electron Microscope (FESEM) available at Rochester core facilities.

III. RESULTS

Results demonstrating filament enhancement of surface-bound actin networks are shown in Figure 2. The figure shows corresponding images of networks in fluorescence and differential intereference contrast (DIC) microscopy. The fluorescence image identifies the location of rhodamine phalloidin while the DIC imaging mode is sensitive to optically dense metallic objects as small as 40nm [10].

In comparing the metallized FGA surfaces (Figure 2; Row A) to untreated controls (Figure 2; Row B), it is clear that the filament density is lower after metal enhancement, but that only the treated network is visible in DIC. Fluorescence quenching is expected in the treated samples because metal plasmon layers are known to quench fluorescence [11]. At the same time phalloidin binds to actin domains that are away from the surface of filaments and this may offer some protection against quenching [12]. One concern was that aggregation alone could create actin bundles large enough to be visible in DIC. To test this we intentionally bundled actin by

treating unenhanced surfaces with polylysine before imaging. Fluorescence images of these surfaces show large actin bundles that are invisible in DIC. Thus while protein loss/aggregation were not fully eliminated by our enhancement technique, the technique does appear to transform the residual surface-bound network into a metallic replica.

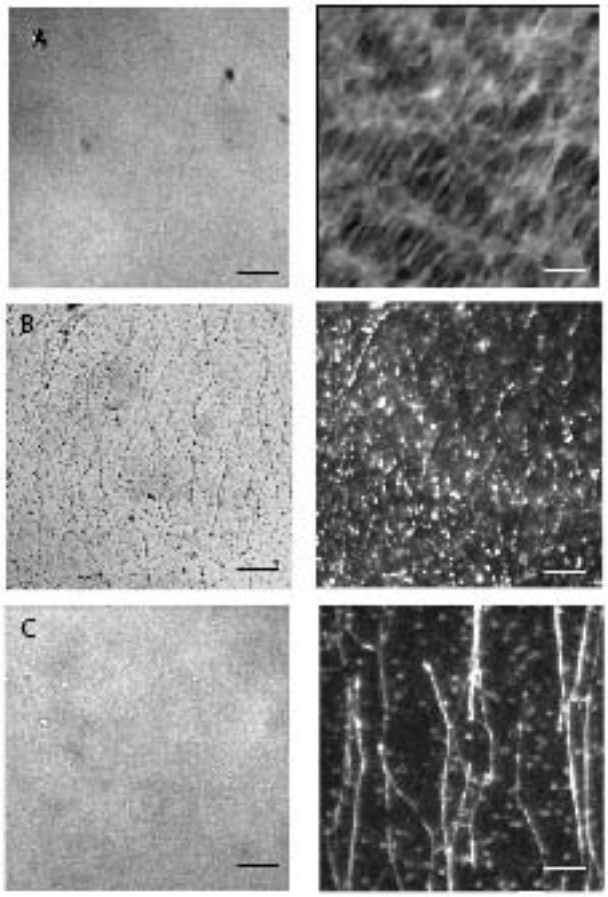

Figure 2. Evidence of actin network surface enhancement. A) A non-enhanced actin filament network visible in fluorescence (right) is invisible in DIC (left). B) An enhanced actin filament network is dimly visibly in fluorescence (right) and prominent in DIC (left). C) Large actin bundles induced by polylysine are visible in fluorescence (right) but not DIC (left). Scale bar in each image is 10 microns.

We attempted to visualize enhanced FGA networks directly by scanning electron microscopy following the same enhancement procedures employed for light microscopy, but with additional washing and drying steps necessary for SEM. Unfortunately these steps destroyed actin networks leaving only filament fragments in FGA samples (Figure 3). By contrast, control samples without FGA contained mostly non-filamentous aggregates consistent with background in the FGA sample. SEM images of filament fragments indicate that the dimensions of individual enhanced filaments can be variable along their length, varying from as thin as 50 nm to more than 500 nm in some locations. We suspect this variability is a result of aggregation occurring hand-in-hand with enhancement.

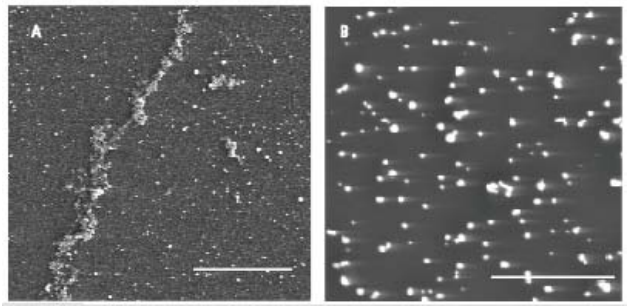

Figure 3. SEM inspection of enhanced surfaces. A) While networks are mostly destroyed during processing for SEM, lone filaments can be found with electron dense aggregates along their length. B) In controls without FGA, the largest features are consistent with background clusters in the FGA samples. Note difference in scale: scale bar on left is 5 microns and on right is 1 micron.

IV. DISCUSSION AND CONCLUSIONS

Here we present methods for the successful metallization of surface-bound actin networks. Similar procedures could someday allow the transformation of patterned actin networks into conducting circuitry. The controlled patterning of actin networks will be challenging, but is feasible given the point-to-point growth of actin filaments seen in biochemical studies [13]. Here we faced challenges in the metallization step, as enhancement solutions cause loss of surface-bound protein even when the surface promotes covalent attachments. Enhancement solutions have also been reported to cause aggregation and loss of DNA arranged on surfaces [14]. In the case of DNA as in our experience with actin, minimizing the times and volumes during surface enhancement provided at least a partial remedy. Optimization of binding chemistries and enhancement times may hold the key to retaining all features of the original network.

The use of biological templates in future circuitry is an intriguing concept, but the expanding abilities of lithography make clear that biomolecule templates are not necessary for nanowire fabrication [15]. Nature, however, has capacities for controlled self-assembly of monomers into diverse, and reversible networks. Subtractive

methods such as lithography cannot have these characteristics and will never rival the material and energy efficiency inherent in bottom-up self-assemblies. Thus at the very least biological systems provide models for the development of synthetic polymers. The more exotic prospect explored here is that biopolymers might be used directly as engineering materials. Actin and its diverse array of regulatory handles is arguably Nature's most versatile nanoscale erector set, and deserves attention in both of these contexts.

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