Supporting information for:

Fabrication of Sealed Nanostraw Microdevices for Oral Drug Delivery

Cade B. Fox,[1] Yuhong Cao,[2] Cameron L. Nemeth,[3] Hariharasudhan D. Chirra,[1] Rachel W. Chevalier,[1,4] Alexander M. Xu,[2,5] Nicholas A. Melosh,[2] Tejal A. Desai[1,3]*

[1] Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA 94158, U.S.A.

[2] Department of Materials Science and Engineering, Stanford University, Stanford, CA 94305, U.S.A

[3] UC Berkeley & UCSF Graduate Program in Bioengineering, UCSF Mission Bay Campus, San Francisco, CA 94158, U.S.A.

[4] Department of Pediatrics, Division of Pediatric Gastroenterology, School of Medicine, University of California, San Francisco, CA 94158, U.S.A.

[5] Current affiliation: Chemistry and Chemical Engineering Division, California Institute of Technology, Pasadena, CA 91125, U.S.A.

**Corresponding author. Email: Tejal.Desai@ucsf.edu. Phone: 415-514-4503.*

Methods

Fabrication of nanostraw microdevices. Unless otherwise noted, all materials were purchased from Sigma-Aldrich (St. Louis, MO). First, two layers of 110 mg/mL 950 kDa PMMA (MicroChem) in anisole were deposited onto a 3-inch-diameter <111> silicon wafer (Addison Engineering) by spin casting at 1350 rpm and curing at 110 °C for 1 min. Microposit S1818 photoresist (MicroChem) was spun cast over the PMMA at 2500 rpm and cured at 110 °C for 1 min. The photoresist was exposed to 225 mJ/cm^2 of UV light through a computer-designed photomask with arrays of opaque annuli (200 μm outer diameters, 100 μm inner diameters, 400 μm pitch). The photoresist was submerged in 351 Developer (MicroChem) diluted 1:3 in dH_2O for 2 min under gentle shaking. The 8 μm PMMA layer was anisotropically etched with oxygen plasma (450 W, 250 mTorr, 6.5 min) by 5.5 μm in regions not protected by photoresist. The remaining photoresist was removed by submerging in Microposit Remover 1112A (MicroChem) for 2 min and then rinsing with dH_2O. A wafer previously spun cast with 75 mg/mL 80 kDa PCL in trifluoroethanol at 1750 rpm was brought into contact with the microdevices on a hot plate at 80 °C, and the wafers were quickly separated. Nanostraw membranes were fabricated as previously described,[1] with minor modifications. Briefly, track-etch polycarbonate membranes with densities of either 10^7 cm^{-2} (AR Brown Global) or 3×10^7 cm^{-2} (GVS, Sanford, ME) and varying pore diameters were coated with aluminum oxide by atomic layer deposition, and the aluminum oxide layer was anisotropically etched by reactive ion etching with BCl_3 and Cl_2 in Argon (300 W, 40 sccm BCl_3, 30 sccm Cl_2, 5 mTorr, 5 min) on both sides of the membrane, waiting until the final fabrication step to expose the nanostraws by etching PC with oxygen plasma. The membrane was then brought into contact with the microdevices at 80 °C, melting the PCL and bonding the membrane to the devices. The membrane was spun cast with two layers

of 75 mg/mL PVA at 2500 rpm, curing at 95 °C for 1 min after each deposition. SU-8 2015 (MicroChem) was then spun cast at 1250 rpm and cured at 95 °C for 5 min. The SU-8 was exposed to 250 mJ/cm^2 of UV light through an opaque photomask with 200 μm transparent circles aligned to the microdevices and then baked at 95 °C for 5 min. The devices were submerged in SU-8 Developer (MicroChem) for 5 min under gentle shaking and dried with a nitrogen gun. The membrane overhang and remaining PMMA between microdevices were removed by etching with oxygen plasma (450 W, 250 mTorr, 40 min). The devices were rinsed in dH_2O, dissolving the PVA and allowing the SU-8 caps to detach. The nanostraws were then exposed by partially etching the surrounding polycarbonate with oxygen plasma at a lower energy (100 W, 250 mTorr, 20 min).

Device profilometry. Device height profiles were measured with an Ambios XP2 profilometer at various fabrication steps to determine nanostraw microdevice dimensions. Specifically, the height profiles of microdevices with etched PMMA after removal of photoresist, etched PMMA coated with PCL, and complete nanostraw microdevices were measured over the center of the devices. The thicknesses of the nanostraw membrane (following etching to expose nanostraws) and the PMMA base layer were also measured via profilometry. The PMMA base layer was scratched to expose the underlying silicon wafer prior to measurement. To account for the thickness of PMMA yet to be etched in remaining fabrication steps, the thickness of the PMMA base layer was added to the height profiles of the etched PMMA devices and the etched PMMA devices coated with PCL. The profile of the base of the nanostraw membrane was determined by subtracting the thickness of the nanostraw membrane from the profile of complete devices.

Quantification of nanostraw density heterogeneity at the cellular scale. Caco-2 cells were cultured as previously described to facilitate formation of a cellular monolayer.[2] SEM imaging indicated that the Caco-2 cells could be approximated as having edge lengths on the order of 10 μm. A nanostraw membrane (inner nanostraw diameter: 60 nm, nanostraw density: 10^7 cm^{-2}**)** was imaged with SEM. The SEM images were divided into 10 μm × 10 μm regions, an approximation of the dimensions of intestinal epithelial cells. The number of nanostraws contained within each of 100 analyzed regions was determined and plotted as a histogram showing the number of regions containing a given number of nanostraws.

Confocal fluorescence imaging to compare the amount of adsorbed FITC-insulin to the amount of in-solution FITC-insulin in device reservoirs. Nanostraw microdevices (inner nanostraw diameter: 60 nm, nanostraw density: 10^7 cm^{-2}**)** were incubated in 10 mg/mL FITC-insulin at 4 °C for 48 h and then washed in PBS for 1 min. The devices were imaged with confocal fluorescence microscopy, collecting images with 2 μm z-steps over the entire device reservoirs. The devices were incubated in 37 °C PBS for 24 h to facilitate drug release and imaged again under identical conditions.

Testing nanostraw microdevice retention of drug following detachment of microdevices from the silicon wafer. Microdevices were incubated in a PBS solution of 10 mg/mL FITC-insulin overnight, rinsed with PBS, and scraped from the wafer with a razor. Microdevices were then loaded into a channel formed by placing a 1.5 × 24 × 0.12 mm adhesive spacer (Grace Bio-labs) between a glass slide and coverslip. The microdevices were incubated at room temperature for approximately 30 min and then imaged with brightfield and fluorescence microscopy.

Determining if device reservoirs become saturated with FITC-dextran after incubation for 48 h. Nanostraw microdevices were incubated in 1 mg/mL FITC-dextran and analyzed with confocal microscopy as performed for the FITC-dextran influx assay, except the timepoints used were 48 and 72 h.

Determining total nanostraw microdevice drug capacity. Devices were loaded with 10 mg/mL FITC-insulin and monitored for drug release as described previously except the mass of drug released was determined at 48 h, after which time no significant drug release was detected.

Figures

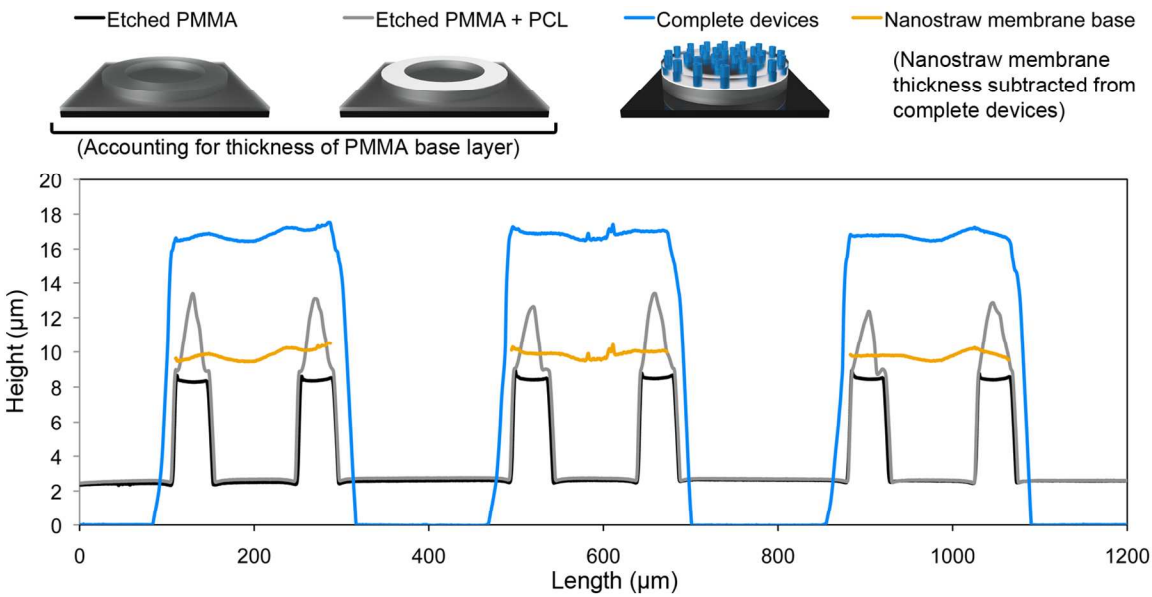

Figure S1. Height profiles of microdevices at various stages of fabrication. Height profiles show thicknesses of approximately 2.5 μm for the PMMA base layer, 7.5 μm for drug reservoirs, 7 μm for nanostraw membranes (following etching to expose nanostraws), and 17 μm for total device thickness (not accounting for nanostraw length). PCL thickness was 5 μm before nanostraw membrane bonding and 1 − 2 μm following compression during bonding.

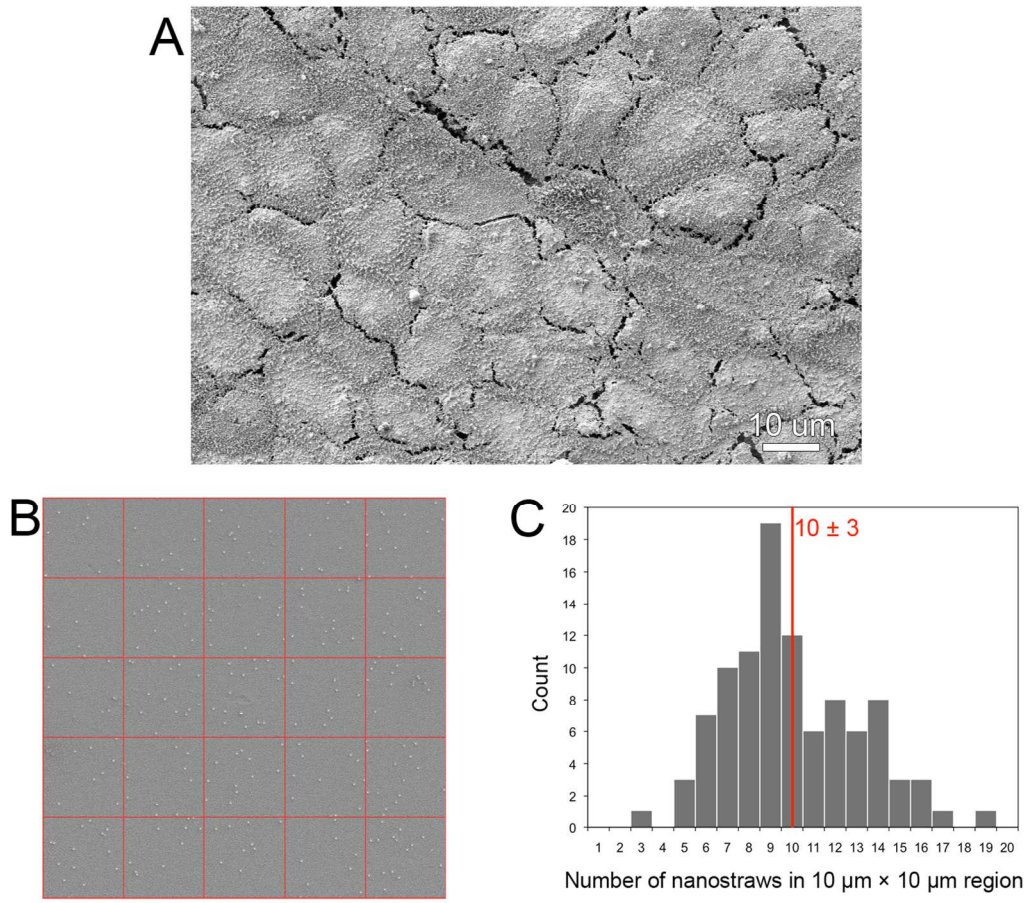

Figure S2. Quantification of heterogeneity in nanostraw density at the cellular scale. A. SEM imaging demonstrated that the cuboidal Caco-2 intestinal epithelial cells can be approximated as having edge lengths on the order of 10 µm. **B.** To determine the heterogeneity in nanostraw distribution at the cellular scale, SEM images of a nanostraw membrane (inner nanostraw diameter: 60 nm, nanostraw density: 10^7 cm^{-2}) were analyzed by quantifying the number of nanostraws within 10 µm × 10 µm regions (outlined in red). **C.** A histogram showing the number of regions containing a given number of nanostraws. There was a mean of 10 ± 3 nanostraws per 10 µm × 10 µm region (n = 100).

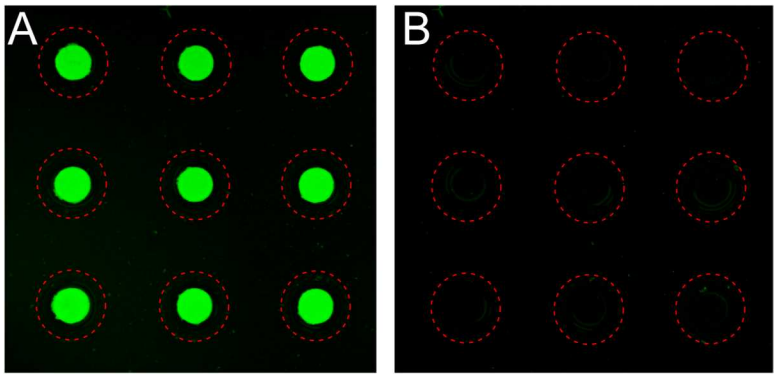

Figure S3. FITC-insulin adsorption to devices is minimal relative to the amount of FITC-insulin loaded within device reservoirs. A. Images of nanostraw microdevices loaded with FITC-insulin and washed in PBS for 1 min. show that the fluorescence intensity of FITC-insulin adsorbed onto the surfaces of the devices (outlined in dotted red lines) is much lower than that of drug in solution within the device reservoirs, demonstrating that the 1 min wash in PBS was effective in removing non-loaded insulin from the external surfaces of the devices. **B.** The devices were imaged under identical conditions after the drug had been released in PBS at 37 °C for 24 hours, and the fluorescence intensity of FITC-insulin remaining adsorbed onto the surfaces of the devices was much lower than the amount of FITC-insulin that was previously loaded into device reservoirs, demonstrating that FITC-insulin adsorption to internal device surfaces is also minimal relative to the amount of loaded drug.

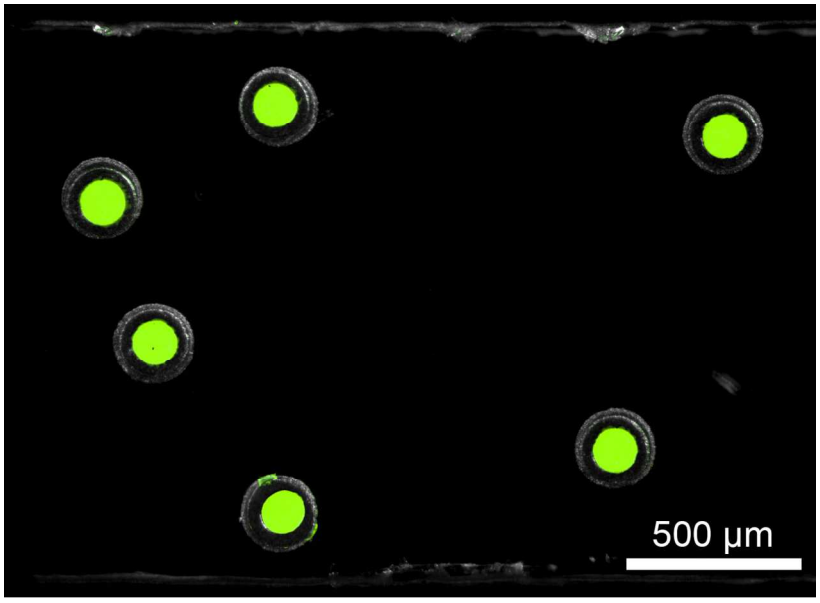

Figure S4. Loaded nanostraw microdevices retain drug after detachment from the silicon wafer. Nanostraw microdevices were incubated in a PBS solution of 10 mg/mL FITC-insulin overnight, rinsed with and submerged in PBS, and scraped from the silicon wafer with a razor. The PBS-suspended microdevices were added to a chamber formed by placing an adhesive spacer between a glass slide and a coverslip and incubated at room temperature for 30 min. The microdevices were then imaged for structure (brightfield signal, shown in grayscale) and FITC-insulin localization (fluorescence signal, shown in green). FITC-insulin remained within the device reservoirs, indicating that the devices remained sealed following detachment from the silicon wafer.

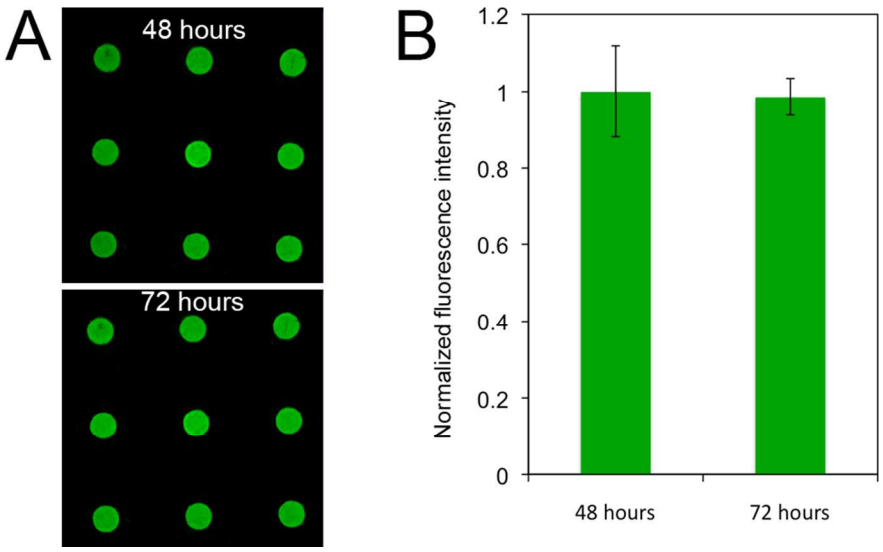

Figure S5. Device reservoirs become saturated with FITC-dextran after incubation for 48 h. **A.** Nanostraw microdevices used for the influx assay were incubated at 37 °C in 1 mg/mL FITC-dextran (10 kDa) for 48 or 72 h and then imaged with confocal microscopy. **B.** The fluorescence intensity values at 48 and 72 h were statistically similar, indicating that the device reservoirs had equilibrated with the external 1 mg/mL FITC-dextran solution by 48 h.

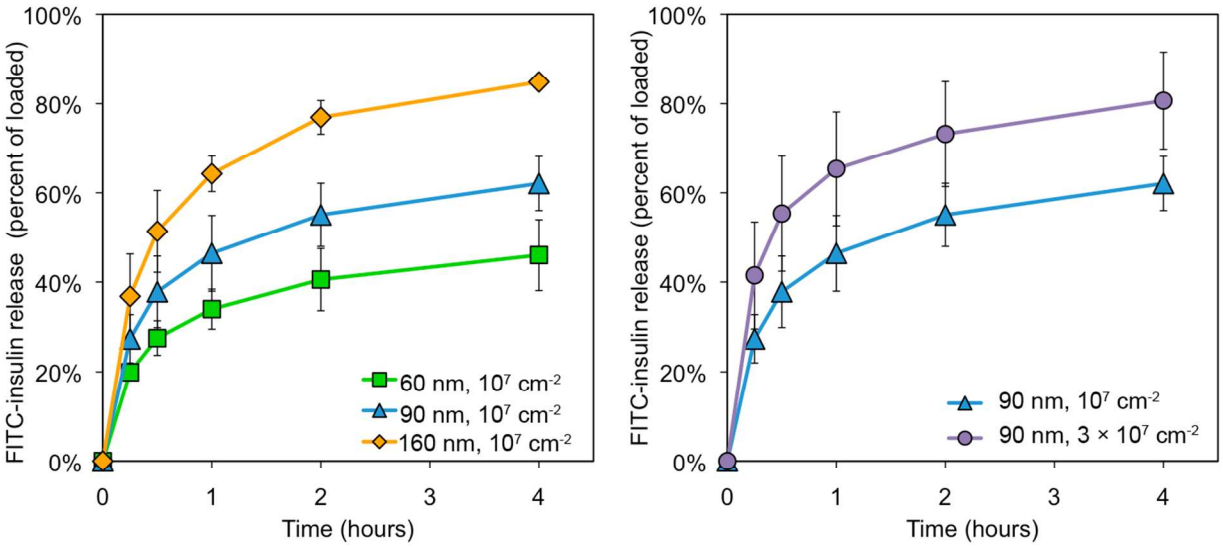

Figure S6. FITC-insulin release over time expressed as a percentage of the total amount of drug loaded into microdevices.

Supporting Information References:

1. VanDersarl, J. J.; Xu, A. M.; Melosh, N. A. Nanostraws for Direct Fluidic Intracellular Access. *Nano Lett.* **2012**, *12*, 3881-3886.

2. Kam, K. R.; Walsh, L. A.; Bock, S. M.; Koval, M.; Fischer, K. E.; Ross, R. F.; Desai, T. A. Nanostructure-Mediated Transport of Biologics across Epithelial Tissue: Enhancing Permeability *via* Nanotopography. *Nano Lett.* **2013**, *13*, 164-171.