Printing of organic and inorganic nanomaterials using electrospray ionization and Coulomb-force-directed assembly

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This letter reports on an additive printing process to deposit organic and inorganic nanomaterials onto desired areas on a surface. The process combines electrospray ionization with Coulomb-force-directed assembly. Electrospray ionization is used to bring the desired nanomaterial into the gas phase while carrier gas, global, and localized electric fields are used to deposit the material onto desired locations on a substrate. Albumin fluorescein isothiocyanate bovine, avidin sulforhodamine, and gold colloids were sprayed from an aqueous solution and patterned with a resolution as high as 100 nm. © *2005 American Institute of Physics*. [DOI: 10.1063/1.2149985]

The ability to print organic and inorganic nanomaterials with a resolution ranging from tens of micrometers to sub-100 nanometers is becoming increasingly important in fields ranging from micro- and nanoelectronics to life sciences. Patterns of organic nanomaterials, including proteins and other macromolecules, find applications ranging from hybrid molecular electronics and biosensors to proteomics, drug discovery, and tissue engineering. Printed inorganic materials are equally important. Many types of techniques have been developed to print nanomaterials onto surfaces. For example, proteins and deoxyribonucleic acid (DNA) can be printed using ink-jet printing,[1,2] microspotting,[3,4] microcontact printing,[5–7] micropatterned agarose stamps,[8] dip-pen lithography,[7,9,10] electrospraying through a dielectric mask,[11] and nanoxerographic-type printing that makes use of localized charge patterns to direct the assembly.[12,13] Nanoxerography overcomes the use of a mask or print head to deliver the materials onto precise locations on a substrate. Materials are transferred onto a prepatterned surface from a powder,[14] nonpolar solution,[12,15] or gas phase[13] based on localized electrostatic force. While a number of different materials have been printed using nanoxerographic methods, it has not been possible to directly print organic and inorganic materials that are dispersed in polar or ionic solutions that screen long-range electrostatic interactions.

This letter demonstrates a unique concept to print materials that are dispersed in polar or ionic solutions. The process uses electrospray ionization, to bring nanomaterials from the liquid into the gas phase, a carrier gas, and global and localized electric fields to direct and deposit them onto desired locations on a substrate. We report our results using albumin fluorescein isothiocyanate (FITC) bovine, avidin sulforhodamine, and gold colloids that have been printed on a charged patterned poly(methylmethacrylate) (PMMA)-coated silicon wafers with a resolution as high as 100 nm.

An illustration of the nanomaterial deposition system is shown in Fig. 1. The system can be divided into two modules—an electrospray ionization module and a nanomaterial assembly module. Both modules were home built. For the electrospray system, we also tested a commercially available system (TSI Inc., Electrospray Aerosol Generator Model 3480, St. Paul, MN) and obtained similar results. Our electrospraying unit is similar to the commercially available system; however, it provides greater control to monitor the electrospray currents, higher flow rates, and the ability to easily modify the system. For a review on electrospraying concepts, we refer the reader to Cloupeau and Prunet-Foch[16] and Ganan-Calvo *et al.*[17]

In brief, our home-built electrospraying system consists of a high-voltage source, pressure regulator, pressure chamber, capillary, electrospray, and neutralization chambers. The pressure chamber houses a centrifuge vial, a high-voltage platinum electrode, and a fused silica capillary that carries the solution out into the electrospraying chamber. The capillary and platinum wire are intertwinned and submersed in the solution. The neutralization chamber contains a Po^{210} alpha source (NRD Model No. P-2042-2000, Grand Island, NY). We tried various capillaries and found fused silica capillaries with inner diameters (i.d.) of 25 μm, 40 μm (TSI Inc., Part Nos. 3900124/3900126, St. Paul, MN) and 50 μm (Polymicro Technologies, LLC, Part No. 2000015, Phoenix, AZ) to work well for our application. The electrospray voltage is increased until the liquid forms a cone shape, which is also know as the cone-jet mode.[18] This is achieved by visually observing the tip of the capillary through the lens while increasing the voltage. A sheath flow of a purified gas mixture of compressed medical grade air and CO_2 is used to prevent corona discharge[19] and to carry the droplets out through an orifice plate into the neutralization chamber. Typical gas flow rates are 0.5 Lpm and 0.2 Lpm, respectively, for air and CO_2 and can be altered to adjust the amount of time the aerosol is in the neutralization chamber and assembly module. A Keithley 6517A electrometer is used to monitor the electrospray current. The electrospray current varies depending on the flow rate, solution, and the electrospray voltage. Typically, electrospray currents in cone-jet mode for the gold colloid solution using a 25 μm i.d. capillary were ~50 nA at 2.5 kV and ~150 nA at 2 kV when electrospraying a protein solution using a 50 μm i.d. capillary. The highly charged primary droplets enter a neutralization chamber and evaporate leaving a nanomaterial aerosol. The neutralization chamber holds a Po^{210} alpha source that interacts with the nitrogen and oxygen molecules in the gas mixture to create ions that neutral-

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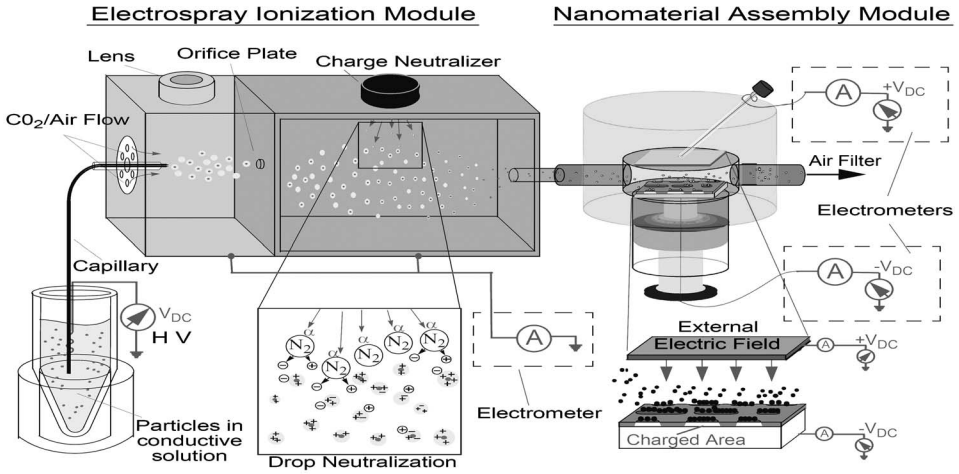

FIG. 1. Nanomaterial deposition system that contains two modules: An electrospray ionization module (left) and a nanoparticle assembly module (right). An aerosol of charged nanomaterials is form as a result of the electrospraying process. The highly charged aerosol becomes partially neutralized by the Po^{210} alpha source. The aerosol flows into the nanomaterial assembly module. A global field is applied to bring nanomaterials of desired polarity into close proximity of the surface. A surface charge pattern is used to deposit the nanomaterials on desired areas on the substrate.

ize the highly charged droplets until they contain only a few (typically less than 3) elementary charges of positive or negative polarities.[20] The nanomaterial travels out of the chamber through a 6 in. long piece 0.17 in. i.d. polyethylene tube to the assembly module.

We introduce the sample to the aerosol through an opening on the bottom of the assembly module. The sample sits flush with the bottom surface of a cylindrical chamber that is 2 cm in diameter and 1 cm tall. To bring charged material of one polarity into close proximity of the surface of the chip, we used a global electric field that is generated by applying a voltage between a 4 cm^2 square copper electrode that is attached to the top of the cavity and a 1 cm^2 bottom electrode that holds the chip. We used two additional electrometers to monitor the amount of charge deposited on the chip and the top electrode.

The sample, a PMMA-coated silicon wafer, carries high-resolution charge patterns to attract oppositely charged nanomaterials. The PMMA thin film was charged using a previously developed technique that makes use of a flexible conductive electrode to inject charge into desired areas on the substrate.[12–15] In our experiments, we used a number of different commercially available organic and inorganic materials. First, we used an aqueous suspension of 100 nm unconjugated gold colloids (SPI Supplies, No. 4804, West Chester, PA). The as-received gold colloids carry a protein layer that prevents agglomeration; the details on the protein type and coverage have not been disclosed to us by the manufacturer. The solution had a concentration of 5.6×10^9 particles/ml which corresponds to a ~ 3 μM solution. The solution was not processed further. As organic materials, we tested two common fluorescently tagged proteins: (i) Albumin bovine with FITC (Green, No. A9771, Sigma, USA) and (ii) Avidin Sulforhodamine 101 (Texas Red, #A2348, Sigma, USA). We used a 1:1 ratio of acetonide: Deionized water as a buffer for the albumin bovine and avidin to prepare 1 and 5 mM solutions. We further added 0.1% of formic acid to increase the ion concentration.

Figure 2 shows a representative scanning electron microscope image of a pattern of 100 nm gold colloids that were assembled on negatively charged 100 nm wide lines. The results show that the charged particles assembled onto the charged lines with a good selectivity. In the illustrated example, we applied a positive potential of 3 kV to the capillary while the orifice plate was grounded to form a primary aerosol of predominately positively charged gold colloids. The measured electrospray current was 50 nA. We ran the experiment for 2 h to electrospray 30 μL of the colloidal gold solution, which are approximately 1.6×10^8 particles.

Figure 3 shows fluorescent micrographs taken of different types of proteins that have been printed onto charge patterned substrates. Figure 3(a) shows positively charged albumin FITC bovine that has been assembled onto negatively charged 2 μm wide lines. The albumin FITC bovine was electrosprayed in positive ion mode (2.5 kV positive capillary potential) to generate a mainly positively charged protein aerosol. A positive potential of 300 V was applied to the top electrode in the assembly module, while the sample was kept at ground to direct the positively charged proteins to the sample surface. Figure 3(b) shows positively charged avidin that has been assembled using the same conditions as above onto higher-resolution 200 nm wide negatively charged lines. Figure 3(c) shows albumin FITC bovine that has been assembled onto negatively charged squares with a linewidth of $\sim$200 nm. Figures 3(d)–3(f) show a single chip after sequentially depositing different proteins onto the same area. In our sequence, we deposited the two proteins with different polarities onto selected surface areas on the sample. The sample contained positively charged 1 μm wide parallel lines that were separated by 1 μm wide uncharged areas. In the first step, we assembled negatively charged avidin that was sprayed in negative ion mode by applying a -2 kV potential to the electrospray solution and a -300 V potential to the top electrode in the nanomaterial assembly module to

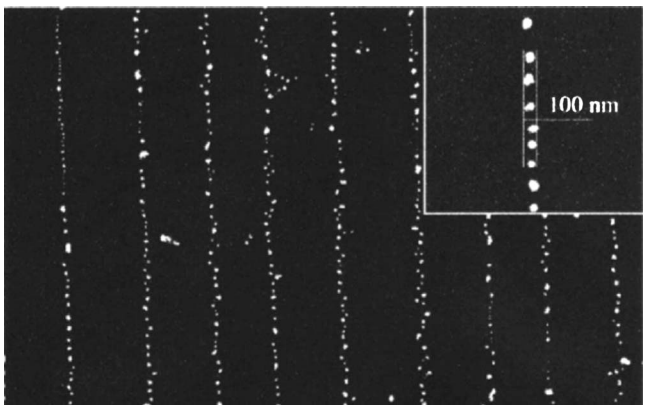

FIG. 2. Scanning electron microscopy image of 100 nm protein-coated gold colloids assembled in 100 nm wide lines by electrospray ionization and Coulomb force directed assembly.

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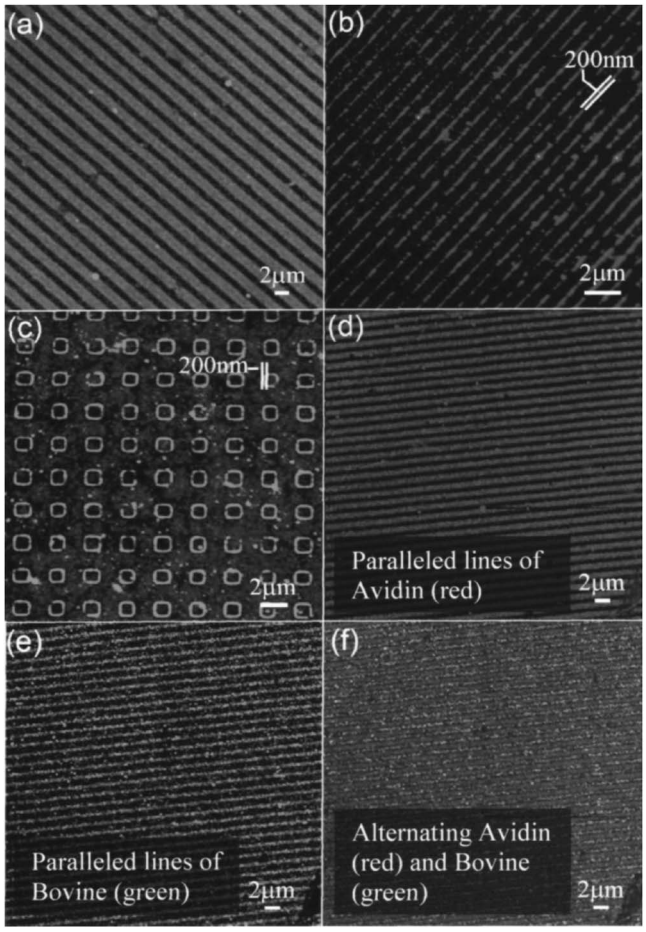

FIG. 3. Representative fluorescent optical microscope images of printed proteins—albumin FITC bovine (green) and avidin sulforhodamine (red). (A and B) show 2 μm and 200 nm wide parallel lines, respectively. (C) A square pattern with 200 nm line widths. (D and E) The sequential assembly of the two proteins into separate line patterns on the same chip as directed by the global and local fields: Negatively charged avidin (D) that assembled onto the 1 μm wide positive charge pattern and positively charged albumin bovine (E) that deposited in between the charged lines. (F) An overlay of (D) and (E).

deflect the negative charged proteins to the substrate. As expected, the negatively charged proteins assembled onto the positively charged areas showing a strong red fluorescence [Figure 3(d)] after a 7 min deposition time. The capillary was then flushed with buffer solution for 15 min to make sure the capillary was clean. Albumin FITC bovine was then loaded, and the polarity of the electrospray apparatus and the nanoparticle assembly module were reversed to produce positively charged protein particles. The albumin FITC bovine was then printed in between the charged lines by operating the electrospray system in positive ion mode for an additional 7 min [Figure 3(e)]. This procedure resulted in a pattern of two different proteins that are separated according to the charge patterned substrate. Figures 3(d) and 3(e) were taken with a high-resolution single-photon confocal microscope, the pictures were captured in black and white and then recolored to represent the colors that we observed. The assembly times to create the various patterns [Figs. 3(a)–3(f)] ranged between 5–14 min. The electrospray voltage varied from 1.8 kV to 4 kV.

In our experiments, we found 15 μL of the 1 mM avidin solution to be sufficient to coat selected areas over a 1 cm^2 sized chip. We have not yet compared this number with other techniques, but note that the required material is small. The amount of material that is deposited can be adjusted by altering the exposure time or the concentration of the material that is suspended in the electrospray solution. It is possible to print as little or as much material as desired, as long as there is enough charge on the substrate to continue to attract or repel the material to be patterned in selected areas.

In conclusion, we have demonstrated nanoxerographic printing of inorganic and organic materials from the gas phase produced by electrospraying. We believe that the method can be adapted to deposit a large array of nanomaterials. There is also a vast variety of measurements that combines electrospray ionization with mass-spectroscopic measurements. Proteins, DNA, and viruses can be electrosprayed and analyzed by electrospray ionization-mass spectrometry systems and are shown to be functionally active.[21–23] The reported printing process could be combined with these systems to assemble nanomaterials of different sizes or compositions onto desired areas on a substrate. Another extension would be to use biased surface electrodes arrays to enable the integration of nanomaterials in large arrays in a programmable fashion.

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