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Multilayer BioDerivatized Nanoparticle Composites by Electric Field Directed Self-Assembly

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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. Up to 47 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 hierarchal assembly of 3D nano, micro, and macrostructures for a variety of electronic/photonic, nanomaterials, energy (photovoltaics, fuel cells, batteries) and biosensor applications.

Keywords: electric field, self-assembly, higher order structures, nanofabrication, nanoparticles, 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 [1]. 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 (enzymes) or nanostructures from the adverse effects of the electrolysis products generated at the electrodes. A second feature of electronic array devices is that they can be designed in a wide variety of shapes and sizes. To date, arrays have been fabricated in sizes from 2 mm x 2 mm to over 2.5 cm x 2.5 cm, with 25 to 10,000 electrodes and with electrode structures which range in size from 10 microns to several millimeters. A third feature is that sophisticated CMOS control elements can be integrated into the underlying silicon structure of electronic microarrays which allows precise control of currents and voltages to each of the individual microelectrodes on the array. 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 [2-4]. In the earlier DNA nanoparticle work, two different sets of streptavidin nanoparticles and the electronic microarray binding sites were derivatized with target and complementary 24mer and 51mer DNA oligonucleotides (Figure 2A-C). Through a rapid series of DC electric field (electrophoretic) directed depositions, accelerated hybridizations and washing steps, a layered nanostructure material was assembled using complementary DNA oligonucleotides as a structural binding material. The 400 site CMOS microelectrode array device used in the experiments allows individual control of activation time, DC current, and voltage levels and microelectrode polarity

(positive, negative, and neutral) (see Figure 2A). The complementary DNA derivatized nanoparticles were specifically concentrated and hybridized (in zwitterionic histidine buffer) to the target DNA sequences bound to the porous hydrogel surface above the microelectrodes. The ability to independently control all 400 sites on the microarray allows for parallel combinatorial testing of a wide range of DNA binding conditions to determine the optimal parameters for hybridization and nanoparticle layering. The basic procedure for electric field directed hybridization and layering of the DNA nanoparticle on the electronic microarray is shown in the Figure 3D-3I scheme. By alternating the deposition between nanoparticles derivatized with complementary and target DNA sequences, a multilayered nanoparticle structure was formed using hybridized DNA as a specific binding agent. Previously developed combinatorial methods used to determine optimal conditions for biotin/streptavidin nanoparticle layering [3] were again used to determine the optimal conditions for the DNA nanoparticle layering, i.e., the optimal addressing times and DC current levels [4]. We now show the fabrication of enzyme derivatized nanoparticles into multilayer structures.

RESULTS AND DISCUSSION

In order to determine optimal conditions for enzyme 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. Figure 4 shows scheme for coupling of bi-derivatized enzyme nanoparticle layers. 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

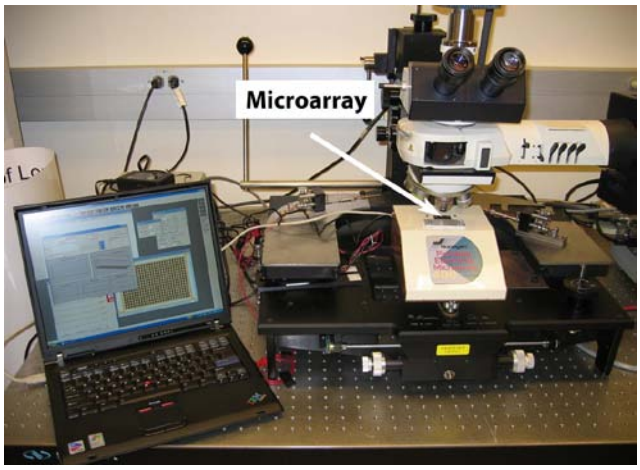

Figure 1 - Shows the electric field nanofabrication system for carrying out heterogeneous integration, nanoparticle layering and assisted self-assembly on the 400 site CMOS microarray device. The CMOS array controller system (with a 400 site CMOS microarray) is mounted on a standard micromanipulator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

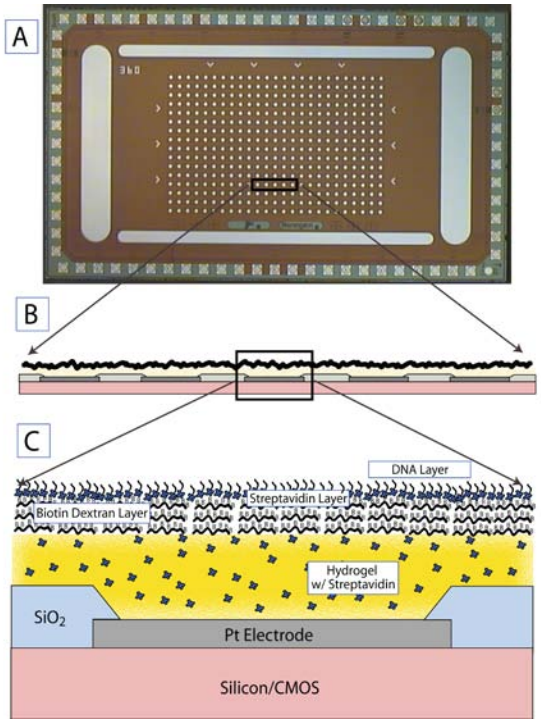

Figure 2 - (A) Shows the 400 site CMOS microelectrode array device. (B) shows a cross section of the 400 site microarray which includes: a silicon base, CMOS control circuitry, three platinum microelectrodes (55 um diameter). (C) shows an expanded view of the cross

section with the overlaying polyacrylamide and streptavidin permeation gel layer; a biotin-dextran layer and a final streptavidin layer to which the biotinylated 24mer oligonucleotide target sequences or 51mer oligonucleotide target sequences are attached.

the chemiluminescent oxidation of luminol, which generates light that can be detected (potential for biosensor applications). Figure 5 shows a cross section of an activated site on which forty 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. Most importantly, the coupled enzyme activity was retained in the final structures.

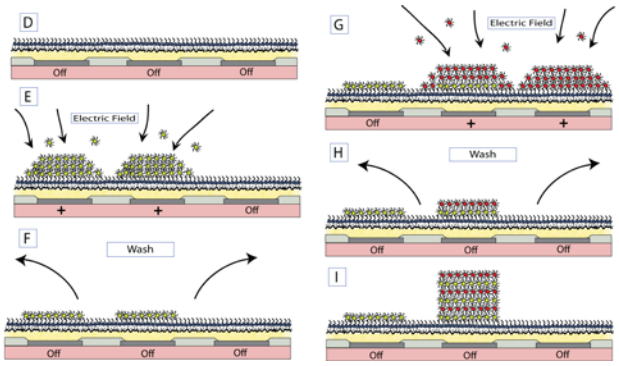

Figure 3 - 3D – 3I shows the initial DNA nanoparticle layering steps, where a solution containing fluorescent DNA nanoparticles with the complementary DNA sequence would be place on the microarray. The positively biased test sites on the microarray will attract and concentrate the negatively charged DNA nanoparticles to the surface. The DNA nanoparticles will hybridize to the target DNA sequences on the microarray surface, and the unattached DNA nanoparticles are washed away. In the next steps, a solution containing DNA nanoparticles with the target sequence is placed on the microarray, the positively biased test sites on the microarray attract and concentrate the negatively charged DNA nanoparticles which now hybridize to the layer of DNA nanoparticles with the target sequence. Alternate addressings and hybridizations of DNA nanoparticles with target and complementary sequences is then carried out.

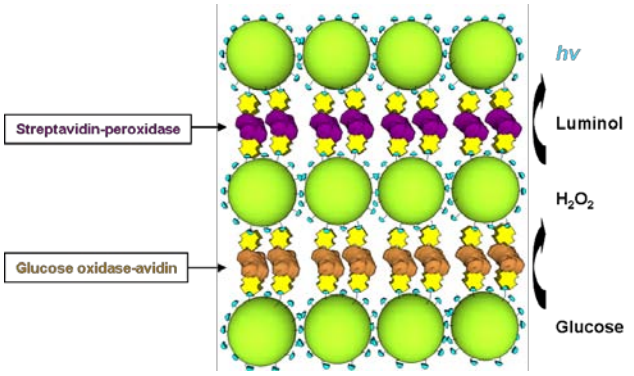

Figure 4 - Coupling of bienzyme nanoparticle layers. The incorporation of both streptavidin-peroxidase and glucose oxidase-avidin into the same layer structure allows for chemical coupling of the layers. The oxidation of glucose by glucose oxidase produces hydrogen peroxide which is then a substrate for the chemiluminescent oxidation of luminol, which generates light that can be detected.

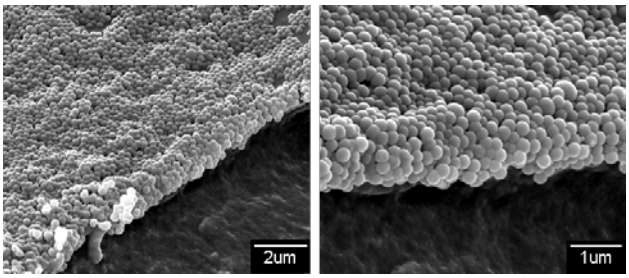

Figure 5 - Figure 8. SEM images of 200nm biotin nanoparticles layered with glucose oxidase-avidin at introduced cuts showing the layering of nanoparticles.

CONCLUSIONS

We believe the results of this study carry significant implications for the future use of enzyme, DNA and biotin/streptavidin nanoparticles and other derivatized nanocomponents for directed self-assembly nanofabrication into higher order structures. First, the electric field process for the directed self-assembly of nanoparticles was significantly faster than the passive process. Second, the electric field directed process requires the use of only minimal concentrations of bioderivatized nanoparticles, which would not be viable for passive process which require much higher concentration of nanoparticles. This use of minimal concentrations of nanoparticles is possible because of the use of zwitterionic histidine buffer and the enormous concentration effects on the activated sites that drive the hybridization reactions to completion. Third, the integrity of the biomolecular binding reactions and the ionic flux was maintained throughout the large number of process steps (20 – 40 depositions). Fourth, the higher order layered nanoparticle structures can be ultimately removed from the microarray by a relatively simple lift-off procedure. Thus, an electric field array device can serve as a manufacturing platform for making higher order 3D structures. Fifth, the overall process demonstrated the nanofabrication of higher order structures from a relatively heterogeneous group of materials i.e., biotin-dextran polymers, streptavidin and two different sets of DNA nanoparticles and various enzymes. Sixth, electronic microarray devices have intrinsic ability for near instantaneous x-y reconfigurability i.e., the field geometry can be switched in less than a second allowing a

maskless (X-Y) patterning fabrication process to ultimately be developed. Seventh, electronic microarray devices can be designed in a wide variety of shapes and sizes. Finally, electric field processes using DNA derivatized nanoparticles represents a unique synergy of combining the best aspects of "top-down" and "bottom-up" technologies into a viable nanofabrication process. It also represents a bio-inspired logic for self-assembly and hierarchal scaling of nanocomponents into integrated microscopic structures. Some important potential applications for this process include miniaturized chemical and biosensor devices, "micronsize" dispersible chem/bio sensors for environmental and bioagent detection, lab-on a-chip devices and in-vivo diagnostic/drug delivery systems.

REFERENCES

[1] C. Gurtner, E. Tu, N. Jamshidi, R. Haigis, T. Onofrey, C.F. Edman, R. Sosnowski, B. Wallace, M.J. Heller, *Electrophoresis* **2002**, 23, 1543-1550.

[2] Dehlinger DA, Sullivan B, Esener S and Heller MJ, "Directed hybridization of DNA nanoparticles into higher order structures", NanoLetters, 8, pp. 4053-4060, 2008.

[3] Dehlinger DA, Sullivan B, Esener S, Hodko D, Swanson P and Heller MJ, Automated Combinatorial Process for Nanofabrication of Structures Using Bioderivatized Nanoparticles, J. Assoc. Lab Automation, v.12, #5, pp.267-276, October 2007.

[4] Dehlinger DA, Sullivan BD, Esener S and Heller MJ, Electric Field Directed Assembly of Biomolecular Derivatized Nanoparticles into Higher Order Structures, SMALL, V3, #7, pp. 1237-1244, 2007