Chapter 15

Micromolding for the Fabrication of Biological Microarrays

Ashley L. Galloway, Andrew Murphy, Jason P. Rolland, Kevin P. Herlihy, Robby A. Petros, Mary E. Napier, and Joseph M. DeSimone

Abstract

The PRINT® (pattern replication in non-wetting templates) process has been developed as a simple, gentle way to pattern films or generate discrete particles in arrays out of either pure biological materials or biomolecules encapsulated within polymeric materials. Patterned films and particle arrays can be fabricated in a wide array of sizes and shapes using Fluorocur® (a UV-curable perfluoropolyether polymer) from the nanometer to micron scale.

Key words: PRINT® process, Molding, Biological microarray, Nanoarray fabrication, Proteins, Oligonucleotides, Gene delivery, Nanoparticles

1. Introduction

Arrays of proteins and nucleic acids have found many applications from fundamental research to medical devices and drug delivery (1, 2). Proteins and DNA arrays used in high-throughput analyses allow rapid analysis of gene or protein expression either for diagnostics or fundamental biological research (1). Also, patterned 2D and 3D films of biomolecules can be used as substrates for cell growth and tissue engineering (3).

Novel techniques developed for material science have now found utility in these life sciences applications with the ability to produce micro- and nanopatterned films and arrays. Beyond patterned surfaces, adaptations of soft lithography have recently been developed that can lead to the formation of discrete particles with controlled size and shape. Even arrays of pure biological molecules or mixtures of biological molecules in polymers can be used to generate discrete, size- and shape-controlled particles for drug delivery (4).

Ali Khademhosseini et al. (eds.), *Biological Microarrays: Methods and Protocols*, Methods in Molecular Biology, vol. 671,
DOI 10.1007/978-1-59745-551-0_15,

1.1. Background of Patterned Surfaces

The majority of soft lithographic methods used for preparing arrays of biological materials employ polydimethylsiloxane (PDMS) molds. Crosslinked PDMS is a good material for these applications due to its low toxicity, high flexibility, gas permeability, and low surface energy. Microcontact printing or microtransfer molding uses patterned PDMS brought into contact with a polymer, protein, or DNA solution followed by transfer onto a suitable substrate (1, 3). The channels or cups of a mold can be filled using capillary forces between the fluid and the mold, which creates a pattern of the molded material on a suitable substrate after evaporation or curing step (3, 5). Also of note are the step and flash techniques using fluorinated glass stamps to create a patterned film of DNA or proteins entrapped in a curable matrix, which is then subjected to oxygen plasma to remove excess polymer between the features (6).

1.2. Breakthrough for Particle Generation

While PDMS molds are utilized with great success in preparing patterned arrays, alternative mold materials, such as crosslinked perfluoropolyethers (PFPE) (Fluorocur® molds, Liquidia Technologies, Inc.) can provide distinct benefits in creating patterned films and discrete particle arrays. PFPE materials (7, 8) have three clear-cut advantages over PDMS based materials: (1) PFPE's have an extremely low surface energy, thus allowing for complete filling of the mold cavities without wetting the land area above the cavities. This results in the formation of discrete particles without an inter-connecting flash layer. (2) PFPEs are non-swelling to organic liquids; therefore, providing greater flexibility in design of particle arrays, enabling one to engineer in surface chemistries, degradation characteristics, and deformability. (3) PFPEs naturally have Teflon™-like characteristics allowing for an easy removal of the particle arrays from the PFPE mold. These characteristics, along with Fluorocur resin's ability to replicate features down to the nanometer scale make it an ideal material for use in molding.

The PRINT® (pattern replication in non-wetting templates) platform technology utilizes the molding advantages of PFPE to offer precise engineering of feature size, shape, composition, and functionality (4, 9–16) Unlike many other nanoparticle and nanoarray fabrication techniques, the PRINT process is versatile and mild enough to be compatible with virtually any biological materials. In fact, the PRINT technology is so gentle, that it can be used to mold polymer micelles, carbon nanotubes, and even biological material such as adenovirus with 0.4 nm resolution (10). Moreover, the inherent mild conditions of the PRINT process can be leveraged to fabricate nanoarrays of pure biological materials (4) or blends of GRAS materials and proteins or oligonucleotides (9, 14, 17). The beauty of the PRINT technology is its flexibility which allows for the solidification of molded materials by lyophilization,

solvent evaporation, photocuring, thermal curing, or crystallization inside the individual cavities of a Fluorocur mold (4, 9–17). These discrete objects inside a mold can be harvested onto a substrate, yielding a nanoarray of biological or organic materials. The PRINT technology has been used to make discrete particles in an array out of pure biological materials such as insulin and albumin (4). Protein dissolution experiments are identical between native protein and molded protein indicating no crosslinking or aggregation occurring during particle fabrication in the PRINT process (4).

Nanoarrays of particles containing biological material as a cargo have been generated using the PRINT process. Particles in these arrays are typically fabricated from biocompatible materials such as poly(ethylene glycol) (PEG), poly(lactic-co-glycolic acid) (PLGA), or poly(vinyl pyrrolidone) (PVP) combined with a bioactive oligonucleotide (9). Oligonucleotides including ssDNA, pDNA, siRNA as well as proteins have been successfully entrapped as a cargo within PRINT particle arrays. Proof of this encapsulation can be observed by fluorescence microscopy when fluorescently labeled proteins or oligonucleotides are molded in these types of polymer matrices. Functional cell-based assays can then be used to test for nucleic acid activity.

Moreover, the PRINT process allows for direct conjugation (17, 18) of ligands such as proteins, antibodies, carbohydrates, and peptides to the surface of each individual particle in the array. For direct conjugation, nanoarrays must be designed to incorporate a reactive species, such as a primary amine, on the surface of each discrete particle. Surface primary amines can easily be conjugated to a variety of electrophilic molecules, often sold as kits from biochemical vendors. Alternatively, avidin can be attached to the reactive species on the particle's surface followed by exposure to any biotinylated species.

Potential applications of nanoarrays of precisely molded bioactive materials are virtually endless (17) Surface ligands can be used to illicit an immune response, to probe cell-specific interactions, or study cell adhesion. In fact, these surface-modified nanoarrays can be used to probe everyday biological interactions and recognition events. In healthcare, these biological arrays can be used to develop treatments by pathogen detection and characterization, to evaluate and diagnose disease susceptibility and progression with protein–protein and protein–ligand interactions, and to discover potential therapeutic targets faster and more accurately than present techniques (17). In genetics, arrays of biomolecules or bioactive ligands could be used to determine special biomarkers in serum or urine for personalized medicine; genetic identification, and even forensics. If the arrays are harvested, the resulting monodisperse, discrete particles can be used as non-viral gene delivery vectors or shape-specific biosensors (5).

2. Materials

2.1. Materials Needed for Mold Fabrication

Fluorocur® resin was received from Liquidia Technologies, Inc. 2,2-diethoxyacetophenone photoinitiator was purchased from Aldrich and the ELC-4001 UV lamp ($\lambda = 365$ nm at >20 mW/cm^2) was purchased from Electro-lite corporation.

2.2. Particle Components, Solvents, and Reagents for Monomer Synthesis

Major components of particle synthesis include trimethyloylpropane ethoxylate triacry late (MW = 428 g/mol) (Aldrich); poly(ethylene glycol) monomethylether monomethacrylate (MW = 1,000 g/mol) (Polysciences); Poly(ethylene glycol) diacrylate (MW = 400 g/mol) (Aldrich); bis(ethyl methacrylate)disulfide was prepared as previously described (19); 2-aminoethylmethacrylate hydrochloride (AEM. HCl) (Aldrich); and acryloxyethyltrimethylammonium chloride (Aldrich). Components such as fluorescein-*o*-acrylate (Aldrich) and Polyfluor 570 (Polysciences) are incorporated for a fluorescent tag on the particles. DEAP, or 2,2-diethoxyacetophenone (Aldrich) is used as a photoinitiator. In preparing reactive monomers, 1,1′ carbonyl diimidazole (Aldrich) and Poly(ethylene glycol) monomethacrylate (MW = 485 g/mol) (Polysciences) are used. Common solvents for particle synthesis include 2-propanol (Acros Organics), DMSO (Acros Organics), PBS (Ambion), Acetonitrile (Fisher Scientific), Methanol (Fisher Scientific), and *N*,*N*-dimethylformamide (Acros Organics). Biomolecules used as cargo have included Albumin (Sigma), fluorescein-isothiocyanate-labeled avidin (68 kDa) (Sigma), Cy-3-labeled avidin (68 kDa) (Sigma), and DNA oligonucleotide 18 mer, sequence GCT ATT ACC TTA ACC CAG containing a 3′ fluorescein label (synthesized at Lineberger Comprehensive Cancer Center Nucleic Acids Core Facility at UNC).

2.3. Harvesting Materials

Filters used to filter solvents or particles include 0.22-mm PTFE filter (Millipore) and 25 μm pore size filters (Fisher Scientific). Particles can be collected on 100 nm pore size PVDF centricon tubes (Millipore). Removing particles from the mold can be accomplished with poly(cyanoacrylate) (Aldrich) or mechanical force with Large Glass Microscope Slides (Fisher Scientific) and solvents such as acetone (Fisher Scientific), chloroform (Fisher Scientific), or water (Ambion).

2.4. Reagents for Surface Treatment of Particles

Particles with reactive end groups can be post-treated with Alexa-Fluor 488-labeled streptavidin (Invitrogen) or ethanolamine (Aldrich). FITC-biotin (Invitrogen) can then be reacted with avidin on the particle surface.

2.5. Reagents for Particle Degradation

If the particle composition includes disulfides, they can be cleaved with dithiothreitol (Acros).

3. Methods

3.1. Description of the PRINT Process (9)

As mentioned above, the PRINT process (or Particle Replication in Non-Wetting Templates) is the first general method to accurately and gently mold size- and shape-specific features from most any material (9). This process begins with the fabrication of a Fluorocur mold from a patterned surface (Fig. 1), such as a nanopatterned silicon master generated by traditional imprint lithography techniques (9). Fluorocur molds can then be filled with a variety of organic materials comprised solely of biomolecules, mixtures of biomolecules and GRAS materials, or even polymers alone (Fig. 2). This particular example describes the fabrication of nanoarrays comprised of poly(ethylene glycol)-based materials.

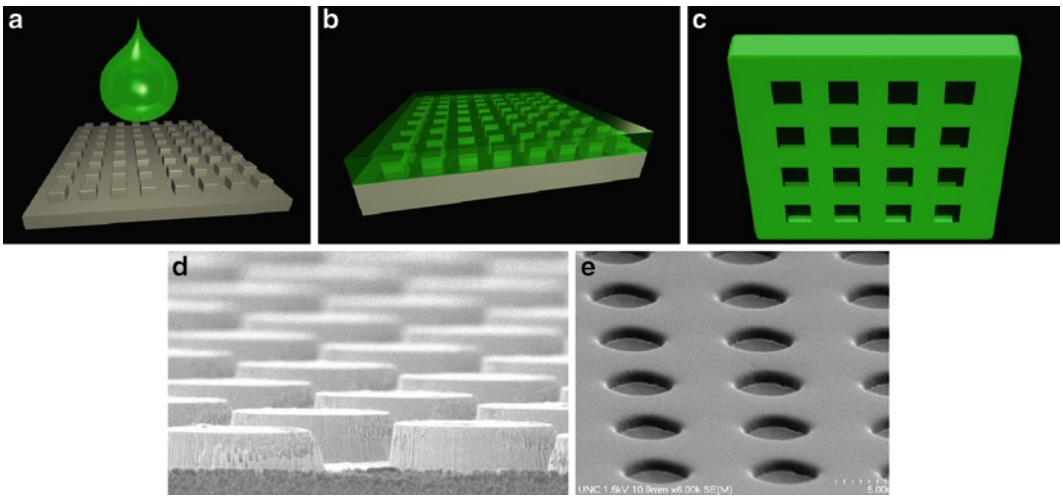

Fig. 1. (**a**) Fluorocur resin is poured over a patterned silicon wafer. (**b**) The resin is solidified on the wafer. (**c**) The mold is peeled away revealing a perfect imprint of the patterned silicon wafer. (**d**) SEM image of a patterned silicon wafer. (**e**) SEM image of a Fluorocur mold generated from the patterned silicon wafer on the *left*.

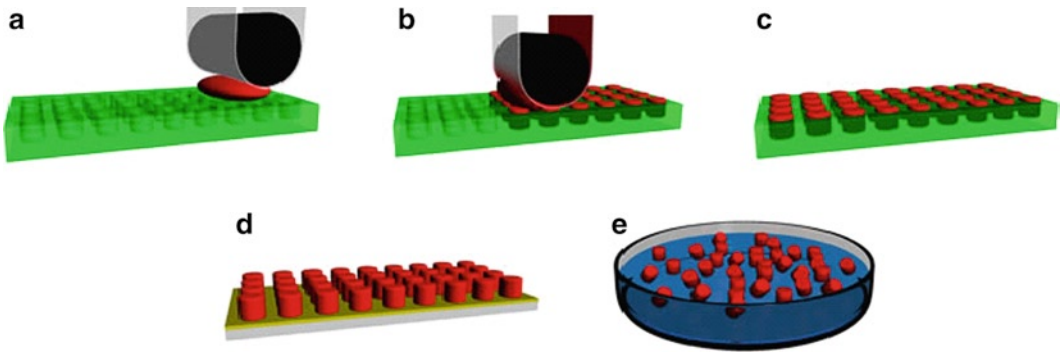

Fig. 2. (**a**) First, the material to be molded is spread evenly across the mold using a roller. (**b**) The excess material is removed from the mold surface, leaving filled cavities without any material on the land area of the mold. (**c**) The liquid in the mold cups is solidified. (**d**) Discrete particles are harvested from the mold, producing a nanoarray on a sacrificial adhesive layer. (**e**) The nanoarray can be disrupted by dissolving the adhesive layer, leaving monodisperse nanoparticles in solution.

3.1.1. Fabrication of a Fluorocur Mold (11)

1. 20 mL of Fluorocur® resin (Liquidia Technologies, Inc.) combined with a photoinitiator, such as 0.1 wt% 2,2-diethoxyacetophenone, is poured over the surface of an 8 in. silicon master containing 200 × 200 nm cylindrical posts and allowed to wet the surface completely.
2. The resin, now completely covering the master, is purged with nitrogen gas for 3 min and then subjected to UV light ($\lambda = 365$ nm at >20 mW/cm^2) while remaining under a nitrogen gas purge for two additional minutes.
3. The cured resin can be easily peeled off of the silicon master, yielding a mold with a perfect imprint of the patterned surface (Fig. 1).

3.1.2. Fabrication of Particles in an Array from a Fluorocur Mold (11, 13, 20)

1. A mixture containing 67% trimethyloylpropane ethoxylate triacrylate (MW = 428 g/mol), 20 wt% poly(ethylene glycol) monomethylether monomethacrylate (MW = 1,000 g/mol), 10 wt% 2-aminoethylmethacrylate hydrochloride (AEM HCl), 2 wt% fluorescein-*o*-acrylate, and 1 wt% 2,2-diethoxyacetophenone was diluted to 10 wt/vol% solution in 2-propanol.
2. This solution is then deposited onto a mold containing 200 × 200 nm cylindrical cavities, laminated with a polymer sheet, and the polymer sheet is removed to yield filled mold cavities.
3. The filled Fluorocur mold is purged with nitrogen for 2 min followed by exposure to 365 nm UV irradiation at >20 mW/cm^2 for an additional 2 min.
4. After curing, the particles are removed from the mold using a medical adhesive such as poly(cyanoacrylate). Images of nanoparticle arrays (9) of various sizes composed of polyethylene glycol based materials are shown in Fig. 3.

3.2. Methods for Fabrication of Protein Arrays and Particles (4)

1. A 25 wt% of albumin was prepared by dissolving 25 mg lyophilized powder with 75 μL H_2O.
2. This solution was spotted directly onto a Fluorocur mold (patterned with 200 × 200 nm cylinders) at the contact point of the patterned molded and an unpatterned polyethyleneterephthalate film affixed at the nip point on a laminator.
3. The solution was laminated between the mold and the PET sheet at a speed 0.25 ft/min and a pressure of 50 psi. The PET and mold were separated at the far side of the nip.
4. The solvent was allowed to evaporate from the filled mold by maintaining the arrays exposure to the atmosphere.
5. To harvest discrete particles from the protein array in the Fluorocur mold, 2 mL of a non-solvent (in this case chloroform) was placed on the mold surface and the particles were removed by slowly scraping the surface with a glass slide.

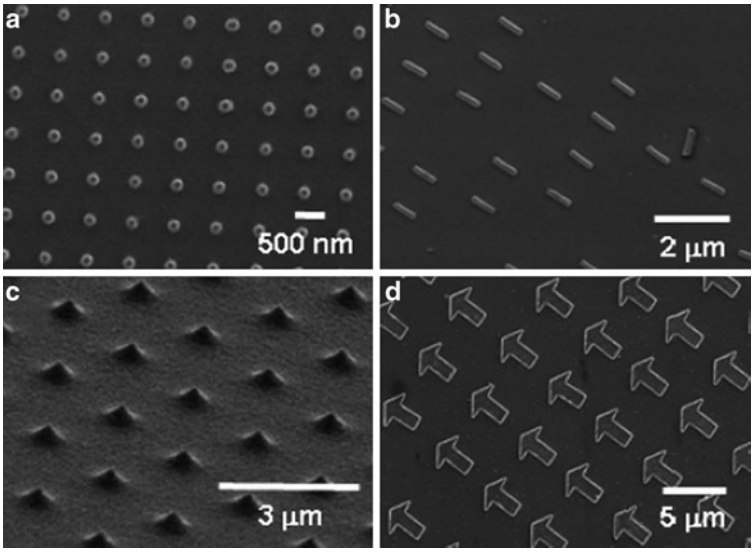

Fig. 3. PEG-based particles in various sizes and shapes (9). (**a**) 200 × 200 nm trapezoidal particles. (**b**) 200 × 800 nm rod-like particles. (**c**) Conical particles with a 500 nm base and a 50 nm tip. (**d**) 3-μm-sized *arrows.*

6. To harvest the discrete protein particles in an array on a glass substrate, a small quantity of cyanoacrylate was laminated between the surface of the filled mold and a glass slide.
7. After the polymerization of cyanoacrylate was completed, the mold was slowly peeled from the surface of the glass yield a patterned array of discrete protein particles.
8. The particles can then be released from the array by dissolving the adhesive (see Fig. 4).

3.3. Fabrication of Organic Nanoparticle Arrays with Encapsulated Protein

Encapsulation of biomolecular cargo such as proteins (9, 14, 17) is straightforward using the PRINT process. In one example, avidin is blended with PEG diacrylate and solidified to yield a protein embedded in a biocompatible polymer matrix. To make this nanoarray (9) follow the steps outlined below:

1. Dissolve 1 mg of Cy-3-labeled avidin (68 kDa) in 1 mL of water.
2. 50 μL of this solution is then mixed with 20 μL of PEG_{400} diacarylate monomer containing 1% photoinitiator.
3. This mixture is concentrated to remove the water completely.
4. Since the concentration in step 3 produces a cloudy suspension, 20 μL of water, the minimum amount of water necessary to obtain a clear solution, is added back.
5. The PEG solution containing avidin is then thinly spread across a mold containing cones with a 500 nm base and a 50 nm tip.

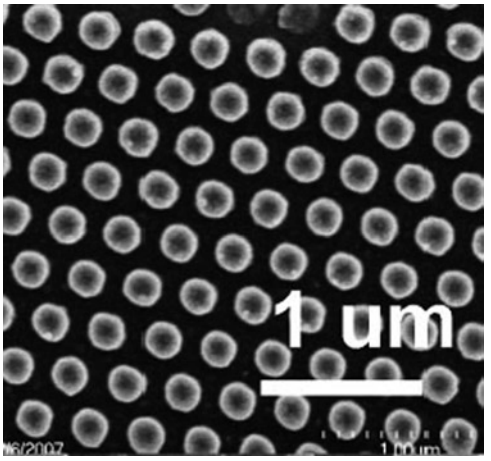

Fig. 4. Albumin particles harvested on a cyanoacrylate layer.

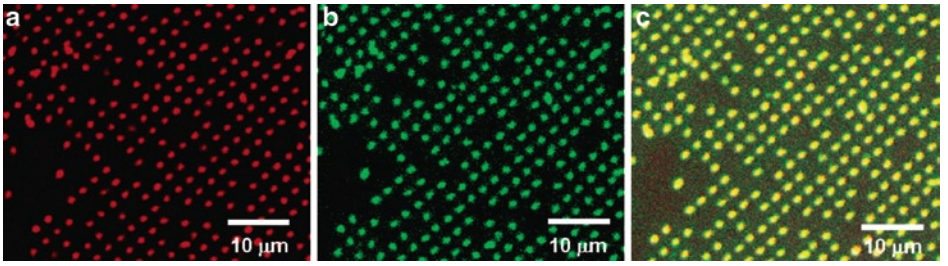

Fig. 5. Images of conical poly(ethylene glycol) particles with a 500 nm base and a 50 nm tip. (**a**) Fluorescence image of Cy-3-labeled avidin encapsulated within the PEG matrix. (**b**) Fluorescence image of FITC-labeled Biotin associated with the array. (**c**) Overlap of the two images in (**a**) and (**b**) showing that the avidin and biotin are co-localized.

6. The mold is sandwiched against a fluorinated surface and pressure (100 N/cm^2) is applied to squeeze out any excess protein/PEG glycol solution.
7. While remaining under pressure, the sample is purged with nitrogen and exposed to UV light at 365 nm for 10 min.
8. Removal of the mold left the avidin-containing nanoarray on the fluorinated substrate (Fig. 5a).

3.3.1. Activity Retained of Avidin Encapsulated in Nanoparticle Arrays (9, 14)

In an effort to demonstrate that the avidin protein maintained its integrity, an array containing Cy-3-labeled avidin-containing poly(ethylene glycol) particles were fabricated from a mixture of PEG acrylates) (3).

1. 70% PEG_{400} diacrylate and 30% PEG_{1000} monomethacrylate were mixed with Cy3-labeled avidin and molded as described above (Fig. 5a).

2. Fluorescein-isothiocyanate-labeled biotin was exposed to the nanoarray of avidin-containing poly(ethylene glycol) particles for 30 min and then washed well with water to remove any unbound biotin.
3. As evident in Fig. 5b, c, the biotin binds with the avidin in the nanoarray and is only localized on the nanoparticles not in the area in between particles.

3.4. Conjugation of Avidin to Nanomolded Poly(Ethylene Glycol) Particles (17, 18)

In addition to fabricating nanoarrays out of pure protein or mixtures of protein and polymers, it is also possible to attach proteins to the surface of biocompatible nanoarrays. In order to fabricate these nanoarrays, a suitable biocompatible material should be chosen that contains a reactive end group that can serve as a point of attachment after array fabrication. In this example, arrays of triacrylate-based particles containing a reactive carbonyl imidazole group are fabricated as described below.

1. Poly(ethylene glycol) monomethacrylate (MW = 485 g/mol) is treated with 1,1′ carbonyl diimidazole to produce the reactive monomer, PEG_{485} carbonyl imidazole monomethacrylate (18) as shown in Fig. 6.
2. A solution of 59 wt% poly(ethylene) $glycol_{428}$ triacrylate is mixed with 40 wt% PEG_{485} carbonyl imidazole monomethacrylate and 1% 2, 2′-diethoxyacetophenone photoinitiator.
3. This mixture is then spotted onto a Fluorocur mold with 200 × 200 nm cavities and covered with a plastic sheet.
4. The plastic sheet is then removed leaving a mold with filled cavities.
5. Next, the filled mold is subjected to a nitrogen purge for 2 min followed by UV exposure ($\lambda = 365$ nm, 20 mW/cm^2) for an additional 2 min.
6. The discrete particles in the mold can be removed onto a cyanoacrylate harvesting layer by first placing a drop of cyanoacrylate onto a glass slide followed by lamination of the slide to the mold surface.
7. After allowing the cyanoacrylate to polymerize (5 min), the mold is removed leaving an array of nanoparticles on the glass slide.

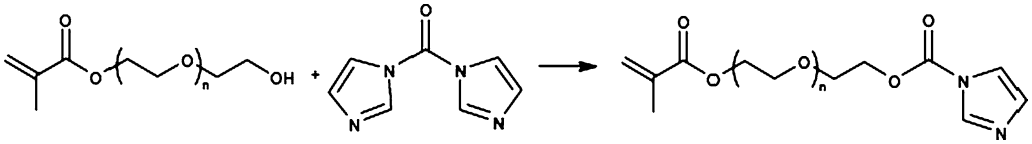

Fig. 6. Reaction of poly(ethylene glycol) monomethacrylate (MW = 485 g/mol) with carbonyl diimidazole to produce the reactive monomer, PEG_{485} carbonyl imidazole monomethacrylate.

8. If desired, the particles can remain in the array for surface functionalization or be released into solution by exposure to acetone.
9. The resulting 200 × 200 nm particles dispersed in DMSO are then exposed to a 0.7 mg/mL solution of Alexa-Fluor 488-labeled streptavidin in PBS (18).
10. After stirring for 14 h at room temperature, the particle solution was treated with ethanolamine to quench any remaining reactive end groups.
11. The particle solution was diluted 3× in deionized water, filtered through a 25 μm pore, and collected on a 100 nm pore size filter membrane.
12. After concentration via centrifugal filtration, the particles are resuspended in fresh water and imaged by fluorescence microscopy to see if the avidin conjugation was successful.
13. Alternatively, the nanoarray could be treated directly with the protein solution for a period of time, and then wash with water to obtain conjugation of the protein to the nanoparticle surface while remaining in the array.
14. If labeling of one side only is desired, it is possible to treat the single exposed side of the nanoarray while the individual particles remain in the Fluorocur mold. Treat the exposed side of the array with the protein solution for a period of time and wash with water to obtain an array of particles with one labeled side. It is of course possible to collect these particles in solution as mentioned earlier in this section.

3.5. Encapsulation of Oligonucleotides in Organic Nanoparticle Arrays

1. A solution of UV-curable monomers and a fluorescently labeled DNA oligonucleotide (18 mer, sequence GCT ATT ACC TTA ACC CAG containing a 3′ fluorescein label) was prepared by adding 2 μg of DNA in 2 μL of H_2O to a mixture of 13.65 mg of bis(ethyl methacrylate)disulfide, 1.53 mg of acryloxyethyltrimethylammonium chloride, 0.075 mg of Polyfluor 570, 0.15 mg 2,2′-diethoxyacetophenone, 2.34 mg of acetonitrile, 2.34 mg of methanol, 9.5 mg of *N*,*N*-dimethylformamide, and 0.4 mg of H_2O.
2. The mixture was spotted directly onto a 2 × 2 × 1 μm patterned Fluorocur mold and then covered with a plastic film.
3. The film was removed from the mold to leave filled mold cavities.
4. The filled mold was then subjected to UV light ($\lambda = 365$ nm) for 2 min under a nitrogen purge that was passed through a gas scrubber filled with *N*,*N*-dimethylformamide prior to entering the curing chamber.

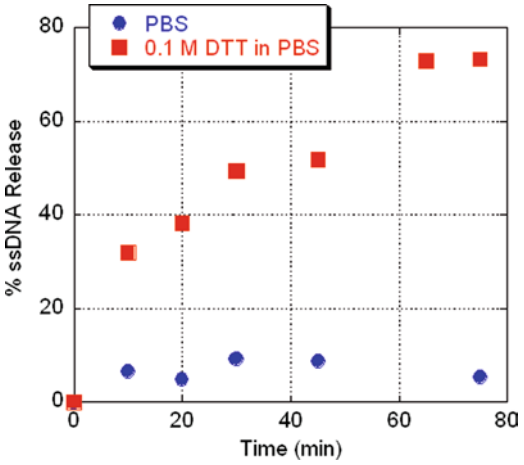

Fig. 7. Percent release of oligonucleotide from degradable disulfide PRINT™ particles.

5. The isolated polymeric particles containing DNA can be removed from the array in the mold by placing ~0.4 mL of filtered acetone (0.22-μm PTFE filter) and scrapping the surface of the mold gently with a glass slide.
6. The particle suspension was transferred to a centrifuge tube, the particles were pelleted, the supernatant removed, and the particles were dried under vacuum.
7. The particles (1.44 mg) were then suspended in 1 mL of H_2O, vortex rigorously, and pelleted out to purify.
8. The DNA can be released from the particles by treatment with 0.1 M dithiothreitol in PBS with 1–2 h. The rate of release in the absence of reductant is minimal (see Fig. 7).

Acknowledgments

The authors would like to acknowledge outstanding scientific collaborations between the Carolina Center of Cancer Nanotechnology Excellence, Liquidia Technologies, and the Chemistry Department at the University of North Carolina, Chapel Hill. Much of this work was carried out by a team of exceptional postdoctoral fellows and graduate students. This work was supported by NIH U54-CA-119343 (the Carolina Center of Cancer Nanotechnology Excellence), NIH F32-CA-123650 (Ruth L. Kirschstein National Research Service Award), PPG P01-GM059299-07 (Pharmacodynamics of Genes and Oligonucleotides), STC Program of the NSF (CHE-9876674), the William R. Kenan Professorship at the University of North Carolina at Chapel Hill, and through a supported research agreement with Liquidia Technologies.

References

1. Voldman, J.; Gray, M. L.; Schmidt, M. A. Microfabrication in Biology and medicine. *Annu. Rev. Biomed. Eng.* 1999, *1*, 401–425.
2. Truskett, V. N.; Watts, M. P. C. Trends in imprint lithography for biological applications. *Trends Biotechnol.* 2006, *24(7)*, 312–317.
3. Kane, R. S.; Takayama, S.; Ostuni, E.; Ingber, D. E.; Whitesides, G. M. Patterning proteins and cells using soft lithography. *Biomaterials* 1999, *20*, 2363–2376.
4. Kelly, J. Y.; DeSimone, J. M. Shape-specific, monodisperse nano-molding of protein particles. *J. Am. Chem. Soc.* 2008, *130(16)*, 5438–5439.
5. Torres, C. M. S.; Zankovych, S.; Seekamp, J.; Kam, A. P.; Cedeno, C. C.; Hoffman, T.; Ahopelto, J.; Reuther, F.; Pfeiffer, K.; Bleidiessel, G.; Gruetzner, G.; Maximov, M. V.; Heidari, B. Nanoimprint lithography: an alternative nanofabrication approach. *Mater. Sci. Eng. C.* 2003, *23*, 23–31.
6. Glangchai, L. C.; Caldorera-Moore, M.; Shi, L.; Roy, K. Nanoimprint lithography based fabrication of shape-specific enzymatically-triggered smart nanoparticles. *J. Control Release* 2008, *125*, 263–272.
7. Rolland, J. P.; Hagberg, E. C.; Denison, G. M.; Carter, K. R.; DeSimone, J. M. High resolution soft lithography: Enabling materials for nanotechnologies. *Angew Chem. Int. Ed. Engl.* 2004, *43(43)*, 5796–5799.
8. Rolland, J. P.; Van Dam, R. M.; Schorzman, D. A.; Quake, S. R.; DeSimone, J. M. Solvent-resistant photocurable "liquid teflon" for microfluidic device fabrication. *J. Am. Chem. Soc.* 2004, *126*, 2322–2323.
9. Rolland, J. P.; Maynor, B. W.; Euliss, L. E.; Exner, A. E.; Denison, G. M.; DeSimone, J. M. Direct fabrication and harvesting of monodisperse, shape-specific nanobiomaterials. *J. Am. Chem. Soc.* 2005, *127(28)*, 10096–10100.
10. Maynor, B. W.; Larue, I.; Hu, Z.; Rolland, J. P.; Pandya, A.; Fu, Q; Liu, J.; Spontak, R. J.; Sheiko, S. S.; Samulski, R. J.; Samulski, E. T.; DeSimone, J. M. Supramolecular nanomimetics: Replication of micelles, viruses, and other naturally occurring nanoscale objects. *Small* 2007, *3(5)*, 845–849.
11. Gratton, S. E. A., Pohlhaus, P. D.; Lee, J.; Guo, J.; Cho, M. J.; DeSimone, J. M. Nanofabricated particles for engineered drug therapies: A preliminary biodistribution study of PRINT™ nanoparticles. *J. Control Release* 2007, *121(1–2)*, 10–18.
12. http://www.chem.unc.edu/people/faculty/desimone/group/research_print.htm.
13. Gratton, S. E. A.; Ropp, P. A.; Pohlhaus, P. D.; Luft, J. C.; Madden, V. J.; Napier, M. E.; DeSimone, J. M. The effect of particle design on cellular internalization pathways. *Proc. Nat. Acad. Sci. USA* 2008, *105(33)*, 11613–11618.
14. Euliss, L. E.; DuPont, J. A.; Gratton, S. E. A.; DeSimone, J. M. Imparting size, shape, and composition control of materials for nanomedicine. *Chem. Soc. Rev.*, 2006, *35*, 1095–1104.
15. Herlihy, K. P.; Nunes, J.; DeSimone, J. M. Electrically driven alignment and crystallization of unique anisotropic polymer particles. *Langmuir* 2008, *24*, 8421–8426.
16. Petros, R. A.; Ropp, P. A.; DeSimone, J. M. Reductively labile PRINT particles for the delivery of doxorubicin to HeLa cells. *J. Am. Chem. Soc.* 2008, *130(15)*, 5008–5009.
17. PCT# WO2008\045486, Liquidia Technologies, Inc. "Nanoparticle Compositions for the Controlled Delivery of Nucleic Acids."
18. International Patent application # WO 2008/127455, Liquidia, Technologies, Inc. "Nanoarrays and methods and materials for fabricating same."
19. Li, Y.; Armes, S. P. Synthesis and chemical degradation of branched vinyl polymers prepared via ATRP: use of a cleavable disulfide-based branching agent. *Macromolecules* 2005, *38*, 8155–8162.
20. Gratton, S. E. A., Williams S. S.; Napier, M.; Pohlhaus, P. D.; Zhou, Z.; Wiles, K. B.; Maynor, B. W.; Shen, C.; Olafsen, T.; Samulski, E. T.; Desimone, J. M. The Pursuit of a scalable nanofabrication platform for use in the material and life science applications. *Acc. Chem. Res.* 2008, *41(12)*, 1685–1695.