Nanoscale replicas

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Supramolecular Nanomimetics: Replication of Micelles, Viruses, and Other Naturally Occurring Nanoscale Objects**

Benjamin W. Maynor, Isaac LaRue, Zhaokang Hu, Jason P. Rolland, Ashish Pandya, Qiang Fu, Jie Liu, Richard J. Spontak, Sergei S. Sheiko, Richard J. Samulski, Edward T. Samulski, and Joseph M. DeSimone*

Naturally occurring supramolecular objects, such as proteins, micelles, and viruses, exhibit sophisticated morphological shapes or surface motifs that conventional synthetic and fabrication techniques cannot reproduce. These structures owe their interesting shapes and shape-related properties largely to noncovalent chemical interactions that can produce unique, “evolutionarily designed” shapes with nanometer precision. These “self-assembly”-driven approaches can be tremendously successful in controlling nanoscale shape in organic and inorganic materials, but the chemical structure of each component must be carefully designed and precisely synthesized to ensure that the desired morphology is obtained. Even if the chemical design of the material is sufficient to promote the desired assembly process, factors such as temperature, solution purity, and kinetic limitations can significantly, and often detrimentally, affect the resulting morphology of “bottom-up” self-assembled materials. Alternatively, traditional micro- and nanofabrication approaches can reproducibly fabricate precise, regular shapes out of a variety of robust materials, but the morphologies are typically limited to geometric shapes and therefore cannot easily mimic the structural complexity that can be created by self-assembled organic and biological structures, such as viruses. In this work, we report a nanofabrication method that is able to reproduce shapes normally associated with self-assembly using robust nanoscale replication methods, thereby combining the morphological sophistication of the natural world with the scalable processing technologies associated with lithography.

Specifically, we use extremely low surface energy, minimally adhesive, low-viscosity, ambient-temperature photo-curable perfluoropolyether (PFPE) elastomers to replicate naturally occurring objects ("master templates"). The naturally occurring motif is replicated in the PFPE elastomer by pouring the curable PFPE resin over the master template and photopolymerizing the resin into a flexible "mold" that transfers the details of the master morphology into a crosslinked fluoropolymer (cf. Figure 1 A). Due to the extremely low surface energy (8–10 dyne cm⁻¹ depending on chemical structure) of the PFPE precursor resin, it will spontaneously spread on materials with a critical surface tension greater than 8–10 dyne cm⁻¹, which includes almost all substrates found in nature, including organic materials. In addition, owing to the chemical inertness and low solubility parameter of the resin, these PFPE molds can then be used to make replicas of naturally occurring masters from a variety of materials with good fidelity. To demonstrate the ability of this strategy to mimic self-assembled structures that are freely lying on a substrate, we chose to replicate block-copolymer micelles and virus particles. In sharp contrast to the simple geometric structures typically produced by molding technologies using man-made microfabricated master templates, this process permits high-definition replication of a wide variety of self-assembled fragile and even metastable transient nano-objects, which may have applications in sensing, materials science, and medicine.

By dissolving amphiphilic block copolymers, such as poly(styrene)-block-poly(isoprene) (PS-b-PI), in a solvent that is a thermodynamically good solvent for one block and a poor solvent for the other block (e.g., n-heptane), one observes the aggregation of block-copolymer molecules into micellar structures. Depending on the molecular and physical properties of the system, this diblock copolymer exhibits a variety of well-defined morphologies including

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spheres, cylinders, vesicles, and toroids. We have created master templates of these motifs by dispersing micellar solutions of PS-b-Pi copolymers in heptane onto mica substrates. A variety of master structures including 50-nm spheres, 1–10-μm-long cylinders and large (microscopic) vesicles have been prepared in this fashion. PFPE molds have been generated from these masters and used to replicate the self-assembled micelle morphologies into thin films of other materials (cf. Figure 1 and Figure S1 of the Supporting Information). Because these micelles are lying on the mica substrate, we are only able to mold and replicate one side of the micellar structure. It is important to recognize that these micelles, kinetically stabilized by a glassy PS core, are particularly fragile structures (see Supporting Information) that are held together only by non-covalent forces and are freely lying on the substrate surface.

In addition to being able to replicate micelles, this new molding technique is also able to capture metastable morphologies. Figure 1H–J shows the molding and high-fidelity replication of metastable PS-b-Pi toroidal micelles that are formed during the transformation from cylindrical micelles to vesicles. Although this motif has been observed in other block-copolymer systems, the replication of these toroids shows that it is possible to capture a metastable structure in a PFPE mold and replicate it into other materials that are stable over a much broader range of conditions. In Figure 1 J, we demonstrate replication of these toroids into robust crosslinked triacrylate resin. Notably, the “hole” in these toroidal structures measures less than 20 nm in diameter. Etching techniques could potentially be used to transfer these structural details to another material to generate nanoscale structures with interesting optical, electronic, or magnetic properties. Alternatively, these toroidal PFPE molds could be used in conjunction with particle replication in nonwetting templates (PRINT) to generate monodisperse, donut-shaped organic nanoparticles. Micropatterned surfaces have found great utility in fundamental cell-biology studies and in emerging biotech-

Figure 1. PS-b-Pi micelle replication. Depending on the block-copolymer composition, self-assembly of PS-b-Pi in heptane results in micelles with well-defined shapes. A) Schematic image depicting self-assembly of micelles and their deposition onto substrates (brown/white), molding (green/black) and replication (blue/magenta); B) spherical micelle master, prepared by self-assembly of a 39 kDa-b-94 kDa PS-b-Pi copolymer and solution deposition onto mica; vertical scale = 100 nm. C) PFPE mold of a spherical micelle master; vertical scale = 20 nm. D) Triacrylate replica of spherical micelles; vertical scale = 130 nm. E) Cylindrical micelle master, prepared by self-assembly of a 40 kDa-b-10 kDa PS-b-Pi copolymer and solution deposition onto mica; vertical scale = 300 nm. F) PFPE mold of a cylindrical micelle master; vertical scale = 200 nm. G) Triacrylate replica of cylindrical micelles; vertical scale = 300 nm. H) Toroidal micelle master, prepared by self-assembly and deposition of a 21 kDa-b-4 kDa PS-b-Pi copolymer and solution deposition onto mica; vertical scale = 45 nm. Inset: larger atomic force microscopy (AFM) image showing a collection of toroidal micelle nano-objects; vertical scale = 150 nm. I) PFPE mold of a toroidal micelle; vertical scale = 25 nm. J) Triacrylate replica of a toroidal micelle master; vertical scale = 60 nm.
technology areas such as drug delivery, tissue engineering, and cell biology. Here, we introduce a new platform for designing novel biointerfaces, where “biological shapes”, rather than geometric shapes, can be fabricated by molding biological materials with low-surface-energy PFPE materials and replicating these shapes into other materials. Specifically, we replicate adenoviruses to demonstrate the ability to reproduce complex biological motifs. The ability to replicate biological structures may provide crucial insight into the importance of shape in biology and lead to advantageous new platforms for imaging and immunotherapies, particularly if chemical cues can simultaneously be incorporated with these biologically derived morphologies. Figure 2 shows the molding and replication of dispersed adenoviruses on silicon surfaces. Again, there is no systematic degradation of the replica structures (Figure 2C) when compared to the master (Figure 2A). After molding, there does not appear to be any visible degradation of the virus particles on the master. In order to quantify the effect of PFPE molding on the adenovirus particles, we have measured the dimensions of virus particles before and after PFPE molding. Before molding, a survey of virus particles results in an average height of 60.4 ± 5.0 nm. After polymerization and removal of a PFPE mold, the virus particles on the master possess an average height of 62.4 ± 4.1 nm. These measured heights indicate that the morphology of the virus particles is largely unperturbed by PFPE molding, which suggests that PFPE molds are indeed noninteracting with biological materials. We have performed high-resolution transmission electron microtomography (TEM) using a single-tilt series to assess the similarity of the viral replicas to the known structure of adenovirus provided by previously reported cryo-electron microscopy reconstructions. Figure 2D displays a nonparametric TEM reconstruction of the adenovirus replicas formed from a 1 kDa PFPE precursor mold that exhibits subvirus structures reminiscent of the known adenovirus structure shown in Figure 2E. This “fine structure” is conserved from replica to replica. Although the atomic force microscopy (AFM) and TEM images indicate that the adenovirus master and the viral replica are of comparable size, there appears to be some systematic distortions of the replica structure relative to the adenovirus cryo-electron microscopy image, which is not surprising given the different preparatory procedures and image-processing conditions used for each image (see Supporting Information).

Although the observed magnitude of the feature heights of the masters and corresponding replicas may vary slightly due to factors such as AFM imaging errors, sample heterogeneity, or compressions of the elastomeric mold, there is no systematic breakdown in the replication efficiency when molding sub-100-nm, relatively fragile objects (see Table 1 for the average diameters and heights of masters, molds, and replicas). It is important to note that the size dispersion of the features in the masters is preserved in the replicas, which provides strong evidence that we are forming faithful impressions of the self-assembled masters. Despite the

| Table 1. Average diameters and heights of natural masters and replicas. |
|-----------------------------|-----------------|-----------------|-----------------|
|                             | Diameter [nm]   | Height [nm]     |
| Cylindrical micelle         | 110 ± 6         | 29 ± 1          | 25 ± 1          |
| Spherical micelle           | 74 ± 5          | 20 ± 2          | 22 ± 1          |
| Toroidal micelle            | 60 ± 4          | 12 ± 1          | 13 ± 1          |
| Adenovirus master           | 88 ± 7          | 60 ± 5          | 48 ± 3          |
| CNT master                  | Tip-resolution limited | 2.4 ± 1.6 | 1.7 ± 0.6[a] |

[a] 4 kDa precursor. [b] 1 kDa precursor

Figure 2. Transmission electron microtomography (TEM) images depicting molding and replication of adenovirus particles. A) AFM image of an adenovirus master, prepared by depositing adenovirus particles onto a silicon surface; vertical scale = 100 nm. B) AFM image of a PFPE mold formed from an adenovirus master; vertical scale = 50 nm. C) AFM image of a triacrylate/bisphenol A dimethacrylate adenovirus replica; vertical scale = 100 nm. D) TEM reconstruction of a triacrylate/bisphenol A dimethacrylate adenovirus replica. E) Cryo-electron microscopy reconstruction of adenovirus (reprinted with permission from Ref. [23].)
narrow size distribution of the micelles and inherent monodispersity of virus particles, we feel a more detailed population analysis would provide interesting insight and this effort is currently underway.

In previous work, we demonstrated that PFPE elastomers are capable of error-free replication of 70-nm features which at the time were some of the smallest three-dimensional (3D) structural details ever generated using nanoscale molding with soft, conformable materials. In theory though, the fundamental limit of replication in these materials is significantly lower and is dictated primarily by the molecular-scale granularity of the PFPE elastomers. To demonstrate this concept, we have used surface-grown carbon nanotubes (CNTs) as masters for molding and replicating with PFPE elastomers. Previously, there have been reports of the molding and replication of CNTs using highly crosslinked silicones. We observe similar replication fidelity using PFPE elastomers. Figure 3 shows a carbon-nanotube master composed of single-walled (diameter 1 nm) and multi-walled (diameter 2–5 nm) nanotubes and replicas produced from 1 and 4 kDa PFPE precursors. The replication fidelity is superior for the 1 kDa PFPE precursor than for the 4 kDa precursor due to the lower molecular weight between crosslinks (mesh size). This finding is similar to line-edge roughness issues encountered as a function of molecular weight in photolithography. Similar improvements in the resolution of nanomolding processes by using lower-molecular-weight precursors have been observed for other materials such as silicones. In order to demonstrate the ability to independently control surface roughness and mechanical properties, we have synthesized chain-extended PFPE precursors that maintain similar granularity at the molecular scale and surface energy, but with significantly altered mechanical properties (see Table 1 of the Supporting Information).

The level of replication reported here reflects the unique characteristics of the PFPE molding materials: they are photocurable at ambient conditions, minimally adhesive, nonswelling and conformable materials that arise from very low surface energy precursors with unparalleled wetting and spreading properties that enable nanoscale molding of objects, in some cases below 2 nm in height. These PFPE molding materials also enable the molding and replication of objects comprising isolated, weakly adhering and, in some cases, metastable “soft” nanoscale objects. These studies demonstrate the successful integration of imprint fabrication with “bottom-up” self-assembly for integrated nanopatterning, which may have applications in many diverse areas such as sensors, implantable biomaterials and medical therapies.

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carbon nanotubes · molding · replication · supramolecular chemistry

Figure 3. CNTs. A) AFM image of a carbon-nanotube master; vertical scale = 100 nm. B) PFPE mold of the nanotube master fabricated by photopolymerization of 1 kDa PFPE precursor; vertical scale = 30 nm. C) Triacrylate replica derived from the 1 kDa PFPE mold; vertical scale = 50 nm. D) PFPE mold of the nanotube master fabricated by photopolymerization of 4 kDa PFPE precursor; vertical scale = 10 nm. E) Triacrylate replica derived from the 4 kDa PFPE mold; vertical scale = 100 nm.


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Replicas of naturally occurring structures, including viruses (see image, left), micelles (center), and carbon nanotubes (right) are made from fluoro-polymer molds templated from naturally occurring objects. The successful integration of imprint fabrication with bottom-up self-assembly is demonstrated for integrated nanopatterning, which may have applications in many diverse areas such as sensors, implantable biomaterials, and medical therapies.