

## **SUPPORTING INFORMATION**

### **Collection of analytes from microneedle patches**

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### **Abstract**

This Supporting Information section contains detailed methods on fabrication of microneedles patches as well as additional figures that support the results presented in the main paper.

## Supporting Materials and Methods

### Materials

Poly(methyl vinyl ether-alt-maleic acid) (PMVE/MA, MW = 1,980 kDa), poly(ethylene glycol) (PEG, MW = 10 kDa) and sulforhodamine B were obtained from Sigma-Aldrich (St. Louis, MO). Sylgard 184 Silicone Elastomer Kit (poly(dimethyl siloxane)) was obtained from Dow Corning (Midland, MI). Poly(tetrafluoroethylene) (Teflon) filters (DW232P) were obtained from DeWal Industries (Narragansett, RI). Polystyrene sheets were obtained from McMaster-Carr (Atlanta, GA). Microcentrifuge tubes were obtained from Corning Life Sciences (Tewksbury, MA). V-bottom and flat-bottom multi-well microplates Thermo Scientific (Waltham, MA).

### Fabrication of microneedles by single-casting method

To prepare microneedle patches, a polymer casting solution was made by dissolving 15 g of PMVE/MA in 77.5 mL of DI water, which was stirred for 12 h at 80°C. After cooling, 7.5 g of PEG was added, and the mixture was stirred for 12 h at room temperature (24°C).

One hundred microliters of the polymer casting solution was then poured on a poly(dimethyl siloxane) (PDMS) micromold having a 10 x 10 array of conical cavities measuring 600 µm in length, 300 µm in diameter at the base and tapering to tip with a radius of curvature of ~10 µm, prepared as described previously (1). Vacuum was applied to the mold for 30 min, which forced the casting solution to fill down into the mold's cavities. The mold was then transferred to a chemical hood and stored there for 24 h at room temperature to dry the patch. Finally, the dried hydrogel patch was stored for 24 h or 72 h at 80°C to crosslink the PMVE/MA and PEG.

A rigid polystyrene disk measuring 4 mm in radius was applied to the backside of the microneedle patch to form its backing by one of two methods. In one method, double-sided adhesive tape was cut into disks measuring 4 mm in radius and attached to one side of the polystyrene disks, which were in turn affixed to the backside of the fully-dried microneedle patches while still in their molds. In the other method, the polystyrene disks were exposed to an atmospheric air-plasma for 4 min. The disks were then immediately applied to the backside of the microneedle patches while still wet in their molds (i.e., after filling the molds under vacuum) and dried for 24 h at 80°C.

### Fabrication of microneedles containing sulforhodamine B by double-casting method

A double-casting method was developed to incorporate sulforhodamine B as a model analyte in the microneedles, but not in their base. A polymer-dye casting solution was prepared by adding sulforhodamine B to a concentration of 1 mM in the polymer casting solution described above.

To prepare the microneedle patches, Teflon filter disks were affixed on top of PDMS microneedle molds using double-sided adhesive tape. One hundred microliters of the polymer-dye casting solution was dispensed on top of the filter and the mold was centrifuged for 10 min at 5,500 x g. The filter was then removed, leaving casting solution only in the mold cavities and

not on the surface of the mold. The casting solution was dried in the molds for 24 h at room temperature

Next, the polymer casting solution (with no sulforhodamine) was cast onto the mold, exposed to vacuum for 30 min, dried for 24 h at room temperature for 24 h and crosslinked for 72 h at 80°C, as described above. Polystyrene disks were also affixed as described above to provide a rigid backing.

### **Measurement of ISF extraction from rats in vivo**

Microneedle patches were prepared by the single-casting method, as described above. Negative-control patches with blunt-tipped microneedles were also prepared by that method using molds with blunt tips.

Microneedle patches were applied to Wistar rats (~300 g, Charles River Laboratories, Wilmington, MA) that were anesthetized with isoflurane. The patches were applied to the skin manually and were removed after one hour. The skin was then stained with gentian violet (Humco, Texarkana, TX) to label sites of skin puncture. This study was approved by the Georgia Tech Institutional Animal Care and Use Committee.

Microneedle patches and negative-control patches were weighed before and after application to the skin. The average mass increase in negative-control patches was subtracted from the average mass increase in microneedle patches to determine the net mass increase in microneedle patches. This value is reported as the mass of ISF taken up by the microneedle patches.

### **Reference**

(1) Lee, J.W; Park, J.H; Prausnitz, M.R. Dissolving microneedles for transdermal drug delivery. *Biomaterials*. **2008**, 29, 2113-2124.

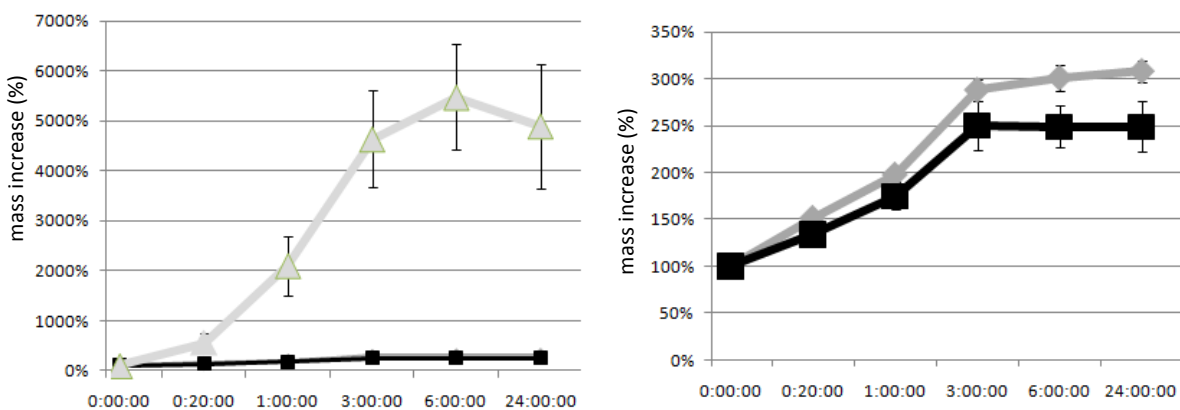


Figure S1. Swelling of microneedle patches during incubation in water. (a) Swelling of microneedle patches crosslinked before drying in the micromold (black squares) and microneedle patches crosslinked after drying in the micromold (gray triangles). Drying in both cases was for 24 h at 80 °C. (b) Swelling of microneedle patches crosslinked for 24 h (black squares) and 72 h (gray diamonds) after drying in the micromold (n = 6 replicates  $\pm$  standard deviation).

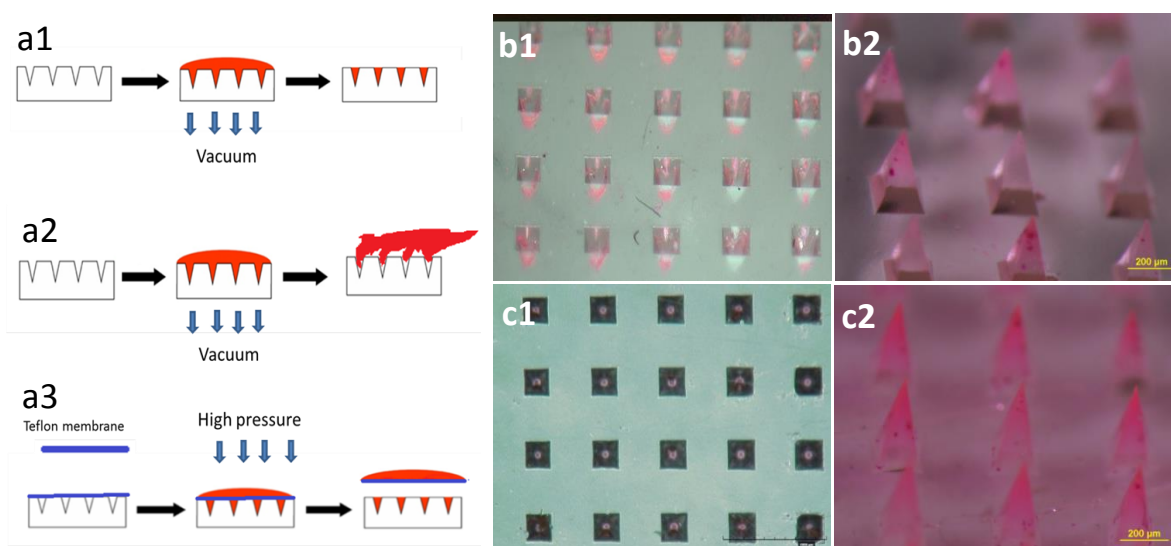


Figure S2. Molding of microneedle patches. In the fabrication process, a polymeric casting solution is cast onto a micromold, vacuum is applied to pull the solution into the cavities of the mold and then excess solution is removed from the mold surface using a razor blade. If a low viscosity casting solution is used, then the excess solution can be removed from the mold surface while leaving the mold cavities filled with casting solution (a1). In contrast, if a high viscosity solution is used, then the process of removing the excess solution from the mold surface also removes solution from the mold cavities (a2). The addition of a hydrophobic (i.e., Teflon) mask with holes aligned with the mold cavities allowed the casting solution to remain in the mold cavities even after removing the excess solution from the mold surface (a3). When casting was done with a highly viscous solution (15% PMVE/MA and 7.5% PEG) containing a pink dye (1 mM sulforhodamine), very little dye remained in the micromold cavities (b1) and resulting microneedles (b2) when the hydrophobic mask was not used, whereas much more dye remained in the micromold cavities (c1) and resulting microneedles (c2) when the hydrophobic mask was used.

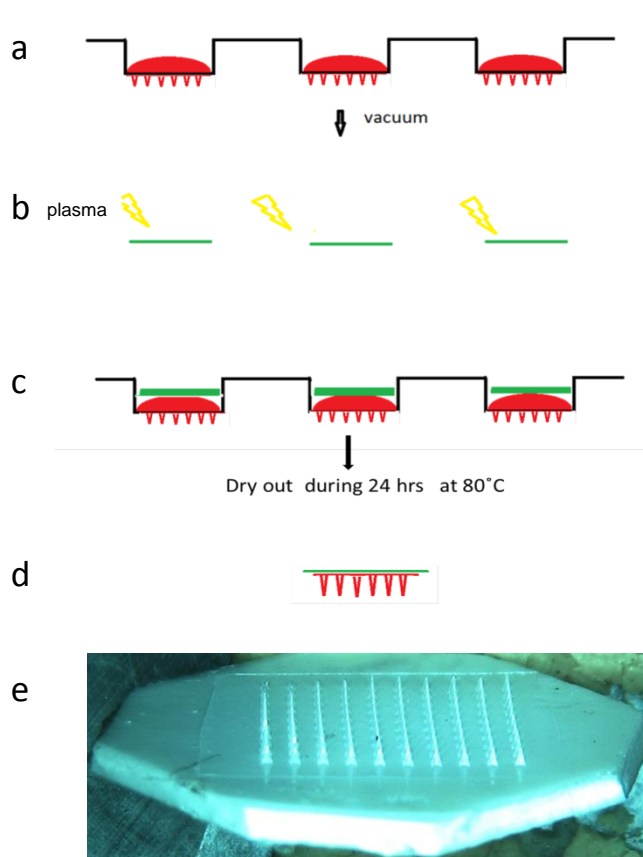


Figure S3. Attachment of polystyrene disk exposed to plasma to form hard backing of microneedle patch. (a) Polymeric solution is cast into microneedle molds. (b) Polystyrene disks are treated with air plasma for 4 min. (c) Plasma-activated disks are applied to the backside of microneedle molds in contact with high-viscosity polymeric casting solution. (d) After drying and crosslinking the polymer for 24 h at 80°C, the microneedle patches attached to the polystyrene backings are removed from the mold. (e) Magnified view of a microneedle patch attached to a polystyrene backing.

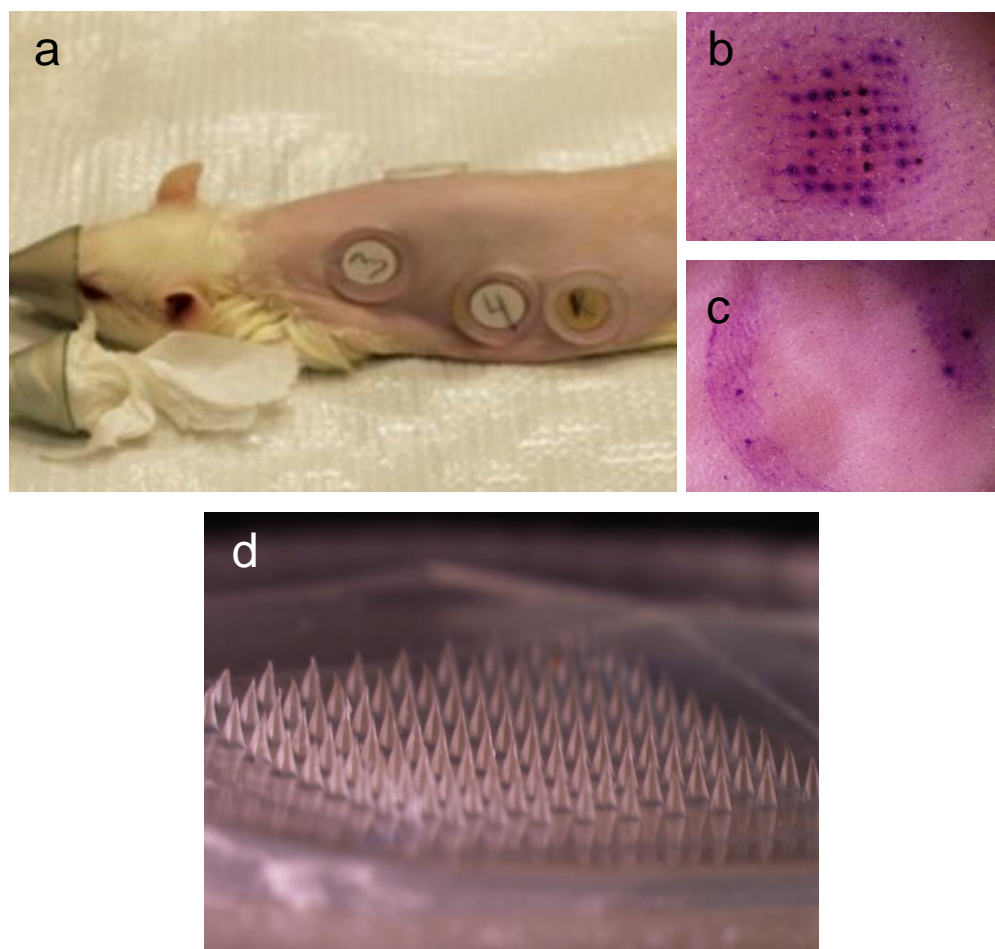


Figure S4. Microneedle patch application to rats for ISF collection. (a) Microneedle patches applied to the shaved skin on an anesthetized rat. (b, c) Magnified view of rat skin after microneedle patch application and removal, and staining with a dye (gentian violet) that labels sites of skin puncture. Image (b) shows skin after application of a sharp-tipped microneedle patch, showing microneedle puncture into skin. Image (c) shows skin after application of a blunt-tipped negative-control patch, showing lack of skin puncture. (d) Microneedle patch (sharp-tipped) after application to skin showing that the microneedles remained intact after use.

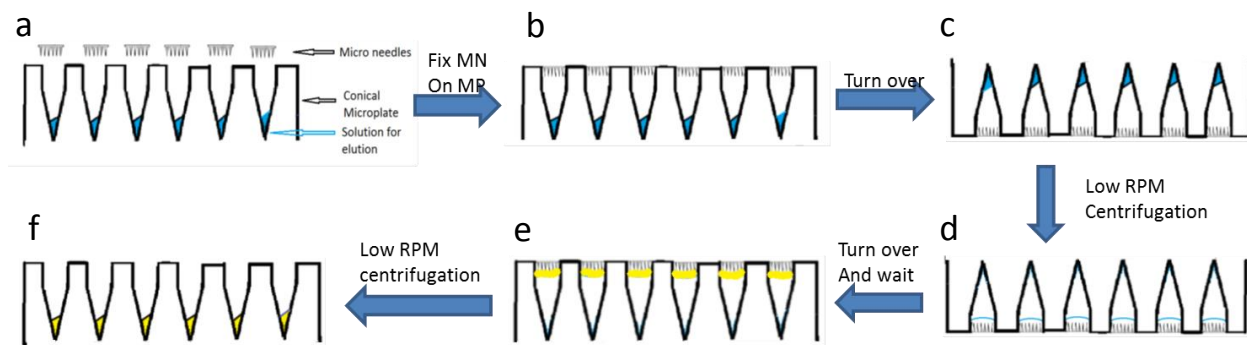


Figure S5. Collection of biomarkers by integration of microneedle patch on top of V-bottom multi-well microplate. (a) Solution for elution is dispensed into the wells of a V-bottom multi-well microplate. (b) Microneedle patches containing biomarkers are affixed to the tops of the wells to provide a water-tight seal. (c) The microplate is turned over and (d) subjected to low-speed centrifugation to contact the elution solution with the microneedle patches. (e) The microplate is turned over again and (f) subjected to low-speed centrifugation again in order to collect the elution solution containing biomarkers eluted from the microneedle patches at the bottom of the wells for subsequent analysis.



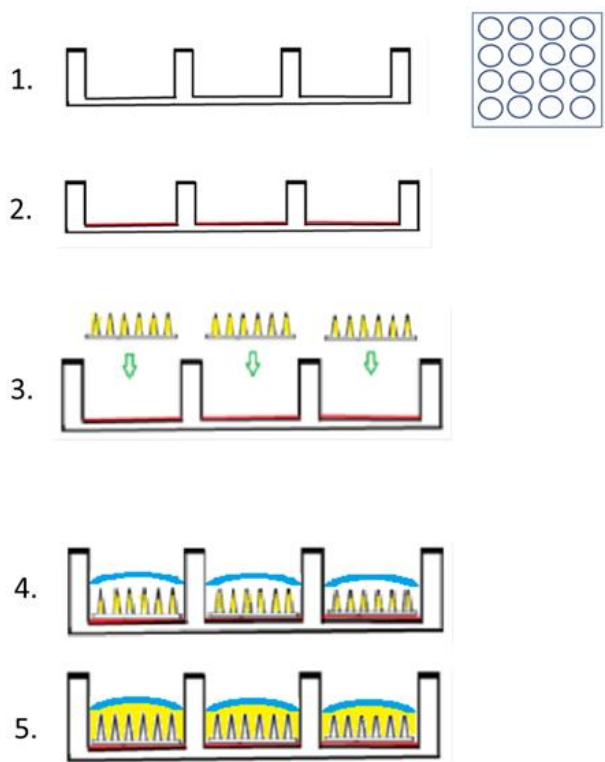


Figure S6. Collection of biomarkers by affixing microneedle patches at the bottom of flat-bottom multi-well microplate. Microneedle patches containing biomarkers are affixed to the bottom of flat-bottom microplate wells using double-sided adhesive tape (1 – 3). An elution solution is placed in the wells to elute biomarkers from the microneedle patches for in situ analysis in the wells.