

# Intact transfer of layered, bionanocomposite arrays by microcontact printing

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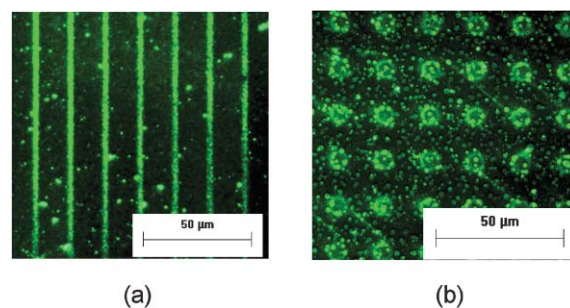
A novel approach is presented that allows high-quality, 3D patterned bionanocomposite layered films to be constructed on substrates whose surface properties are incompatible with existing self-assembly methods.

Elastomeric patterning methods, such as microcontact printing ( $\mu$ CP),<sup>1</sup> microcontact molding ( $\mu$ CM),<sup>2</sup> and microfluidics,<sup>3</sup> allow a two-dimensional pattern to be fabricated on a surface. In  $\mu$ CP, a topographically patterned, elastomeric stamp is used to transfer a patterned layer of molecular ink to a surface. Based on the chemical contrast between the inked features and the ink-free background, additional layers can then be selectively deposited on either the features or background by directed self-assembly,<sup>4</sup> resulting in 3-D, patterned, nanocomposite arrays.

Biological activity can be imparted to such arrays by incorporating proteins or other biomolecules.<sup>5,6</sup> The biological activity can be augmented by co-immobilization of macromolecular adjuvants, such as dendrimers or polyelectrolyte multilayers (PEMs). Dendrimers are radially symmetrical polymeric molecules that are grown by sequential addition of branched monomers to the outer shell.<sup>7</sup> Dendrimers can serve as functional frames to encapsulate small molecules needed by the proteins. PEMs are thin films<sup>8</sup> formed when two oppositely charged polyelectrolytes are alternately adsorbed onto a surface one layer at a time. PEMs are robust, easy to fabricate, and have tunable architectures (*i.e.* film composition and physical and chemical microstructure). Polyelectrolytes can be used to immobilize hydrophobic membrane proteins onto hydrophilic substrates, entrap ionic or polar small molecules needed by the proteins, and act as an ion-selective barrier to screen out interfering molecules. PEMs have further been extended to colloids,<sup>9</sup> inorganic nanoparticles,<sup>10</sup> polymer-on-polymer stamping (POPS)<sup>11</sup> and selective electroless metal depositions.<sup>12</sup>

Layer-by-layer assembly of 3-D structures onto patterns deposited by  $\mu$ CP can be hindered by a lack of chemical contrast between the features and background, making it difficult to deposit additional layers cleanly onto only the features or the background. This problem can be particularly challenging when a layer of amphiphilic molecules (*e.g.* proteins and some dendrimers) is to be deposited, because amphiphilic molecules can adsorb to both hydrophilic and hydrophobic surfaces.

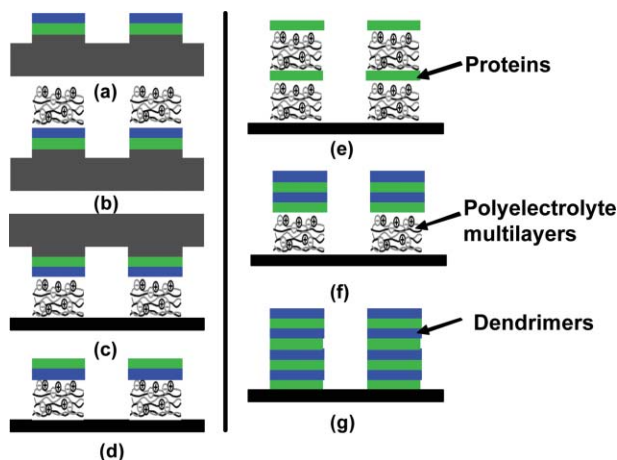
In this paper amphiphilic biomacromolecular patterning by directed self-assembly (see Fig. 1) is compared with a novel approach that overcomes the above-mentioned difficulties in establishing well-defined, 3-D, layered bionanocomposite patterns



**Fig. 1** Arrays of amphiphilic proteins obtained on a patterned substrate using directed self-assembly.

containing alternating layers of polyelectrolytes, dendrimers, and amphiphilic proteins. The approach entails combining spin self-assembly<sup>7</sup> and layer-by-layer self-assembly<sup>4,13</sup> to pre-establish a multilayered structure on an elastomeric stamp, and then using  $\mu$ CP to transfer the 3-D structure intact to the target surface. While  $\mu$ CP was recently used to transfer preformed PEMs to a substrate,<sup>14</sup> this paper presents for the first time conclusive evidence of the formation of bionanocomposite layered structures on a micropatterned stamp and subsequent transfer of the structures intact to a target substrate.

An example of the approach used is shown in Fig. 2. A patterned polydimethylsiloxane (PDMS) stamp was spin-coated



**Fig. 2** (a)–(d) Schematic representation of the procedure used for printing: (a) stamp spin-coated with protein and dendrimer solutions; (b) PEMs being grown on the stamp; (c) layered bionanocomposite arrays being transferred to the substrate; (d) patterned substrate. (e)–(g) Some examples of the different 3-D structures possible using this technique.

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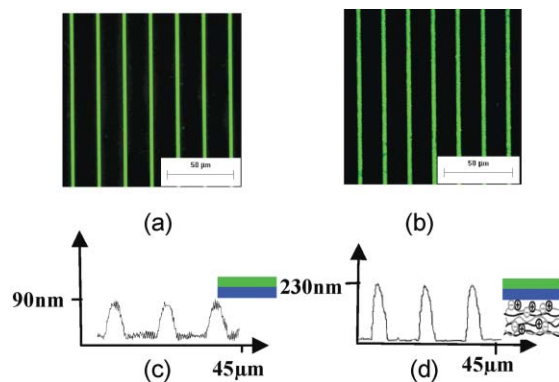
twice, first with a secondary alcohol dehydrogenase (SADH) protein solution and then with a polyamidoamine-organosilicon (PAMAMOS) dendrimer solution [see Figs. 2(a)–2(d)]. This stamp then served as the template for the growth of PEMs before these patterns were transferred to a glass slide coated with PEMs. Slight modifications of this approach can result in many different 3-D architectures [see Figs. 2(e)–2(g)]. As examples, different enzymes can be sandwiched between PEMs to catalyze sequential reactions, proteins immobilized on dendrimers can be trapped between PEMs, and alternating layers of enzymes and dendrimers can be separated by PEMs.

Strong polyelectrolytes, sulfonated poly(styrene) (SPS) and poly(diallyldimethyl ammonium chloride) (PDAC), were used to fabricate multilayer platforms using glass slides as the substrates. The concentration of SPS was 0.01 M, and the concentration of PDAC solution was 0.02 M as based on the molecular repeat unit of the polymer. All polyelectrolyte solutions contained 0.1 M NaCl. A Carl Zeiss slide stainer equipped with a custom-designed ultrasonic bath was connected to a computer to perform layer-by-layer assembly.<sup>4</sup> Multilayers were grown on a glass slide by alternatively dipping them in PDAC and SPS solutions. Second-generation (G2) PAMAMOS-DMOMS (2,1) dendrimers (DMOMS = dimethoxymethylsilyl) were obtained as a 20% w/w methanolic stock solution from Dendritech, Inc. (Midland, MI). This solution was further diluted to the desired concentrations with methanol.

Several factors were evaluated in optimizing the stamping process, including the plasma treatment of the PDMS stamps, the type and concentration of the solvents used to make the ink solution, and the contact times. The PDMS stamps were first treated with oxygen plasma to increase the fidelity of pattern transfer. Different methods were then used to ink the PDMS stamps: spin inking, cotton-swab inking and dip inking. The spin-inking method provided the most efficient transfer of the ink onto the PEM surface. Contact times were varied from a few seconds to 30 min. Optimal stamping conditions were found to be a 1%wt solution of protein spin-coated at 2500 rpm for 20 s, and a 1%wt solution of PAMAMOS dendrimers spin-coated at 2500 rpm for 20 s.

Fluorescence microscopy was used to establish the existence of alternating protein and dendrimer layers in the multilayered films after deposition. In one case [Fig. 3(a)], only the protein was fluorescently labeled. In the other case [Fig. 3(b)], only the dendrimer was labeled. Fluorescence observed in both figures confirms the presence of both protein and dendrimer layers. AFM was then used to confirm incorporation of multiple PEM bilayers into the multilayered films. Fig. 3(c) shows the cross-sectional topography of a patterned film containing sequential layers of protein and dendrimers deposited onto a PEM-coated substrate. Fig. 3(d) shows the topography of a patterned film containing sequential layers of protein, dendrimers and PEMs (50 bilayers of PDAC/SPS). The heights of the patterns were approximately 90 nm without PEMs, and approximately 230 nm with 50 PEM bilayers. Based on these data, the average height of each PDAC/SPS bilayer was estimated to be about 2.8 nm, which is in agreement with published values.<sup>4</sup>

We believe electrostatic interactions between the enzymes, dendrimers and PEMs are responsible for stabilizing the multilayered structures. Weak polyelectrolytes change their



**Fig. 3** (a) Fluorescence image of the patterned films of protein, dendrimer and PEMs with fluorescently labeled protein as the topmost layer. (b) Fluorescence image of the line patterns of fluorescently labeled dendrimers sandwiched between patterned proteins and PEMs. (c) AFM image of the patterned film of protein and dendrimers, with protein as the topmost layer. (d) AFM image of the patterned film of protein, dendrimers, and PEMs (50 bilayers), with protein as the topmost layer. All the bionanocomposite arrays were deposited onto PEM-coated substrates.

conformation or charge density with pH. Thus, the shape and stability of the resulting 3-D structures formed with weak polyelectrolytes often vary with pH. To avoid such effects, we used the strong polyelectrolytes SPS and PDAC, whose charge density is relatively unaffected by pH. Further versatility of these interfaces is provided by the unique ability of the PAMAMOS dendrimers to cross-link and encapsulate nanoparticles and transition metal ions. Cross-linking could also enhance the physical stability of these 3-D nanostructures.<sup>7</sup>

Our novel approach, in which bionanocomposite arrays are pre-established on a stamp and then transferred intact to the target substrate, is based on *topographical contrast* between the feature and background regions of the pattern, rather than *chemical contrast*. Thus, the new method offers significant advantages over the conventional, directed self-assembly approach in cases when the chemical contrast is marginal or when amphiphilic or zwitterionic molecules (*e.g.* proteins) are involved. In such cases, adsorption is likely to occur on both the background and feature regions, leading to poor resolution. This effect is clearly illustrated by the much cleaner patterns seen in Fig. 3, where the SADH-containing pattern was transferred intact, than in Fig. 1, where SADH was adsorbed from solution onto both the PAMAMOS dendrimer features and the SPS background.

This research significantly extends the range of surfaces and layering constituents that can be used to fabricate 3-D, patterned, bionanocomposite structures. Such structures have a broad range of potential applications, including fabricating protein-containing microarrays for screening drug candidates, studying mechanisms of protein-mediated cell adhesion,<sup>15,16</sup> diagnosing disease states,<sup>17</sup> constructing biosensors, and investigating interactions between proteins and other molecules.

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