Electric Field Directed Fabrication of Multilayer BioNanoparticle Based Composites

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ABSTRACT

An electronic microarray has been used to carry out directed self-assembly of higher order 3D structures from biotin/streptavidin, DNA and enzyme derivatized nanoparticles. Structures with up to fifty layers of alternating biotin and streptavidin, DNA and enzyme nanoparticles were fabricated using a 400 site CMOS microarray system. In this process, reconfigurable electric fields produced by the microarray were used to rapidly transport, concentrate and accelerate the binding of 40 and/or 200nm nanometer bio-derivatized nanoparticles to selected sites on the microarray. The nanoparticle layering process takes less than one minute per layer. The nanoparticle addressing/binding process was monitored by changes in fluorescence intensity as each nanoparticle layer was deposited. The final multilayered 3-D structures are about two microns in thickness and 50 microns in diameter. Most recently, we have successfully fabricated enzyme-nanoparticle layers with streptavidin-alkaline phosphatase, glucose oxidase-avidin, and streptavidin-HRP. Forty seven layers were addressed with 200nm nanoparticles and enzyme activity was retained in the assembled structure. This work represents a unique example of combining "top-down" and "bottom-up" technologies into a novel nanofabrication process. Such a process will be useful for the assembly of a variety of electronic/photonic, nanomaterials, energy and biosensor applications.

Keywords: electric field, self-assembly, nanofabrication, nanoparticles, biosensors, nanomaterials, composites

INTRODUCTION

One of the grand challenges in nanotechnology is the development of fabrication technologies that will lead to cost effective nanomanufacturing processes. In addition to the more classical top-down processes such as photolithography, so-called bottom-up processes are also being developed for carrying out self-assembly of nanostructures into higher order structures, materials and devices. To this end, considerable efforts have been carried out on both passive and active types of Layer-by-Layer (LBL) self-assembly processes as a way to make three dimensional layered structures which can have macroscopic x-y dimensions. Nevertheless, limitations of passive LBL and as well as active assembly processes provide considerable incentive to continue the development of better paradigms for nanofabrication and heterogeneous integration. Electronic arrays have several important features that make them attractive for assisted self-assembly nanofabrication. First, a permeation layer or porous hydrogel is used to cover the microelectrode structures on the array. The permeation layer is usually impregnated with streptavidin which allows biotinylated DNA (antibodies, nanoparticles, etc.) to be bound at the selected site. This layer also allows relatively high DC electric field strengths to be used for rapid electrophoretic transport of molecules and nanostructures, while protecting the more sensitive DNA, proteins or nanostructures from the adverse effects of the electrolysis products generated at the electrodes. Finally, significant size reduction in the electronic array controller system provides a relatively compact control unit that can be run with a laptop computer (Figure 1). Using a 400 site CMOS microarray device and controller system we have demonstrated rapid and highly parallel assisted self-assembly of biotin and streptavidin and DNA derivatized nanoparticles into higher order structures [1-4]. We now show the fabrication of enzyme derivatized nanoparticles into multilayer structures.

RESULTS AND DISCUSSION

In order to determine optimal conditions for derivatized nanoparticle layering, experiments were carried out at addressing times of 5 seconds, 15 seconds and for 30 seconds. For each of the addressing time experiments, ten columns (16 sites) were activated with DC current levels that ranged from 0.025 uA to 0.4 uA, in increments of 0.025 uA. The activation of all 160 sites (at different current levels) was carried out in parallel. For each addressing time experiment (5 seconds, 15 seconds and 30 seconds) the addressing process was carried out forty times with alternating 40 nanometer red fluorescent streptavidin nanoparticles and green fluorescent biotin nanoparticles. In these experiments, the alternate columns were not activated. By the relative fluorescent intensity of the activated sites, the best conditions for nanoparticle layering appear to be at the 5 second and 15 second addressing times in the 0.30 to 0.40 uA current level range. At the lower current levels (<0.30 uA) the overall fluorescent intensity for the layers begins to decrease. At the longer 30 second addressing time, the nanoparticle layers become visibly damaged. Under real time epifluorescent microscope observation some of these fractured layers could actually be observed to flap when the sites were activated. Scanning electronic microscopy was used to examine the forty layer nanoparticle structures in more detail. Results for biotin and streptavidin, and DNA derivatized nanoparticle multilayer structures has been published in references [2-4]. A multilayer composite structure composed of biotin and strepatavidin

nanoparticles is shown in Figure 2. The scheme for coupling of bi-derivatized enzyme nanoparticle layers is now shown in Figure 3. The incorporation of both streptavidin-peroxidase and glucose oxidase-avidin into the same layer structure allows for chemical (enzyme reaction) coupling of the layers. The oxidation of glucose by glucose oxidase produces hydrogen peroxide which is then a substrate for the chemiluminescent oxidation of luminol, which generates light that can be detected (potential for biosensor applications). Figure 4 shows a cross section of an activated site on which addressings of nanoparticles was carried out. A number of nanoparticle layers can be seen from the top nanoparticle layer down to what appears to be the lower surface of the permeation layers, the coupled enzyme activity was retained in the final structures.

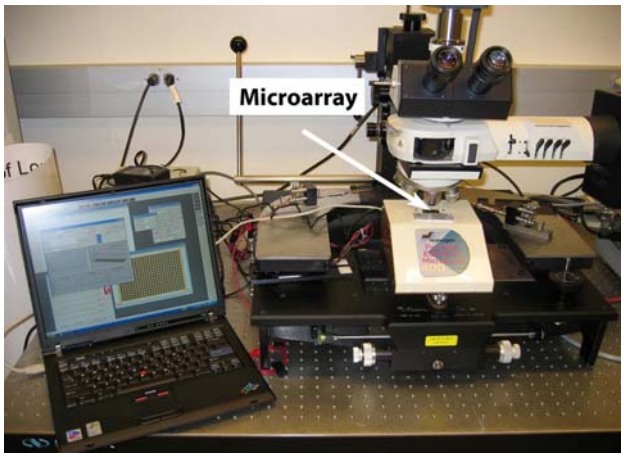

Figure 1 - Shows the electric field nanofabrication system for carrying out heterogeneous integration, nanoparticle layering and assisted self-assembly on the 400 site CMOS microarray device. The CMOS array controller system (with a 400 site CMOS microarray) is mounted on a standard micromanipulator probe station with an epifluorescent microscope and imaging system.

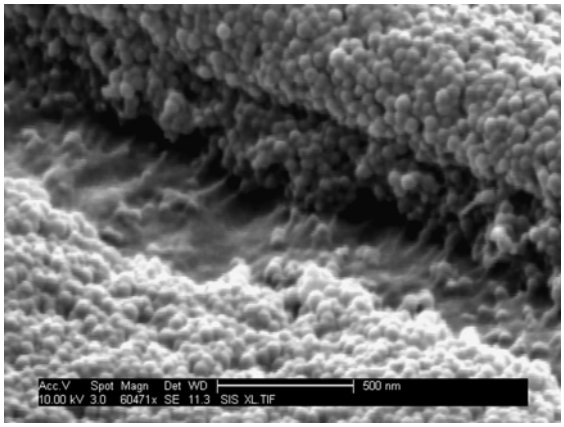

Figure 2 - Shows an SEM image of a cross section from one of addressed sites on the microarray. A number of biotin-streptavidin nanoparticle layers can be seen between the top nanoparticle layer all the way down to the surface of the permeation layer.

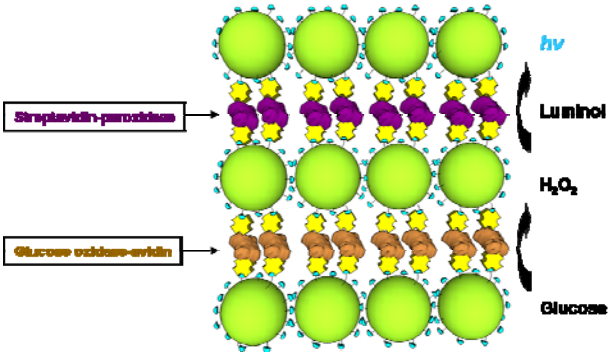

Figure 3 - Coupling of bienzyme nanoparticle layers. The incorporation of both streptavidin-peroxidase and glucose oxidase-avidin into the same layer structure allows for chemical coupling of the layers. The oxidation of glucose by glucose oxidase produces hydrogen peroxide which is then a substrate for the chemiluminescent oxidation of luminol, which generates light that can be detected.

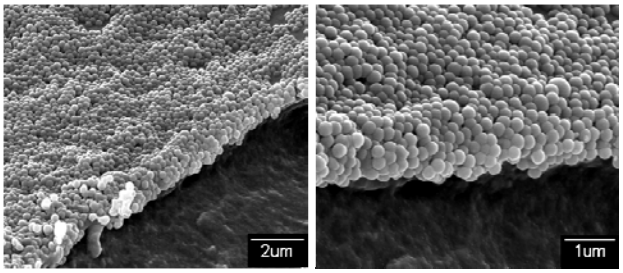

Figure 4 - SEM images of 200nm biotin nanoparticles layered with glucose oxidase-avidin at introduced cuts showing the layering of nanoparticles.

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