Supporting Information (SI)

Plasmonic Microneedle Arrays for *in situ* Sensing with Surface-Enhanced Raman Spectroscopy (SERS)

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Experimental Section:

Chemicals and Materials. Hydrogen peroxide (H_2O_2) solution 30%, sulfuric acid (H_2SO_4), hydrochloric acid (HCl), ammonium hydroxide (NH_4OH), chloroauric acid ($HAuCl_4$ $3H_2O$, 99.9%), cetyltrimethylammonium bromide (CTAB), sodium borohydride ($NaBH_4$, 99.99% trace metals basis), silver nitrate ($AgNO_3$, 99%), ascorbic acid (99%), 4-mercaptobenzoic acid (4-MBA), sodium hydroxide, agarose, and 1x phosphate buffered saline (PBS) tablets (pH 7.4), Dulbecco's phosphate buffered saline were purchased from Sigma-Aldrich and used without further purification. Silicon wafers were purchased from WaferNet, Inc. Gold pellets (99.999% pure) were purchased from Kurt J. Lesker. Silica microspheres (dia. 0.39 μm) were purchased from Bangs Laboratory, Inc. Milli-Q water with a resistivity of 18.2 MΩcm was used in all preparation. Norland Optical Adhesive (NOA) 65 was purchased at Norland Product Inc. PDMS molds of pyramidal features with the 300 μm height, 200 μm base, and 500 μm tip to tip distance in a 25 X 90 array were purchased from Micropoint Tech, Singapore. Human skin (human baby foreskin) was obtained from the Skin Tissue Engineering Core Facility in Feinberg School of Medicine at Northwestern University.

Instrumentation. UV-vis extinction spectra were obtained using the Perkin Elmer LAMBDA 1050 is a high-performance UV/Vis/NIR double beam, double monochromator, ratio-recording spectrophotometer. Scanning electron micrographs (SEM) were taken with a LEO Gemini 1525 microscope using an InLens detector and Hitachi SU8030, operating at an acceleration voltage of 5.00 or 2.00 kV. The working distance was varied between 3 and 7 mm. NOA prepolymer was cross-linked using UVP CL-1000L at 365 nm wavelength. Sample surfaces were treated with ozone using Harrick plasma generator. The SERS measurements were collected using an inverted microscope (Nikon TE300) with a 20x objective (Nikon, NA=0.45), where a CW laser with excitation wavelength of 785 nm (Innovative Photon Solutions) was focused on the sample. A long-pass filter for 785 nm (Semrock) was used and the scattered light was dispersed using 600 grooves/mm gratings. The SERS signal was detected using a liquid nitrogen-cooled CCD (Action 300i, Spec-10). SERS spectra were processed using Origin Pro Lab and MATLAB. pH measurements of the Britton-Robinson buffer and Dulbecco buffer solutions were obtained using Accumet (Fisherbrand) pH meter. Optical coherence tomography (OCT) images were obtained using Thorlabs Ganymede 220C1 OCT system.

Au film-over-nanosphere (AuFON) fabrication. Circular Si wafer with 25 mm diameter was first cleaned by incubating the wafers in Piranha solution (3:1 $H_2SO_4:H_2O_2$) for 1 hour and rinsed with copious amount of Milli-Q H_2O. Then the wafers were base-treated by sonicating for 1 hour in 5:1:1 H_2O: H_2O_2:NH_4OH and rinsed copiously with Milli-Q water to make hydrophilic wafer surface. The wafers were stored in Milli-Q water for future use. 390 nm SiO_2 microspheres was centrifuged 3 times at 5000 rpm for 5 min and the supernatant was removed and replaced with Milli-Q. The SiO_2 solution was then diluted to 5% with Milli-Q water and 14-15 μL were drop-casted onto the Si wafer and air-dried. After drying, 150 nm Au was thermally deposited at a rate of 1 Å/s under ~ 10^{-7} Torr (PVD-75, Kurt J. Lesker). LSPR of AuFON was measured by a fiber light spectrometer (Ocean Optics) with a flat Au 150 nm film deposited on a glass coverslip as a mirror reference.

Functionalizing AuFON surface with 4-MBA AuFON surface was functionalized with 4-MBA by incubating the substrate in 10 mM 4-MBA in ethanol for 45 minutes and rinsing with ethanol afterwards.

Synthesis of Gold nanorods. Gold nanorods were synthesized using a silver-assisted seed-mediated method.[1–3] Au seeds were prepared by adding 0.25 mL of $HAuCl_4$ (0.01 M), 9.75 mL of 0.1 M CTAB solution, and 600 µL of ice-cold $NaBH_4$ (0.01 M) with vigorous stirring. After 1 hour of seed aging, 24 µL of seeds were then added to growth solutions, which contains 9.11 mL of CTAB solution (0.1 M), 0.13 mL of silver nitrate solution (0.01 M), 0.5 mL of $HAuCl_4$ (0.01 M), 0.19 mL of HCl (1 M), and 0.08 mL of ascorbic acid (0.1 M) with mixing. The seed solution was added to the mixture and left overnight for purification. AuNR solution was centrifuged twice at 10000 rcf for 30 min and the supernatant was removed. The pellets were then dispersed with the same volume of Milli-Q H_2O to give the final concentration of $2x10^{13}$ particles/mL.

Characterization of Gold nanorods. AuNRs were characterized by UV-visible spectrophotometer for determining the LSPR, scanning electron microscope (SEM) and transmission electron microscope (TEM) for visualization, and inductively coupled plasma optical emission spectrometry (ICP-OES) for determining the Au concentration. Size of AuNRs was determined to be 15 nm by 55 nm using TEM images of AuNRs. The concentration of AuNRs stock solution was determined as $2x10^{13}$ particles/mL by using the volume of AuNRs, the density of Au, and the concentration of Au (from ICP-OES).

Plasmonic NOA microneedle fabrication. NOA 65 prepolymer solution (~1.5 g/sample) was spread on the PDMS mold. In order to remove air bubbles trapped within the mold cavities, mold with NOA on top was kept in a light protected vacuum chamber for 10 min. Then, the prepolymer was crosslinked with UV light exposure (365 nm) for 10 min. The rigid polymer microneedle array was easily removed from the flexible PDMS mold. The large microneedle array was then cut into three pieces. Each sample surface was cleaned with plasma treatment for 20 seconds. Following the plasma treatment, the microneedles array was incubated in 1 mL solution of AuNRs (40 uL from AuNRs stock solution with $2x10^{13}$ particles/mL) and 10 mM 4-MBA using (1:1) (v/v) water:ethanol co-solvent condition. Microneedles array was incubated in this mixture for 24 hours. Finally, the plasmonic microneedles array surface was cleaned with ethanol and dried with nitrogen.

AuNRs stability on the plasmonic microneedles surface under ISF mimicking condition. Plasmonic microneedle array was tested under ISF mimicking condition over a month period. The microneedle array was incubated in 1 mL of PBS solution using a 24 well plate with. The well plate was protected from light and water evaporation throughout the stability test. The buffer solution was replaced with a freshly prepared PBS twice a week. For evaluation of the AuNRs stability, UV-vis extinction spectra were taken every week for a month.

Preparing Britton-Robinson buffer. In a glass bottle, 100 mL of a solution of phosphoric acid, acetic acid, and boric acid with individual concentrations of 0.04 M was prepared with Milli-Q. Then, the solution was titrated with 0.2 M sodium hydroxide depending on the desired pH.[4] The pH of the solution was measured using a pH meter.

Preparing Dulbecco's phosphate buffered saline (DPBS) solutions with different pHs. The pH of DPBS (pH 7.1) was adjusted to pH 6.6 and pH 7.6 by adding 1 M HCl and 1 M NaOH, respectively. The pH of the buffer solution was measured using a pH meter.

Punching agar gel experiment. The microneedle array was punched through an agar gel. Then, the microneedle with agar gel was placed on a microscope stage so that the needles were faced up. The laser was focused through the back of the microneedle and on the tips. The SERS measurements were done on seven different tips. For the mechanical stability test (punching agar gel multiple times), the microneedle array was punched through an agar gel 10 times. The needles went through one part of the agar gel at a time so that no area was punched twice.

pH sensing experiment. The microneedle array sensor was placed in a quartz cuvette (with Britton-Robinson buffer solution) so that the needles were facing up (**Figure S4**). Using the microscope, the laser was focused through the back of the microneedle and on the tips. The SERS measurements were done on seven different tips for averaging. After measuring seven tips, the buffer solution was replaced with the next higher pH buffer solution after rinsing the cuvette and the microneedle with the next buffer solution. The experiment was repeated using AuFON (functionalized with 4-MBA). For the pH reversibility test, the microneedle in a quartz cuvette was placed in buffer solutions with alternating pH values (pH 2 to 12 with 6 cycles).

Removing NOA 65 background from SER spectra. To remove the background Raman signal of NOA 65, normal Raman of NOA 65 microneedles (without AuNRs) was taken by focusing the laser through the polymer and on the microneedle tips (**Figure S5, green**). After baseline correction, the spectrum was then normalized to the dominant NOA peak located at ~1450 cm^{-1} and multiplied by the peak intensity at ~1450 cm^{-1} of the SER spectrum of NOA microneedles with AuNRs functionalized with 4-MBA. The normal Raman of NOA was then subtracted from the SER spectrum of NOA microneedles.

Applying plasmonic microneedle arrays in human skin. After receiving human skin, the tissues were rinsed with DPBS (repeated 3 times). After removing fatty tissues under the skin, the skin was placed on a microscope slide and plasmonic microneedle arrays sensor was pressed into the skin.

OCT measurement. After inserting plasmonic microneedle arrays in human skin, the sensor was fixed in place by taping the sensor edges. Using the OCT system (Thorlabs Ganymede 220C1 OCT), 2D cross-section images of the skin with and without microneedles were obtained (**Figure S7**). In the parameter section, the refractive index was set to 1.524 (refractive index of cured NOA 65 obtained from the Norland Products website: http://www.norlandprod.com/adhesives/noa%2065.html).

Skin pH detection. After receiving human skin, the tissues were rinsed with DPBS (repeated 3 times). After removing fatty tissues under the skin, the skin was placed on a metal mesh boat in a Petri dish filled with 7 mL of DPBS solution with three different pH values (pH = 6.6, 7.1, and 7.6) as shown in **Figure S8**. The purpose of the metal mesh boat was to soak only the bottom of the skin and keep the top part of skin dried. The skin tissues were incubated in the buffer solution for 12 h while replacing the buffer every 2 hours to facilitate the incubation. After 12 h of

incubation, skin tissues were dabbed with clean lab tissue paper. The skin tissues were placed on a microscope slide. Plasmonic microneedle arrays were then inserted in each skin samples (with pH 6.6, 7.1, and 7.6) and SER spectra were acquired while the sensors were inserted in the skin. The laser was focused through the polymer and on the tips.
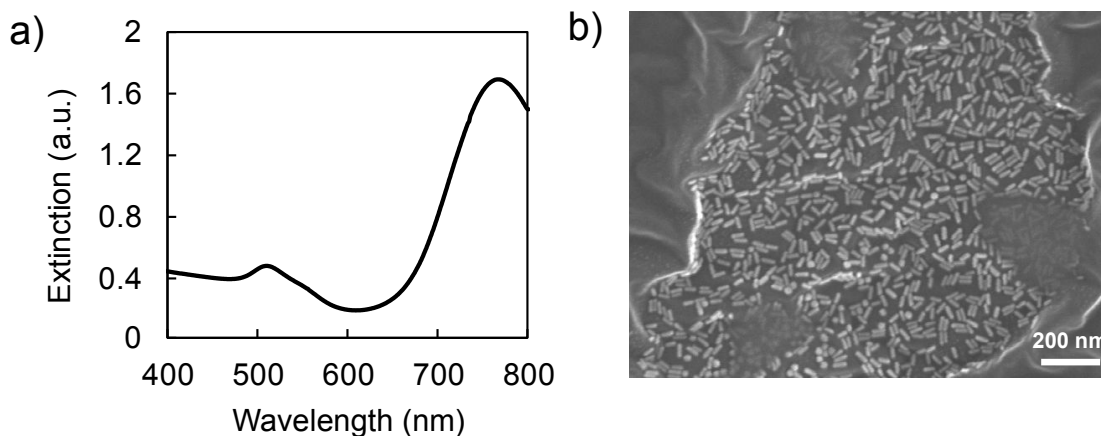
Figure S1. Characterization of AuNRs. a) UV-Vis extinction spectrum of CTAB stabilized AuNR solution demonstrating a transverse surface plasmon resonance at 510 nm and longitudinal resonance at 775 nm. b) SEM micrograph of AuNRs on a conductive surface (carbon tape).
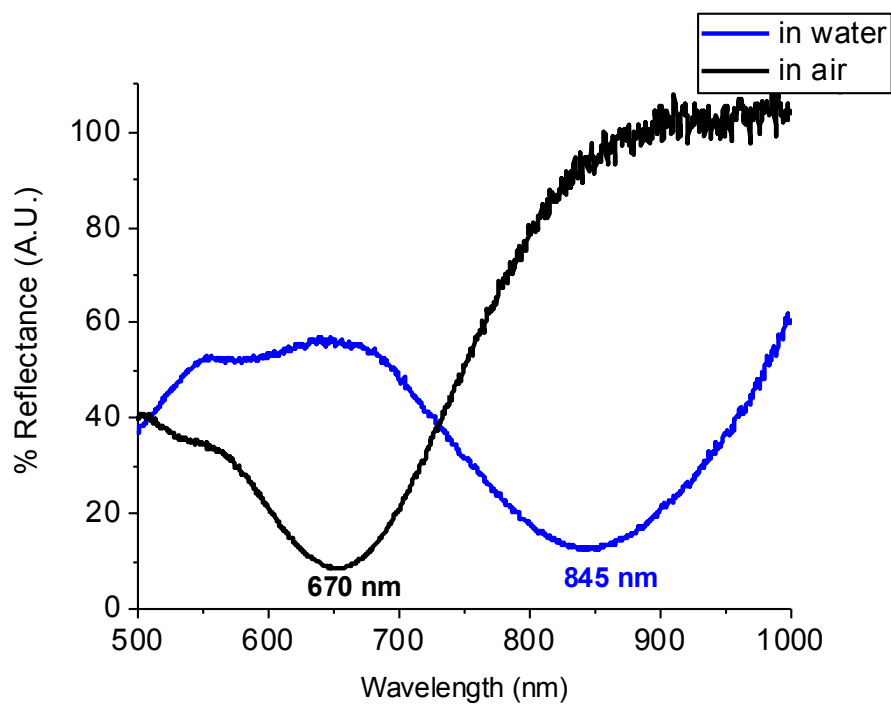
Figure S2. LSPR of AuFON in two different media. LSPR changes from air (black) to water (blue).
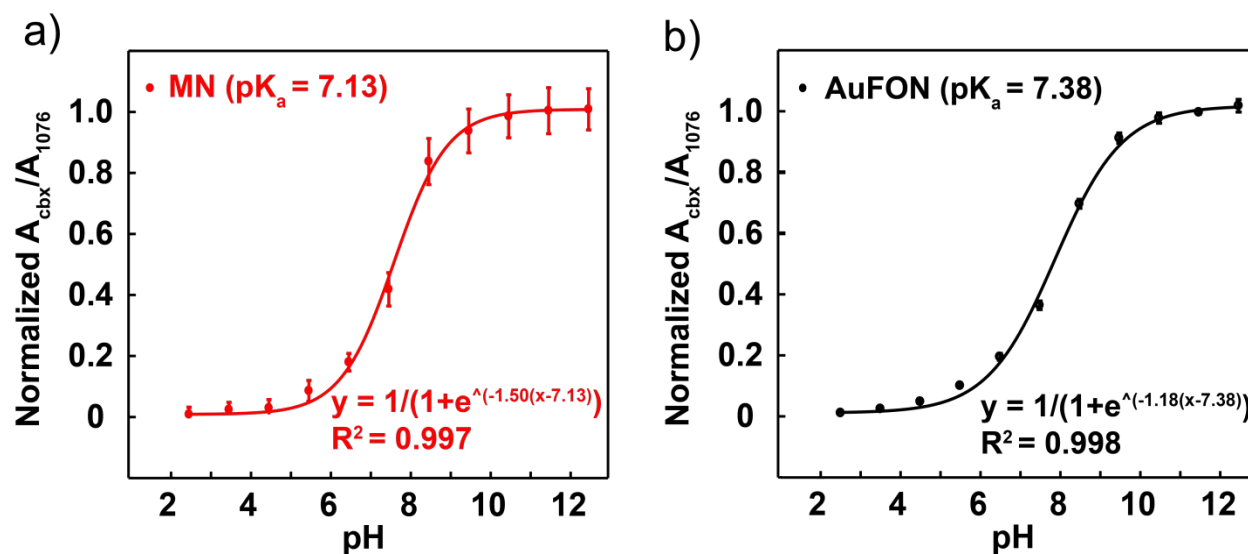
Figure S3. pH calibration curve fitting to a logistic function. S-shaped calibration curves after fitting to logistic functions for (a) plasmonic microneedle array (MN) in the range of pH 2-12 compared with (b) a standard SERS substrate, AuFON.
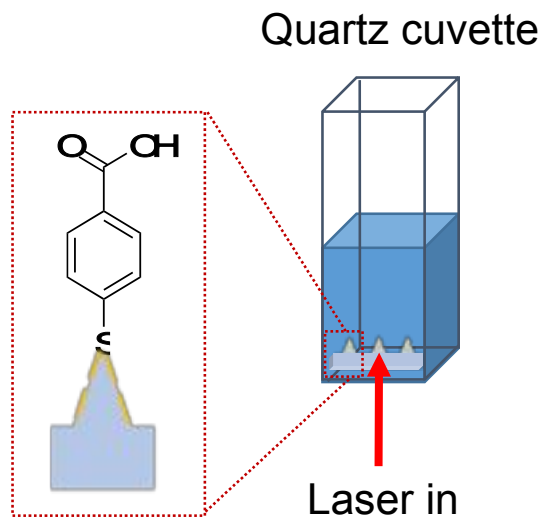
Figure S4. Schematic of pH sensing experiment setting. Plasmonic microneedle array is placed in a quartz cuvette with a Britton-Robinson buffer solution. The laser (785 nm) is focused through the polymer and onto a tip using a microscope for SERS measurement.
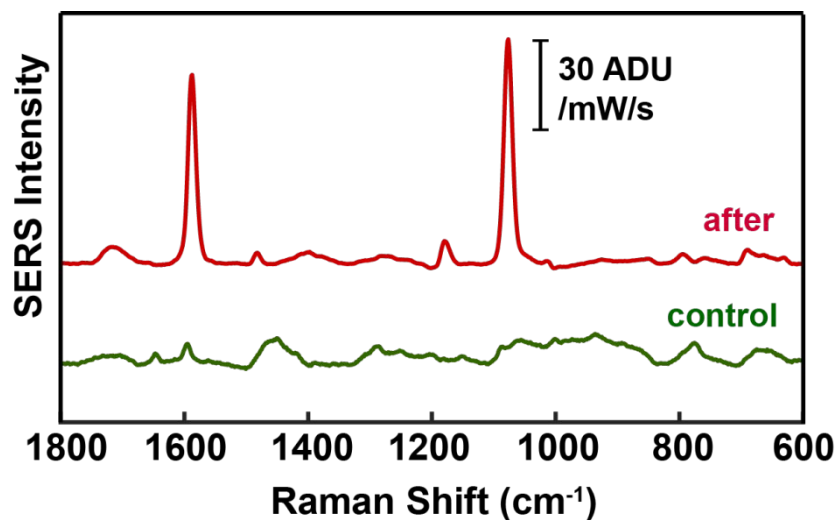
Figure S5. SER spectra of MN array with (red) and without (green) AuNR aggregates functionalized with 4-MBA. Each spectrum is an average of 7 different microneedle tips. The measurement was done by focusing the laser through polymer and on the tips. The parameters for the SERS data acquisitions were: λ_{ex} = 785 nm, 20x ELWD objective, t_{acq} = 1 min, P_{ex} = 1 mW.
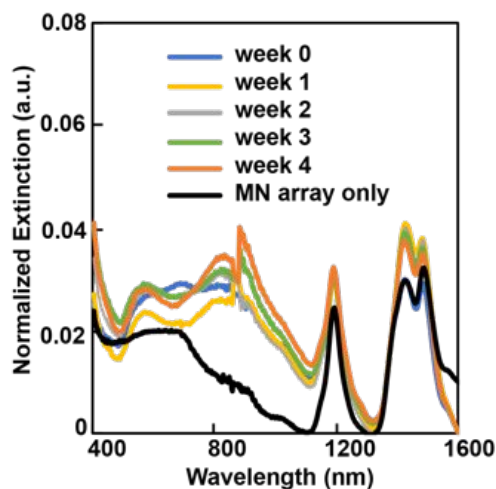
Figure S6. Light extinction spectra of AuNR aggregates on microneedle array incubated in 1xPBS over four weeks.
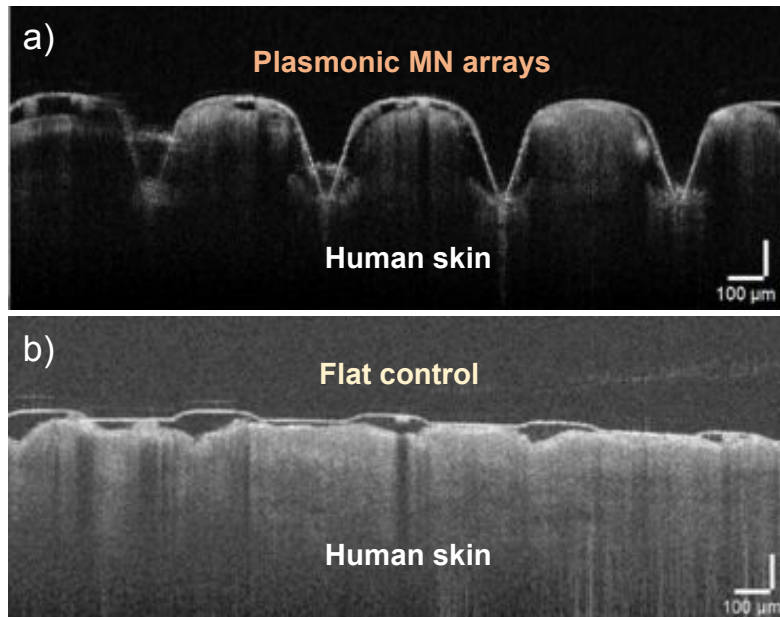
Figure S7. Optical coherence tomography (OCT) image of human skin with a) plasmonic MN arrays and b) flat control.

Figure S8. Image of human skin tissues on mesh boat in Petri dish filled with DPBS with three different pHs (pH 6.6, 7.1, and 7.6).
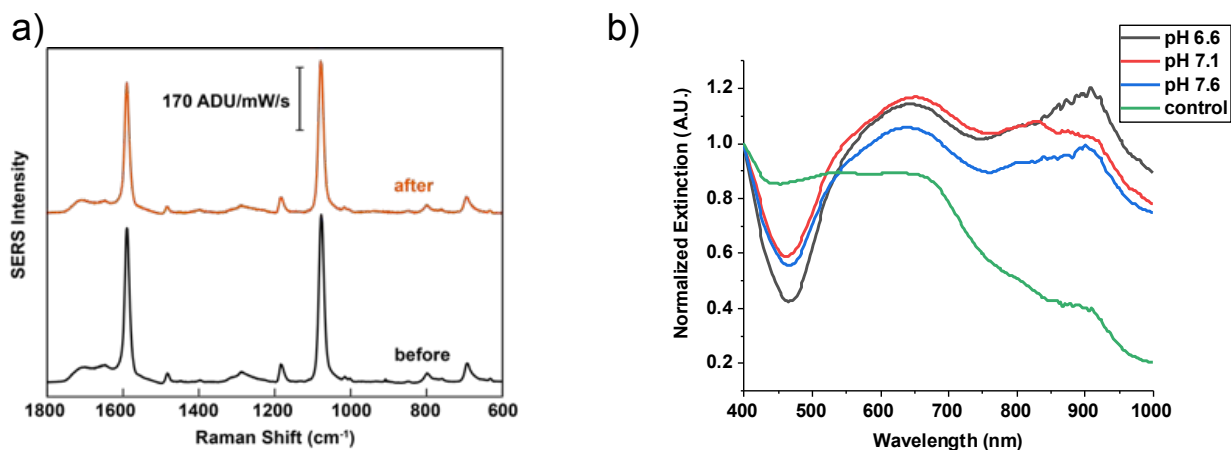
Figure S9. Optical characteristics of plasmonic MN array following *ex vivo* punching test using human skin. (a) SER spectra of plasmonic MN array before (black) and after (orange) one time insertion in human skin (taken in air). Each spectrum is an average of 7 different microneedle tips. The measurement was done by focusing the laser through polymer and on the tips. The parameters for the SERS data acquisitions were: λ_{ex} = 785 nm, 20x ELWD objective, t_{acq} = 1 min, P_{ex} = 1 mW. (b) Normalized extinction spectra of plasmonic MN array functionalized with AuNRs after insertion into human skin tissues with pH 6.6 (black), pH 7.1 (red), pH 7.6 (blue), and MN array control without AuNRs (green).
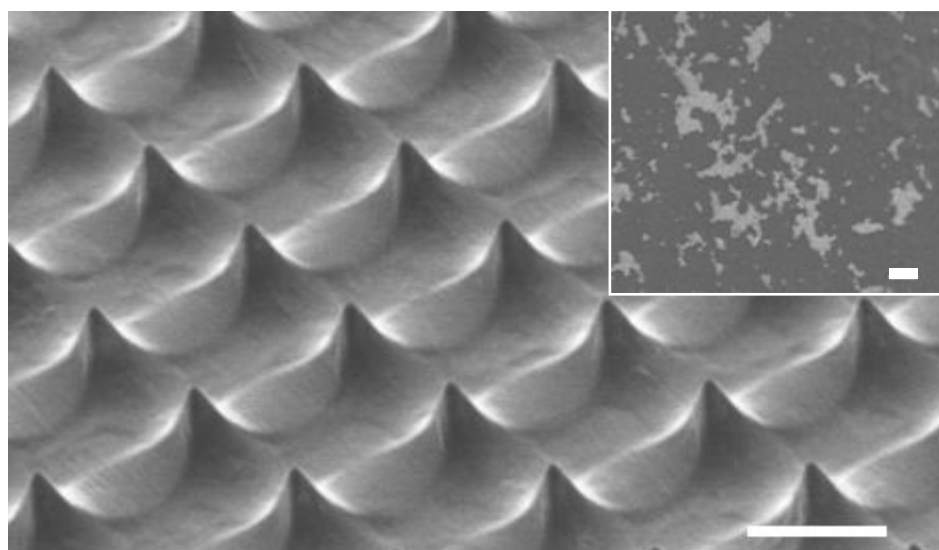

Figure S10. SEM image of MN array with AuNRs functionalized with 4-MBA after puncturing human skin (scale bars are 200 μm and 2 μm (inset)).
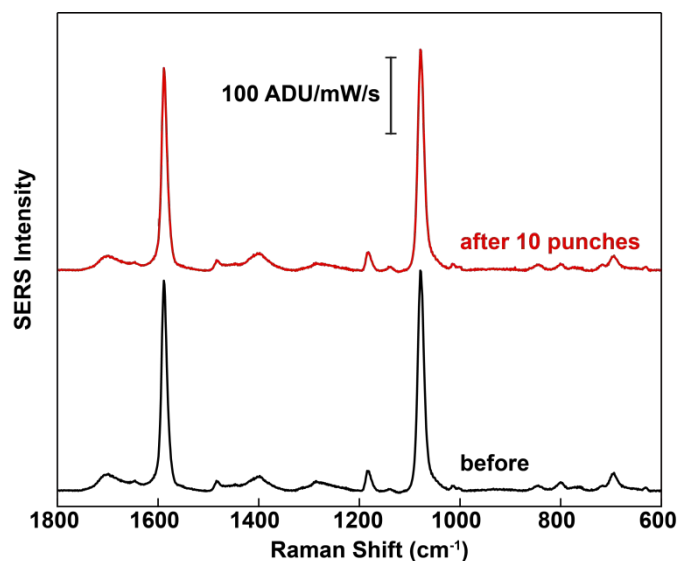
Figure S11. SER spectra before (black) and after (red) 10 punches in human skin. Each spectrum is an average of 7 different microneedle tips. The measurement was done by focusing the laser through polymer and taken in pH 6.6 DPBS solution (filled in a quartz cuvette). The parameters for the SERS data acquisitions were: λ_{ex} = 785 nm, 20x ELWD objective, t_{acq} = 1 min, P_{ex} = 1 mW.

Reference:

(1)   Sau, T. K.; Murphy, C. J. *Langmuir* **2004**, *20* (15), 6414–6420.
(2)   Fernández-López, C.; Mateo-Mateo, C.; Álvarez-Puebla, R. A.; Pérez-Juste, J.; Pastoriza-Santos, I.; Liz-Marzán, L. M. *Langmuir* **2009**, *25* (24), 13894–13899.
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(4)   Britton, H. T. S.; Robinson, A. R. *J. Chem. Soc.* **1931**, 1456, 1456–1462.