

Use of Nanosphere Self-Assembly to Pattern Ultrathin Membranes for the Study of Extracellular Vesicles

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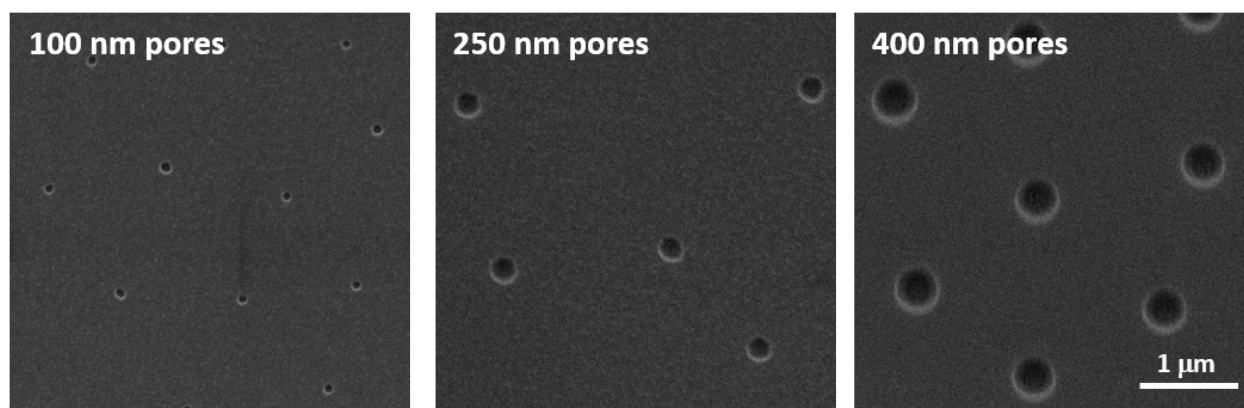


Figure S1. SiO₂ membranes. Top-view electron micrographs taken from three different SiO₂ membranes fabricated following the same procedure described for SiN in the main manuscript.

In this case, the SiO₂ was deposited by plasma enhanced chemical vapor deposition in an Applied Materials P500 tool at 390 °C and a working pressure of 1.2 kPa using tetraethoxysilane (TEOS) as precursor. After SiO₂ deposition, the stack (Si/ZnO/SiO₂) was annealed at 600 °C under N₂ flow for 1 h for stress stabilization. This SiO₂ deposition and subsequent annealing has been previously found to yield a tensile film stress of 150 MPa, stable over time.¹

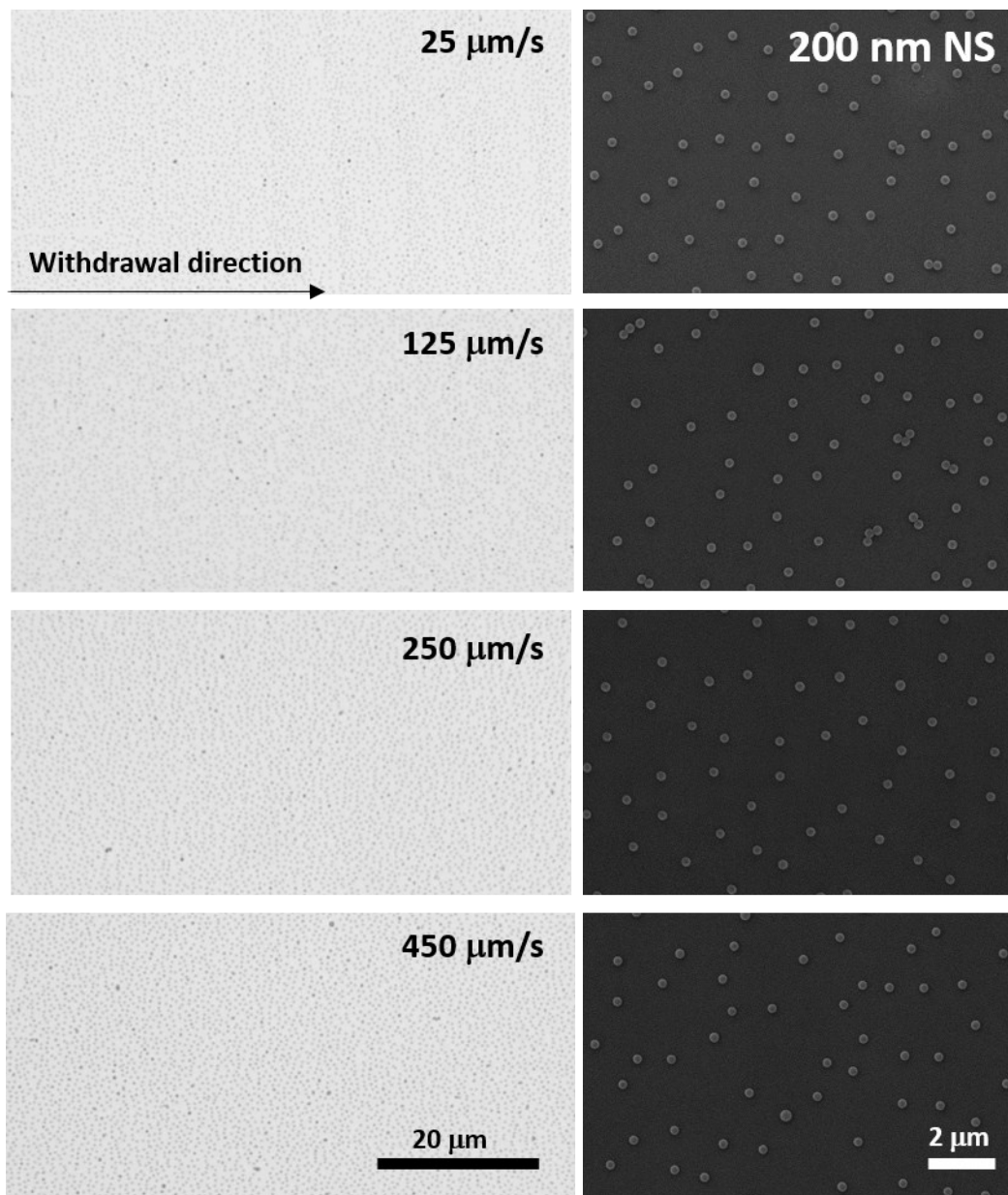


Figure S2. Withdrawal speed optimization. Optical and electron micrographs taken from 200 nm self-assembled monolayers transferred onto a substrate at different withdrawal speeds.

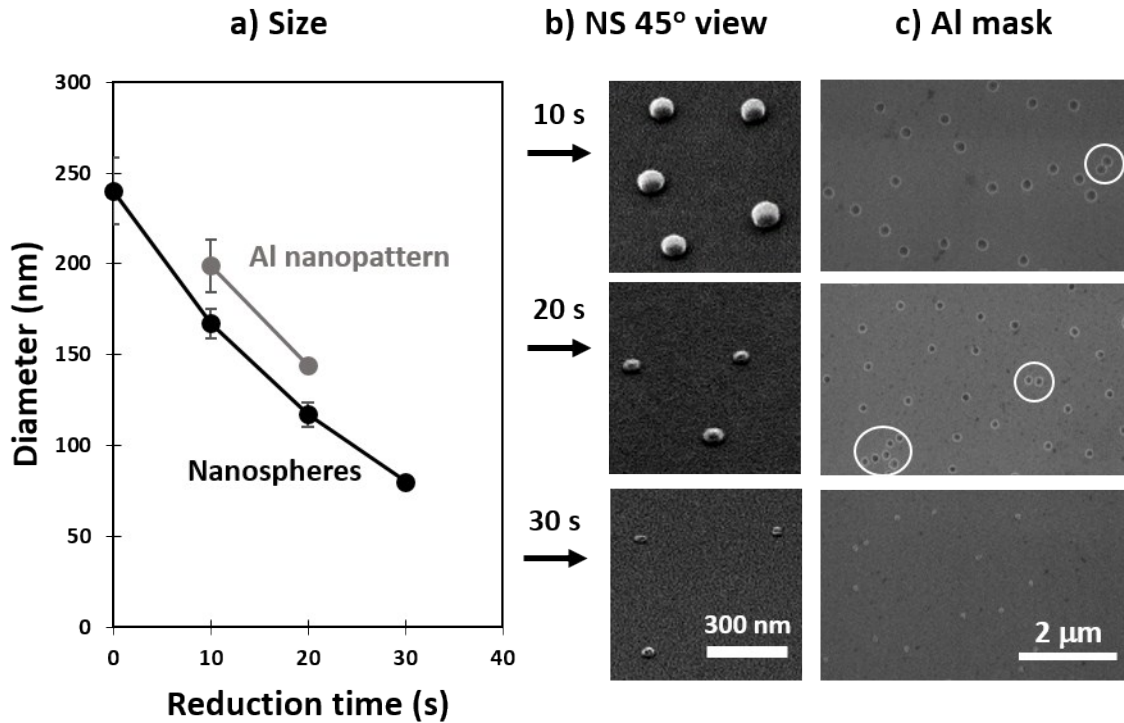


Figure S3. Nanosphere size reduction. a) diameter of the reduced nanospheres (200 nm nominal size) and the resulting nanopores in the Al mask, b) tilted-view of the reduced nanospheres, and c) the resulting pores in the Al mask and unmerging of pores.

The Al mask produced after 10 s of nanosphere reduction presents a doublet (circled in white). When reducing the nanospheres for 20 s two individual pores are observed in close proximity, these constitute a doublet that has been unmerged after reducing the size of the nanospheres.

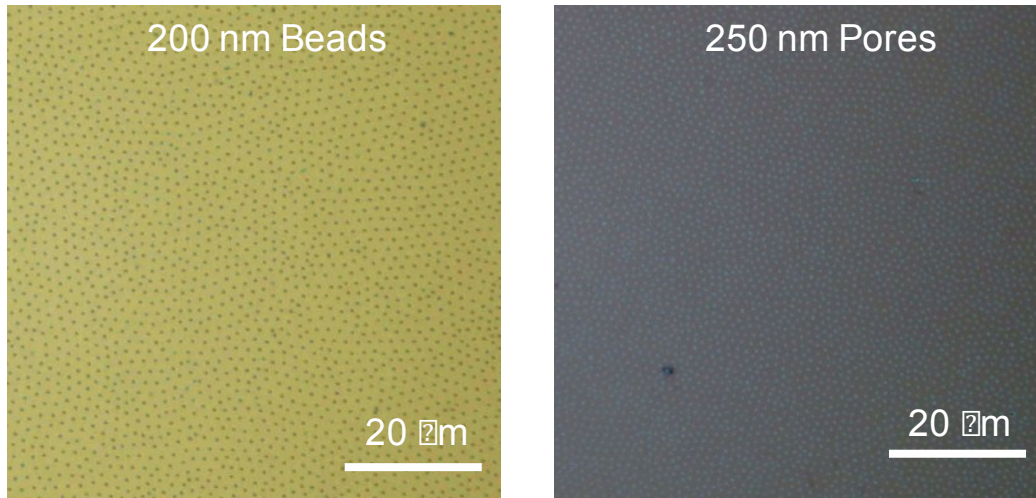


Figure S4. Optical images of beads and corresponding NSL membrane. 100x magnification optical microscopy images of 200 nm beads on a substrate and the resulting 250 nm pores following etching mask fabrication and nanoporous pattern transfer into the SiN membrane.

Supporting video 1. Membrane lift-off: following immersion in 1M HCl for sacrificial film removal, an ultrathin nanoporous silico nitride membrane is released from its supporting substrate, Si wafer, with the use of tweezers.

- (1) Carter, R. N.; Casillo, S. M.; Mazzocchi, A. R.; DesOrmeaux, J.-P. S.; Roussie, J. A.; Gaborski, T. R. Ultrathin Transparent Membranes for Cellular Barrier and Co-Culture Models. *Biofabrication* **2017**, *9*, 15019.