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Squid Suckerin Microneedle Arrays for Tunable Drug Release

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Experimental details

Fabrication of polydimethylsiloxane (PDMS) molds and suckerin microneedle arrays

Besides the 3M microneedle templates, a customized microneedle array template was also employed in the study and was fabricated by a 3D printing method. The 3D model of the microneedle array template was first designed using AutoCAD© 2016 to have a 500 µm base diameter, 50 µm tip diameter, 1000 µm height and a 1000 µm center-to-center spacing. Subsequently, a Digital Light Processing or DLP 3D printer (Titan 1, Kudo3D Inc.) equipped with a Viewsonic® DLP projector was utilized to print the microneedle array template. The individual images used for fabrication was processed by Creation Workshop Envision Labs (St Mankato) into layers of 50 µm, exported and opened as a .zip file in Titan1's control software. The printer XY resolution was set as 50 µm. Four microneedle array templates were printed at the same time to reduce the total operation time. A proprietary resin, 3DM-Castable was used for the fabrication.

Skin penetration efficiency of suckerin microneedle arrays

The rat skin after microneedle penetration was also examined by SEM. The skin was flash-frozen using liquid nitrogen[1] and dried with a freeze drier for 2 days. It was then coated and imaged by SEM similarly as suckerin microneedle arrays. The microneedle penetrated rat skin was also fixed with 5% formalin at 4 °C for 24 h, and subsequently transferred to 15 % sucrose solution to incubate for 24 h. Afterwards, the skin samples were frozen in the embedding matrix with liquid nitrogen and separately cross sectioned using the Microcryostat (Leica) to obtain cross sections of 10 µm. These sections were then

stained with hematoxylin and eosin (H&E) and finally examined by stereomicroscopy to determine the depth of insertion.

Antibacterial activity assessment

The quantitative comparison of antibiotic efficiency between different microneedle arrays was investigated according to previously established protocols.[2] Briefly, agar slurry containing 0.3% agar (w/w) and 0.85% NaCl (w/w) was prepared, autoclaved and equilibrated at 45 °C for further use. The *E. coli* culture with an OD_{600} around 3.0 was diluted with the slurry for 100 times (equal to ~ 3×10^6 cells/mL). In the following step, the microneedle arrays were placed in sterile Petri dish and 200 µl bacteria slurry inoculum was loaded onto the surface of each patch and spread with pipette tips to ensure good contact and entire coverage of the microneedle arrays. The slurry was allowed to gel and transferred to a customized double layer box which contained 200 mL water on the bottom layer to keep a high humidity and incubated at 37 °C for 24 h. The bacteria were then recovered by transferring the samples into 50 mL Falcon tubes containing 10 mL of PBS. The tubes were sonicated for 1 min and vigorously vortexed for 1 minute to facilitate the complete release of bacterial from the microneedle surfaces. The samples were then serially diluted with PBS (1X, 10X, 100X and 1000X) and 100 µl aliquot from proper dilutions of each samples was spread on LB-agar plates (3 plates per dilution). The plates were cultured at 37 °C for 24 h. The number of colony forming unit (CFU) was obtained from the counts of the colonies, taking the folds of dilution into account.

The analysis of antibacterial activity of free suckerin proteins was performed in a similar way. The *E. coli* culture with an OD_{600} around 2.0 was diluted with MHB medium for 100 times (equal to 2-3 × 10^6 cells/mL). Suckerin solution at 27 mg/mL (in 1.1% acetic acid)

was diluted with sterile milli-Q water to 9, 3, 1, and 0.33 mg/mL. Afterwards, 900 µl cell culture was mixed with 100 µl suckerin at different dilutions in 14 mL Falcon tubes for cell culture (three tubes per sample) and the OD_{600} for each tube was recorded. Cells mixed with various concentrations of acetic acid corresponding to that of each suckerin dilution were also cultured respectively. The tubes were then incubated at 37 °C for 24 h before the OD600 was recorded for each tube.

Supplementary Figures

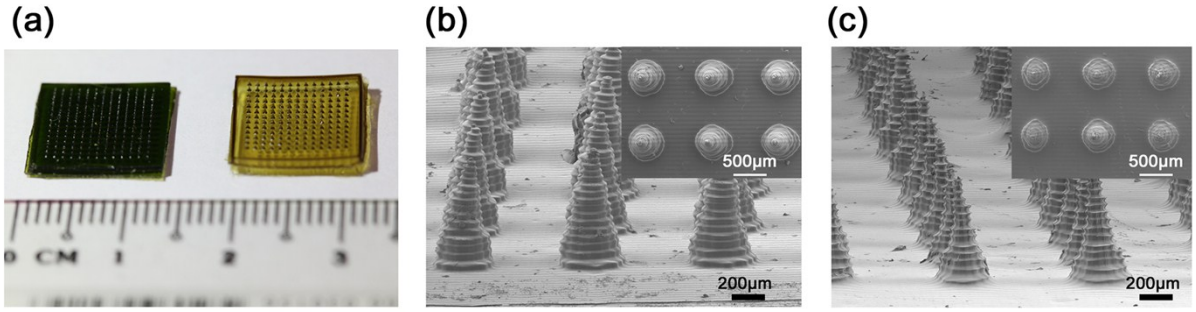

Fig. S1. (a) Photograph of a 3D printed template and of the resulting suckerin microneedle array. **(b)** SEM micrograph of 3D printed template microneedles. The inset shows the top view of the microneedles. **(c)** SEM micrograph of suckerin microneedles prepared from the 3D printed template. The inset shows the top view of the microneedles.

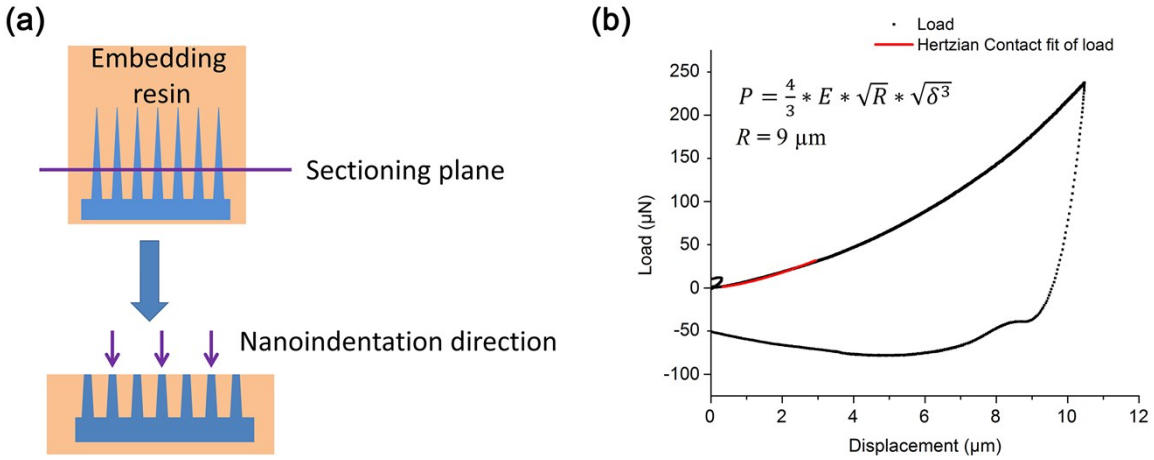

Fig. S2. (a) Schematic illustration showing sample embedding, sectioning and loading direction of suckerin microneedle arrays for nanoindentation testing. **(b)** A representative nanoindentation loading-unloading curve (cono-spherical tip) of a suckerin microneedle array in pH 5 buffer. The elastic modulus was obtained by fitting the initial loading portion of the curve with the Hertzian solution. Negative load values on the unloading portion of the curve are due to adhesion of the tip to the surface upon tip retraction.

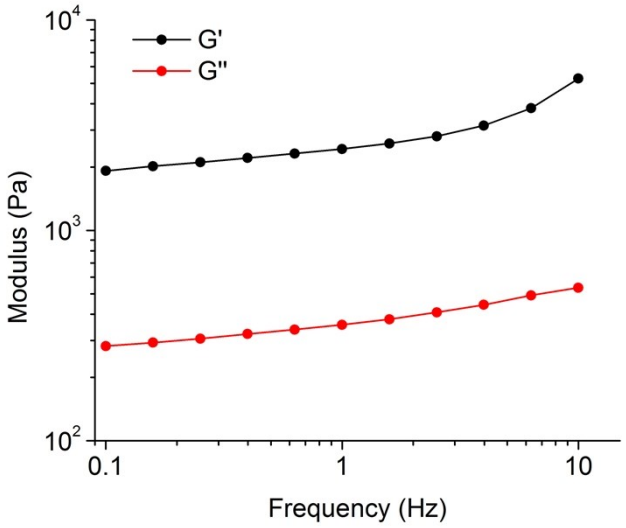

Fig. S3. Mechanical properties (measured by rheometry) of suckerin microneedle arrays incubated in 2M urea solution. The sample was initially measured by amplitude-sweep to identify the viscoelastic regime. Subsequently the frequency-sweep measurements were done at 0.5% strain.

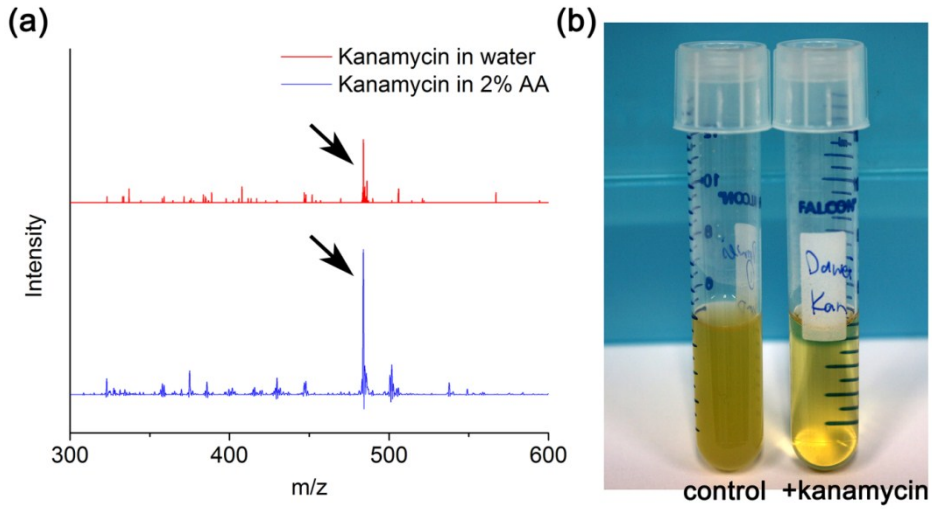

Fig. S4. (a) Maldi-ToF spectra of kanamycin in 2% acetic acid (AA) and water. The molecular weight of kanamycin didn't change in 2% water compared to that in water, suggesting no degradation of the drug. **(b)** Antibacterial effect of kanamycin in 2% acetic acid (right) against *E. coli* cells after an overnight culture at 37 °C. Cells not exposed to kanamycin solution were used as control.

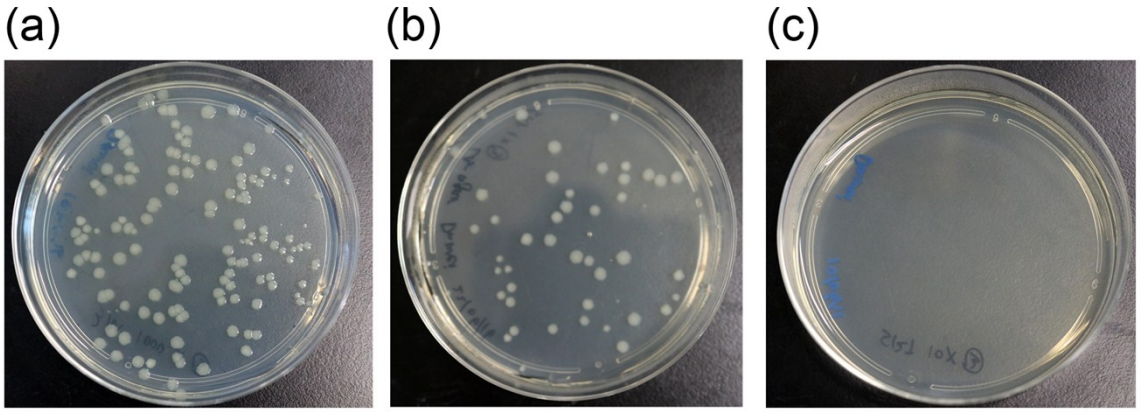

Fig. S5. Representative images of LB-agar plates after the 24 h culture of diluted *E. coli* resuspensions at different folds that were incubated on various microneedle arrays before plating. **(a)** 1000X dilution of *E. coli* incubated on 3M microneedles; **(b)** Original (1X) resuspension of *E. coli* incubated on SRT microneedles; **(c)** Original (1X) resuspension of *E. coli* incubated on SRT microneedles loaded with kanamycin. The colony number is taken as 1 if no colony is found on the plate.

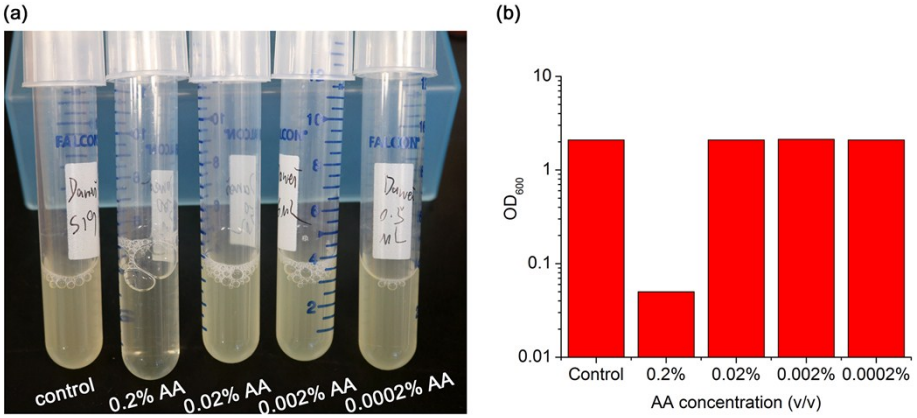

Fig. S6. (a) *E. coli* cells cultured with different concentrations of acetic acid at 37 °C overnight. LB medium was mixed with acetic acid solutions at different concentrations respectively by 9:1 (LB vs acetic acid solution, v/v) and inoculated with *E. coli*. **(b)** OD_{600} of cells cultured in different concentrations of acetic acid.

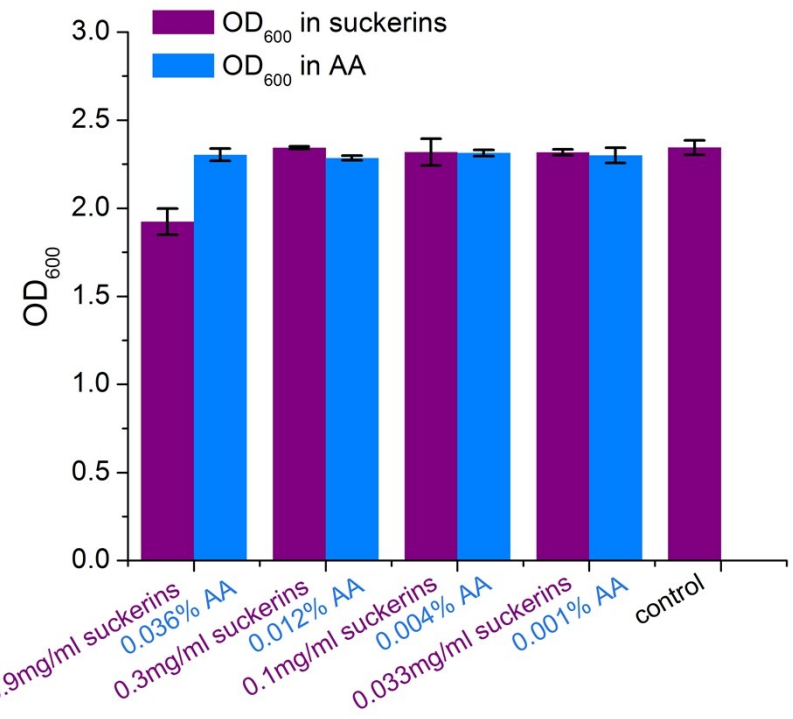

Fig. S7. OD_{600} of *E. coli* cultured in MHB medium with different concentrations of free suckerins and with the same concentration of acetic acid as that in various suckerin dilutions, respectively. AA: acetic acid.

Supplementary References

1. S. P. Sullivan, N. Murthy and M. R. Prausnitz, *Adv Mater*, 2008, **20**, 933-938.
2. A. ASTM Committee E35 on Pesticides, A. C. A. S. E. o. A. Agents and A. International, *Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) in Polymeric Or Hydrophobic Materials*, 2012.