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Shape-Specific, Monodisperse Nano-Molding of Protein Particles

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Herein we report the fabrication of monodisperse protein particles with full control over shape, size, and composition using the PRINT process. Current state-of-the-art protein particle fabrication methods utilize a number of different physical processes, each with their own unique advantages and disadvantages. Commonly used processes include spray−freeze−drying,[1,2] micro (inverse) emulsions,[3] and supercritical solvent[4,5] processes. These traditional processes all yield polydisperse particles having sizes that usually range between hundreds of nanometers[6] to tens of microns, are spherical in shape, and often tend toward aggregation. Recently, techniques used by the microelectronics industry to fabricate patterns on the micro- and nanoscale have been adopted by the life sciences for patterning,[7–9] particle fabrication,[10] and crystallization[11,12] of a wide range of biologically relevant materials.[13] However, a significant improvement in the methodologies used in this field is required because proteins are extremely sensitive compounds that are easily denatured or degraded. Engineered protein particle fabrication with control over shape and size that preserves biofunctionality has not been realized to date.

The fabrication of "smart particles", with independent control over size, shape, composition, cargo encapsulation, surface functionality, and biodistribution,[14] has been achieved using a technique that we developed called PRINT, Particle Replication in Non-wetting Templates. PRINT is capable of generating shape-specific, monodisperse nanoparticles composed of a variety of materials including synthetic polymers, hydrogels, and active pharmaceutical ingredients.[14,15] Herein we report the utilization of PRINT to generate monodisperse, shape-specific protein particles, specifically the generation of neat insulin and albumin particles and albumin particles that contain therapeutic cargos including siRNA and paclitaxel. Paclitaxel containing albumin nanoparticles is of immense interest as a treatment for metastatic breast cancer.[16]

Insulin, albumin, and albumin mixtures with siRNA or paclitaxel were molded using PRINT to yield monodisperse micro- and nano-sized protein particles. A master template was first fabricated by selectively etching a pattern generated by photolithography into a silicon substrate to yield densely packed micro- or nano-sized features on a two-dimensional array (Figure 1A). A fluorocarbon elastomeric mold was created by pouring a photochemically curable telechelic perfluoropolyether (PFPE) onto the patterned master templates and curing it while in contact with the master template to yield a patterned PFPE PRINT mold containing the corresponding 2 μm, 5 μm, or 200 nm features as cavities (Figure 1B). Nano-molding of protein particles was achieved by a lamination technique where an aqueous protein

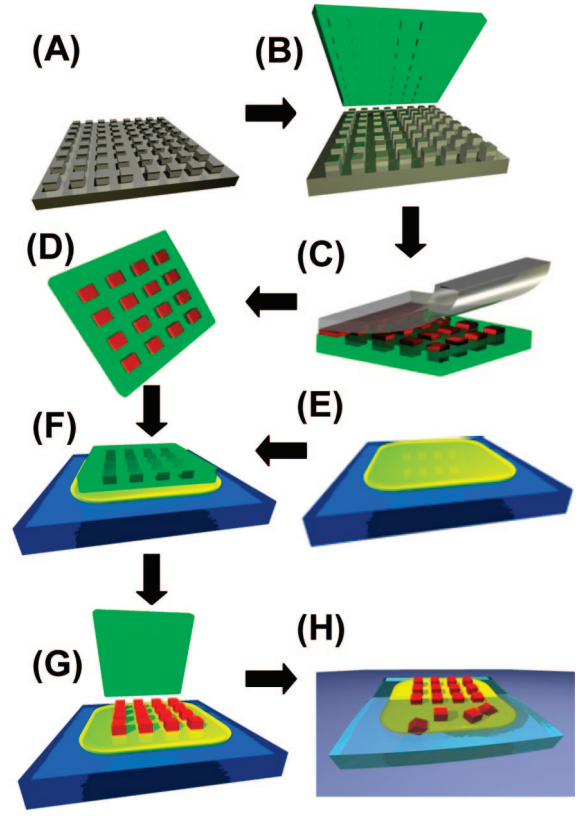

Figure 1. Illustration of PRINT nano-molding of protein particles. Silicon master template (A); mold (green) release from master template (B); nano-molding via capillary fill (protein solution red) with countersheet having a higher surface energy than the PFPE mold (C); filled mold lyophilized (D); glass slide (blue) with harvest film (yellow) (E); filled mold rolled onto harvest film (F); mold release from array of isolated features (G); dissolution of the harvesting film to yield free particles (H).

solution, typically 25 wt % protein in water (S1), was placed between the patterned PFPE mold and another film (countersheet) having a higher surface energy (Figure 1C). In this particular case, a polyethylene sheet was used as the countersheet during the PRINT lamination process. The PFPE mold, aqueous protein solution and polyethylene sandwich structure, was passed through a roller with an applied pressure of 50 psi. As the mold passes under the roller, the high energy film was peeled away, leaving a filled mold of individual cavities containing the aqueous protein solution without the formation of a flash layer between each filled cavity. The filled mold is subsequently frozen and lyophilized overnight to remove the

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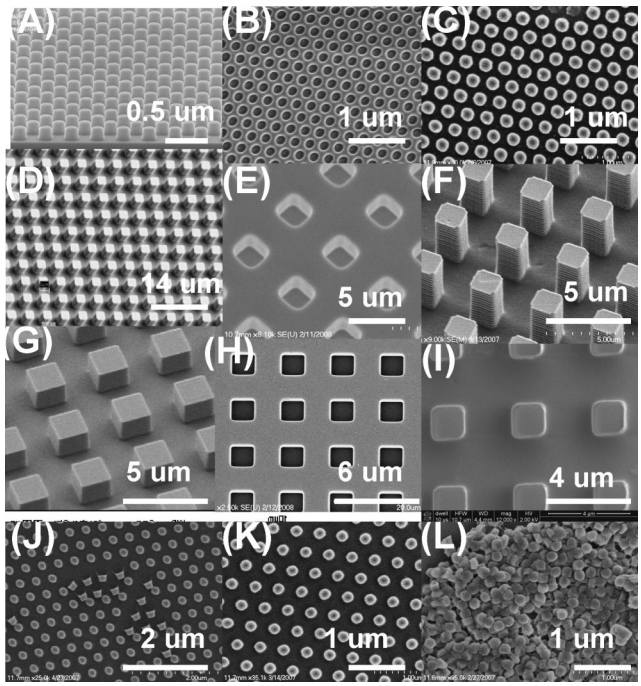

Figure 2. SEM micorgraphs of the PRINT process. (A) SEM micrograph of an etched silicon master template of 200 nm posts; (B) cured PFPE mold of the master template shown in A; (C) 200 nm albumin particles harvested on Povidone; (D) etched silicon master template of 2 μm cubes, aspect ratio 2; (D) cured PFPE mold of the master template shown in D; 2 μm insulin particles harvested on Povidone; (G) etched silicon master template of 5 μm cubes, aspect ratio 1; (H) cured PFPE mold of the master template shown in G; (I) 5 μm albumin cubes harvested on Povidone; (J) 200 nm Abraxane particles harvested on medical adhesive; (K) 200 nm albumin with siRNA particles harvested onto medical adhesive; (L) harvested and dispersed 200 nm albumin particles.

majority of the water (Figure 1D). Protein particles were harvested directly by gently moving a drop of a polar nonsolvent, such as chloroform, across the mold. Harvesting the protein particles in the form of a two-dimensional array on a film, such as medical adhesive grade poly(cyano acrylate) or on an excipient film such as Povidone (poly(vinyl pyrrolidinone)), was achieved by first casting a uniform thin film of the harvesting layer onto a glass slide using a Meyer Rod (Figure 1E). Then the patterned PFPE mold containing the protein particles was run through a roller, pattern side down, onto the liquid harvesting film (Figure 1F). Once the harvesting film was dried (in the case of using a Povidone harvesting film) or polymerized (if using the cyano acrylate harvesting film), the filled patterned mold was peeled away from the harvesting film to yield a two-dimensional array of protein particles (Figure 1G). During this harvesting step, the protein particles were transferred from the patterned PFPE mold onto the harvesting film in an essentially quantitative manner. By dissolving the harvesting film, individual protein particles could be collected (Figure 1H).

PRINT molds represent hundreds of billions of individual volume controlled lyophilization chambers. Shown in Figure 2 are SEM micrographs of master templates, molds fabricated from the templates and protein particles thereof collected on a harvesting layer. Fluorescent dye was added to 5 μm albumin particles to monitor dissolution in water using optical and fluorescent microscopy (Figure 3). Images were taken of particles harvested on Povidone before (Figure 3A,B), during (Figure 3 C,D), and after (Figure 3E,F) addition of water. The nano-molding of these various protein particles does not appear

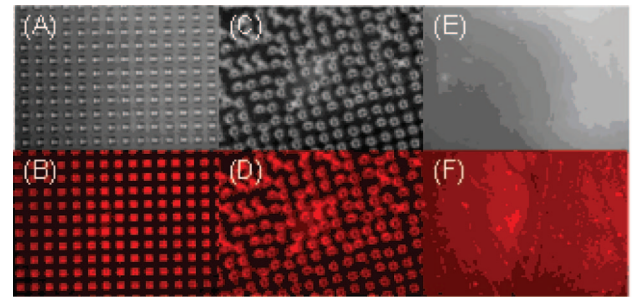

Figure 3. DIC and fluorescent images of 5 μm albumin particles with dye harvested onto Povidone (A,B) after adding water to watch dissolution (C,D) and after full dissolution (E,F).

to cause aggregation or cross-linking of the protein based on dissolution characteristics.

In summary, our approach for nano-molding proteins offers a facile, rapid, and gentle fabrication process for shape-specific, monodisperse particles from any protein. Currently, this approach is being expanded to therapeutic drug delivery platforms and roll-to-roll fabrication processes. Ongoing efforts are investigating numerous other proteins, hormones, antibodies, and enzymes and the physical and biofunctional characterization thereof.

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Supporting Information Available: Protein lyophilization and particle characterization details, albumin with siRNA and Abraxane formulation preparation, and fluorescent microscopy images. This material is available free of charge via the Internet at http://pubs.acs.org.

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