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Porous nanosheet wrapping for live imaging of suspension cells

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Electronic Supporting Information Contents

Supplementary Experimental Section

Flow Chamber Design: The flow chamber is homebuilt using traditional machining out of a poly(methyl methacrylate) (PMMA) block. The chamber consists of two separated parts: an upper lid and a perforated plate. The nanosheet wrapped cover slip (diameter: 25 mm, thickness: 0.12–0.17 mm, Matsunami Glass) was fixed between the two chamber parts with silicone rubber O-rings (width: 2.5 mm, thickness: 1.0 mm) on both sides. The flow chamber was mounted on the microscope stage with bolts, and connected with a syringe pump (SPS series, AS ONE) for perfusion studies. The flow rate of the feed solution in this study is 0.5 mL min^{-1}, corresponding to an apparent wall shear rate of 35 s^{-1}. The shear rate was calculated according to the formula, $\gamma = 6Qh^{-2}w^{-1}$, where Q is the volumetric flow rate, h the height of flow channel (protruding height of the silicone O-ring: 0.3 mm), and w the width of flow channel (16 mm herein).

Particle Image Velocimetry Analysis: In order to measure the effect of nanosheet wrapping on the flows inside the chamber, particle image velocimetry analysis was performed. Fluorescent nanoparticles with diameter of 200 nm (Fluoresbrite® YG Microspheres, Ex = 441 nm, Em = 485 nm) was purchased from Polysciences Inc. (PA, USA). A *ca.* 60 nm thickness nonporous nanosheet loaded with Nile red (20 μM, Ex = 552 nm, Em = 636 nm) was spin-cast on a cover slip as a height reference. The porous nanosheet was also loaded with Nile red. Then, 10 μL of HEPES buffer was placed on a cover slip, wrapped with a fluorescent porous nanosheet, and nanoparticles in HEPES buffer (1.2×10^5 particles μL^{-1}) was perfused through the chamber. Moreover, to meet the conditions in the following test on activation of platelets, the surface of the nonporous nanosheet coated on cover slip and the bottom of porous nanosheet employed for wrapping were treated with 30 mg mL^{-1} BSA (Sigma-Aldrich) as a blocking agent in HEPES buffer at 4 °C for 2 hours. The treated nanosheets were freshly used after three washes with HEPES buffer. The distribution and motion of nanoparticles was recorded with a confocal microscope.

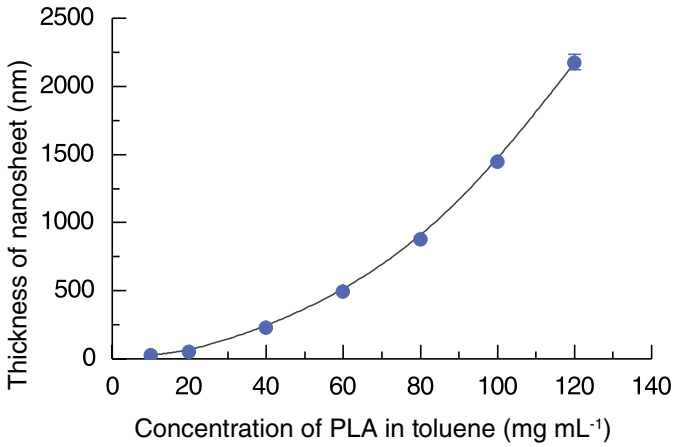

Fig. S1 Thickness of nanosheet prepared by spin-casting, plotted with s.e.m. The thickness of nanosheet is increased with increasing the concentration of PLA in toluene. Herein, the concentration of spin-casting solution is fixed to be 20 mg mL^{-1}, and the thickness of nanosheet is 60 ± 5 nm (mean ± s.e.m.).

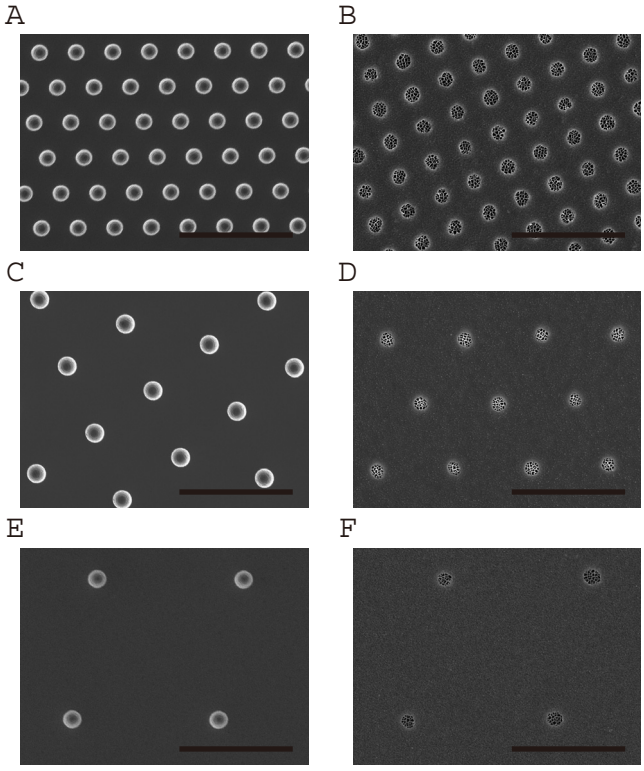

Fig. S2 Fabrication of nanopores on nanosheet by thermal nanoimprinting. (A, C, and E) Scanning electron microscope images of the cone structure of the nickel mold used for nanoimprinting with a pitch distance of 1.5, 3.0, and 6.0 μm, respectively. (B, D, and F) SEM images of the porous nanosheet prepared by the corresponding mold with different pitch distances. Herein, the pitch distance of porous nanosheet is fixed to be 6.0 μm, if not otherwise noted. Scale bars: 5 μm in all panels.

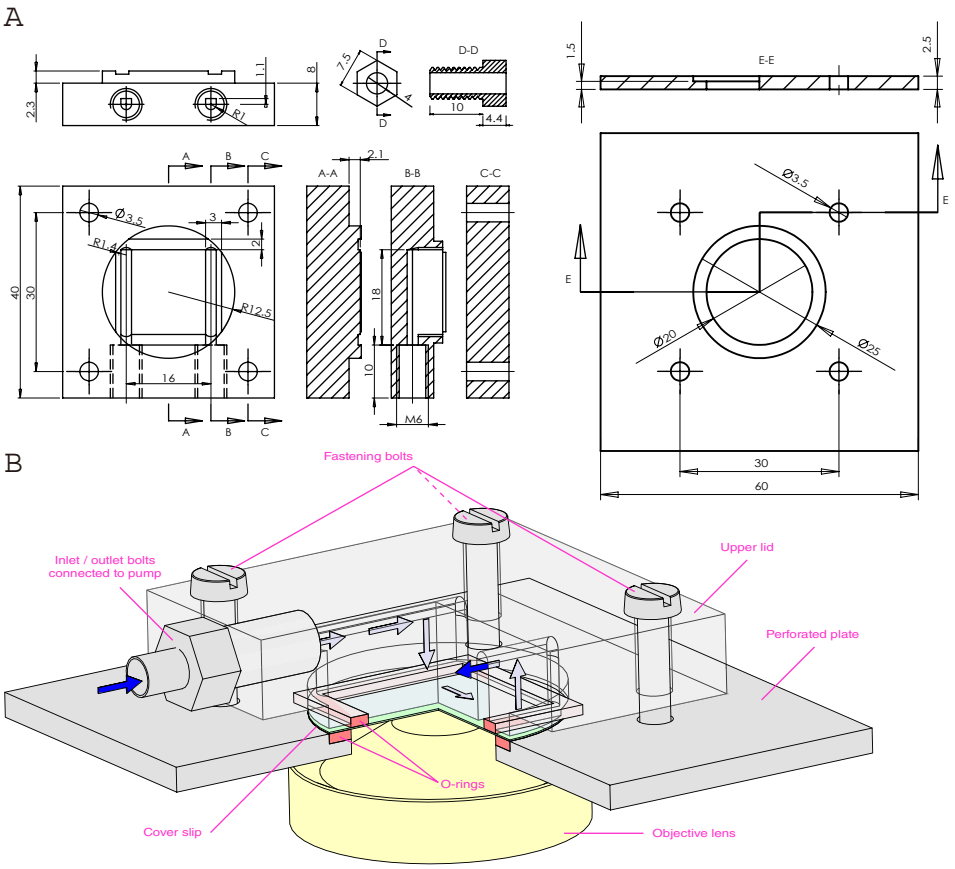

Fig. S3 Design drawing and assembly view of flow chamber. (A) Design draw of the flow chamber (unit: mm). The chamber consists of two separated parts: an upper lid and a perforated plate, which is homebuilt using traditional machining out of a PMMA block. (B) Assembly view of the flow chamber mounted with a cover slip and installed on the microscopy stage for imaging. The inlet/outlet bolts are connected to the syringe pump with flow rate of 0.5 mL min^{-1}. Blue arrows show the flow path of the perfusion. Note that the upper lid is shown semi transparent and a quarter cutaway view is applied for a better visualization.

Captions for Video S1–S11

Video S1 Trypan blue penetrates the pores on nanosheet. Left panel: Trypan blue is dripped on a floating nonporous nanosheet. Right panel: Trypan blue is dripped on a floating porous nanosheet (pitch: 6.0 μm). Video is played in real time.

Video S2 Membrane staining of liposomes without nanosheet wrapping. Left panel: brightfield. Right panel: FM 1-43 red fluorescence. The image field is 212×212 μm^2.

Video S3 Membrane staining of liposomes wrapped with a nonporous nanosheet wrapping. Left panel: brightfield. Right panel: FM 1-43 red fluorescence. The image field is 212×212 μm^2.

Video S4 Membrane staining of liposomes wrapped with a porous nanosheet with a pitch distance of 6.0 μm. Left panel: brightfield. Right panel: FM 1-43 red fluorescence. The image field is 212×212 μm^2.

Video S5 Membrane staining of liposomes wrapped with a porous nanosheet with a pitch distance of 1.5 μm. Left panel: brightfield. Right panel: FM 1-43 red fluorescence. The image field is 212×212 μm^2.

Video S6 Transferrin endocytosis of B cells wrapped with a porous nanosheet (pitch: 6.0 μm). Left panel: differential interference contrast. Right panel: Tf-488 green fluorescence. The image field is 160×80 μm^2.

Video S7 Transferrin endocytosis of B cells wrapped with a nonporous nanosheet. Left panel: differential interference contrast. Right panel: Tf-488 green fluorescence. The image field is 160×80 μm^2.

Video S8 Membrane antigen labeling of B cells wrapped with a porous nanosheet (pitch: 6.0 μm). Left panel: differential interference contrast. Right panel: Ab-594 fluorescence pseudo-colored in purple. The image field is 212×106 μm^2.

Video S9 Activation and aggregation of platelets under a flowing TRAP wrapped with a nonporous nanosheet. Video is taken *in-situ* with brightfield during the perfusion of TRAP. The image field is 140×93 μm^2.

Video S10 Activation and aggregation of platelets under a flowing TRAP wrapped with a porous nanosheet (pitch: 6.0 μm). Video is taken *in-situ* with brightfield during the perfusion of TRAP. The image field is 140×93 μm^2.

Video S11 Activation and aggregation of platelets under a flowing TRAP without nanosheet wrapping. Video is taken *in-situ* with brightfield during the perfusion of TRAP. The image field is 140×93 μm^2.