

## ***Particulate-like Nanoporous Microdevices for Biomedical Applications***

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A robust manufacturing protocol is proposed based on the selected integration of a number of micro- and nano-manufacturing modules to produce particulate-like polymer nanoporous microdevices (NMDs). At this stage, we have successfully developed techniques for fabricating conically-shaped nanonozzle arrays, followed by reinforcing the nanochannels with silica. With these conically-shaped nanonozzles, the electrokinetic transport of rigid particles and flexible DNA molecules was also studied in two important flows: converging flow and diverging flow.

A simple and low cost approach, Sacrificial Template Imprinting (STI), was developed and applied to the fabrication of the polymer nanonozzle array with high convergence ratios. The schematic of the STI process is shown in Figure 1(A). In this approach, a polymer sacrificial template, instead of the master, is used in the fabrication, which can be easily removed after molding and recycled. It avoids generating structural damage or defects during de-molding. Masters with an array of conically shaped nanotip were fabricated by differential wet etching of the distal faces of a coherent fiber-optic bundle composed of GeO<sub>2</sub>-doped silica core and fluorine-doped silica cladding. The tip density is around  $\sim 10^7/\text{cm}^2$  and each tip is 5  $\mu\text{m}$  high with a diameter of 50 nm on the sharp end.<sup>2, 3</sup> A two-step replication was then applied to produce polymer sacrificial templates. A PDMS mold was first served as the transition mold to generate inverted conical nanowells from the fiber-optic master by replica molding. An aqueous solution of a water soluble polymer was cast on the PDMS mold. After drying, the sacrificial template was peeled off and attached onto a flat substrate (i.e., a glass slide). A polymer solution (e.g. 10-20wt% polymethyl methacrylate in toluene) or resin (e.g. PDMS) was spun on the sacrificial template. A thin polymer layer with open channels was formed by controlling the spin conditions to ensure that the film thickness was slightly less than the height of the nanotips on the sacrificial template. After curing the resin or drying the solution, the nanonozzle array was released by dissolving the sacrificial template in water.

Figure 1B and 1C shows SEM images of the polyvinyl alcohol (PVA) sacrificial template and a polymethyl methacrylate (PMMA) nanonozzle array. The conically-shaped nanotips were around 4  $\mu\text{m}$  in height with sharp tips of 50-100 nm in diameter (Figure 1B). This PVA sacrificial template mostly replicated the original optical fiber nanotips, but with a slightly reduced tip sharpness. Figure 1C shows the SEM images of a PMMA nanonozzle array from

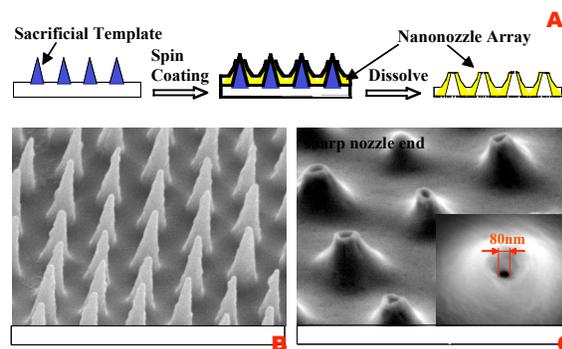


Figure 1. (A) Schematic of the sacrificial template imprinting (STI) for fabricating nanonozzle array with uniform conically shaped nanochannels. SEM images of a polyvinyl alcohol (PVA) sacrificial template (B) and polymethyl methacrylate (PMMA) nanonozzle array (C).

the sharp end. The surface of the polymer layer consists of an array of volcano-shaped nozzles, which is a result of the meniscus of polymer solution (or resin) on the PVA nanotips. Along the tip surface, surface tension is balanced with the gravity force. The final nozzle shape and channel diameter depend on the geometry of nanotips, viscosity of polymer solution or resin and the capillary effect. In Figure 1C, the height of each nanonozzle is  $3\mu\text{m}$  and the aperture size is less than  $1\mu\text{m}$ . The channel diameter is  $80\text{ nm}$  on the sharp end and  $1300\text{ nm}$  on the large end. Thus the convergence ratio of the  $3\mu\text{m}$  long channel is over 30. Nanonozzles with various nozzle heights and sizes on the sharp end were also successfully demonstrated by adjusting polymer concentration in solution (i.e., solution viscosity). Using optic fibers with a smaller diameter and adjusting the etching solution composition, smaller nanochannels with different convergence ratios can be obtained.

Limited by the height ( $\sim 4\mu\text{m}$ ) of the template nanotips, the nanonozzle membrane produced is very thin ( $< 4\mu\text{m}$ ). Moreover, many commonly used polymers are structurally unstable due to their low transition temperatures and aqueous working environment. As a result, the applications of the nanonozzle membrane may be restricted by the membrane's low mechanical strength and dimensional stability. In addition, the tip size ( $50\text{ -}100\text{ nm}$ ) of the template nanotips makes it very difficult to obtain nanonozzles smaller than  $80\text{ nm}$  in diameter. We developed an approach to solve this problem by growing silica on the internal surface of nanonozzles. This was realized via external force-induced dynamic surface reactions. Electro-osmotic flow (EOF) was used to drive molecules or their precursors into the nanonozzles and surface chemistry was used to control the growth of nanostructures on the channel wall. Such a combination provides an efficient way to further tune the channel size and reinforce the polymer nanostructure.

The schematic of the dynamic assembly process is shown in Figure 2A. The nanonozzle array was first treated to anchor a cationic polyelectrolyte, polyallylamine hydrochloride (PAH), onto the surface. The role of PAH is two-fold: it enhances the electro-osmotic flow (EOF) that moves the silica precursor into the conically shaped nanochannels and it also catalyzes silica condensation enhancing the surface reaction rate.<sup>4</sup> A dilute solution of silica source was used to suppress homogeneous nucleation in the bulk and to promote heterogeneous nucleation at the surface.<sup>5</sup> Figures 2B and 2C compare the channel diameter at the sharp end before and after the dynamic synthesis of silica. In this case, the nanonozzle channel with a diameter of  $200\text{ nm}$  is reduced to  $50\text{ nm}$ . The final structure is essentially a polymer/silica composite. Depending on the initial channel diameter, we believe that this technique has the potential to reduce the channel diameter to  $10\text{ nm}$  or less by proper control of EOF and surface reaction. Moreover, this dynamic assembly approach can add other functionalities to the nanochannel surface.

These conically shaped nanonozzles can provide two important flow patterns: converging flow and diverging flow. Both can have much higher permeate flux than analogous flow in cylindrical channels. Meanwhile, such nanochannels can fulfill the requirement of good

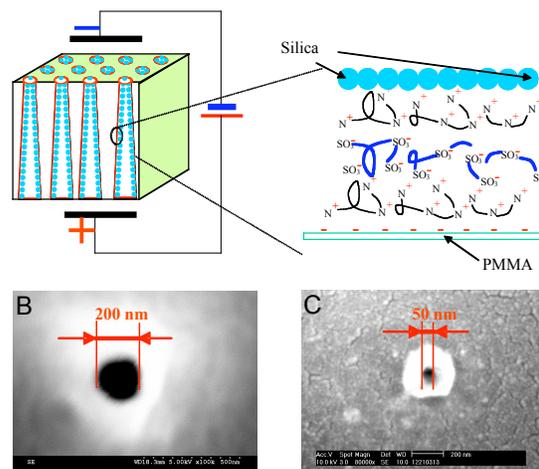


Figure 2. EOF based dynamic assembly of silica in STI nanonozzle array, ( A) process schematic; nozzle size before (B) and after (C) dynamic assembly.

sieving efficiency without the loss of high transport rate, which cannot be accomplished simultaneously using commercial sieving products. Both flow patterns were studied with electrolyte suspensions in such nanonozzle arrays. The analytes include various sizes of rigid colloid nanospheres as well as flexible polymers (i.e., DNA). An external electric field was applied to enhance the transport of electrolyte analytes.

For rigid PS nanospheres, different migration behaviors were observed in converging and diverging flows. The cumulative transport rate of 50 nm nanospheres under converging flow was almost constant after a slight increase at the very early stage. This implied that the rigid nanospheres probably blocked the conical nanochannels and were rapidly stacked, building huge transport barriers to the following nanospheres. But under diverging flow, permeate flux kept increasing, followed the prediction from Fick's law as the gradient decreased, implying that few stacking problems exist in the diverging flow case. Various size nanospheres were investigated to further explore the hindered transport under diverging flow. The cumulative transport rate of 84 nm nanospheres conforms to a linear relationship with time following the single file diffusion (SFD), while the 200 nm nanospheres were nearly 100% retained because of their equal size to the nanochannels. In contrast, large flexible molecules (i.e.,  $\lambda$ -DNA) showed different migration behaviors in these nanonozzle arrays. The  $\lambda$ -DNA used in our experiments had a radius of around 0.7  $\mu\text{m}$  (unstained) under a supercoiled configuration while being fully stretched, with a contour length of 16.3  $\mu\text{m}$  and a hydrodynamic radius of 2 nm. Thus under diverging flow, coiled  $\lambda$ -DNA cannot easily pass the nanonozzle array in a short time. But under converging flow, molecules can transport through much easier because of short moderate stretching along the 3 $\mu\text{m}$  travel distance of the nanonozzles. The tapered channels produce a high velocity gradient over a short distance, providing sufficient forces to ensure stretching of the DNA.

In summary, we have developed a Sacrificial Template Imprinting technique for fabricating polymer-based nozzle-like nanochannel arrays with high convergence ratios. The nanonozzles can be further reduced in size and mechanically reinforced by dynamic assembly of silica within the nanochannels. These polymer nanonozzles with uniform conical fluidic channels can provide two important flow patterns: converging and diverging flow. Electric field enhanced transport was studied in both flow patterns with rigid nanospheres and flexible DNA molecules. For rigid colloid nanospheres, the diverging flow showed a self-cleaning function, while the converging flow easily clogged. But for flexible polymers (i.e., DNA), the converging flow can moderately stretch the DNA chain to achieve an easy pass when the equilibrium size of molecules is much larger than the channel size. Such nanonozzle arrays have great potential in biomedical applications. On the one hand, diverging flow can fulfill the requirements of maximum permeate flux and good sieving efficiency when applied in cell-based drug delivery and biomolecular separation devices. On the other hand, converging nanochannels may be useful to assist flexible molecules (i.e., genes and polymer-biomolecular conjugates) to pass through the cell membrane by moderate stretching of biomolecules.

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