Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Bacteria Repellent Protein Hydrogel Decorated with Tunable, Isotropic, Nano-on-Micro Hierarchic Microbump Array

Table of contents:

1. Experimental Section

Materials

Polystyrene (M_w=650 000), chloroform, ethanol and toluene were purchased from MilliporeSigma. Bovine serum albumin (lyophilized, powder)，glutaraldehyde solution (50%) and LB broth were obtained from fisher scientific. Ecoflex was purchased from Sculpture Supply Canada. All chemicals were used as received. *Staphylococcus aureus* MZ100 is generously provided by Dr. Michael Surette, McMaster University.

Preparing polystyrene honeycomb film

A sealed chamber with a removable lid was set up within saturated NaCl solution and a platform above the liquid level to maintain a stable humid environment. 5 wt% of polystyrene (M_w= 650 k) in chloroform was dropped on a glass slide placed on the platform and the chamber lid was sealed thereafter. The polystyrene solution was allowed to evaporate and turn solid for 20 mins before being taken out, peeled off the glass slide, and stored for imaging and use in further experiments.

Preparing BSA hydrogel film with microbumps

A final concentration of 5% BSA and 1% GA solution was added on to the surface of a plasma-treated polystyrene honeycomb film and left incubating for 30 mins at room temperature to solidify and form a gel. The polystyrene honeycomb film was removed from the BSA hydrogel by submerging the sample in a glass vial of toluene for 20 mins in a shaking incubator. This step was repeated 2 more times with new glass vials of toluene. The sample was dried with Kimwipes. The sample was then stored in MilliQ water for future imaging and experimentation. BSA hydrogel

with flat surface was obtained using a flat polystyrene film as the template where the procedure of removing flat polystyrene film was same as removing polystyrene honeycomb films.

Control the sphericity of microbumps on BSA hydrogel film

To achieve varied sphericity of the microbumps, the polystyrene honeycomb films underwent a combination of either plasma treatment with CO_2 for 10 minutes to increase hydrophilicity or no plasma treatment, and the addition of 5% BSA and 1% GA under vacuum conditions or normal atmospheric conditions.

Synthesizing and characterizing BSA nanogels

The preparation of BSA nanogels followed a classic desolvation method, described by Weber et al.[1] Briefly, 150 mg of BSA was dissolved in 2 mL of 10 mM NaCl solution, and then 10 μL of NaOH was added to adjust the pH to 7-9. 8 mL of ethanol was added continuously dropwise using a syringe pump (1 mL/min) while stirring for 500 rpm at room temperature. After all the ethanol is added, 176.25 μL of 8% GA (1.175 μL GA/mg BSA) was added to the solution to induce particle crosslinking. The reaction was performed while stirring at 500 rpm for 24 hrs. Once the crosslinking reaction was completed, the sample was centrifuged at 25,000xg for 10 mins. The supernatant was discarded, and the nanoparticles were dispersed in water for characterization and further experiments. Average particle size was measured using dynamic light scattering (DLS) using a Malvern Zeta Sizer Nano ZSP. Zeta potential of BSA nanogels was measured in 1 mM KCl.

Controlling the roughness of microbumps on BSA hydrogel films

To achieve varied surface roughness of the microbumps, the polystyrene honeycomb films underwent a combination of plasma treatment with CO_2 for 10 mins or no plasma treatment, the addition of the BSA solution in vacuum conditions or normal atmospheric conditions, the addition of BSA nanogels, and the addition of 5% BSA with 1% GA as the chemical crosslinker.

Preparing Ecoflex film with microbumps

To prepare Ecoflex film with microbumps, a polystyrene honeycomb film was firstly placed inside a well of a twelve-well plate. Ecoflex components A and B were mixed together in a 1:1 ratio and dropped on top of the polystyrene honeycomb film. Then the plate was placed in vacuum for 20 mins. After 10 mins, the 12-well plate was taken out. The honeycomb film was pushed to the bottom of the well and left on the bench for 4 hrs to solidify. After solidifying, the Ecoflex film was taken out of the 12-well plate. The polystyrene film was peeled off to obtain an Ecoflex microbump film and stored at room temperature for future imaging and experimentation.

Preparing BSA honeycomb films

A Ecoflex microbump film was either unstretched or stretched using an apparatus containing twine, clamps, and an empty petri dish. BSA solution was placed on the surface of the Ecoflex microbump film and placed in a vacuum desiccator for 20 mins or until there were visually no more air pockets in the solution. GA was added to the BSA solution to a final concentrations of 1% GA and 5% BSA, and the solution was gently mixed. The sample was put under vacuum for another 30 mins and was then taken out and left on the bench for an additional 1 hr to continue gelation. Once gelled, the BSA hydrogel was peeled and kept in MilliQ water for future imaging and experimentation.

Bacterial adhesion test

Overnight culture of *S. aureus* was diluted 1000× (10 μL of overnight culture in 10 mL of LB media), added to 3 BSA hydrogel films with or without microbumps and incubated at 37 °C, 180 rpm for 48 hrs. Then the hydrogels were taken out and washed for electron microscopy. 8 images were counted for bacteria quantification.

Hydrogel characterization

The morphologies of the honeycomb films and microstructured hydrogels were coated with a 15 nm layer of gold and imaged by a scanning electron microscopy (TESCAN VP, SEM) under 10 kV. The hydrogels were processed critical-point drying before imaging. The water contact angles of hydrogel surfaces were measured by a contact angle instrument (KRUSS, Drop Shape Analysis System DSA 10) with water droplets (5 μL) dispensed by automated syringe.

2. Supplementary figures

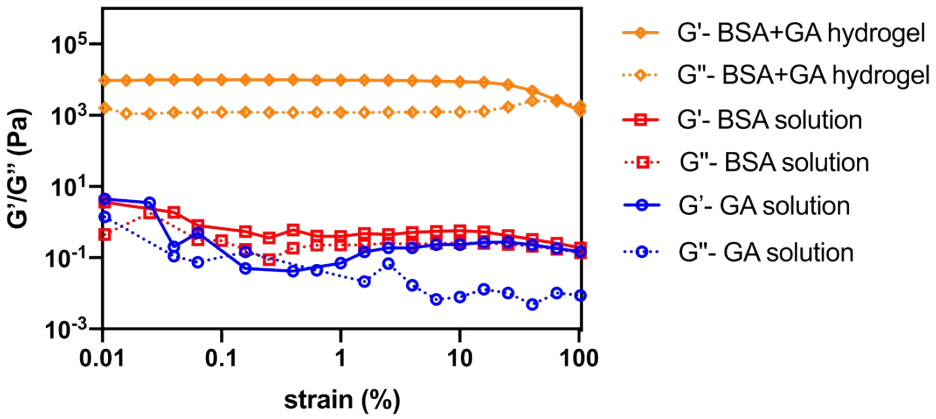

Figure S1. Strain sweep test indicating the storage modulus (G', filled) and loss modulus (G", unfilled) for BSA hydrogel and the pre-gelling components (BSA solution and glutaraldehyde solution)

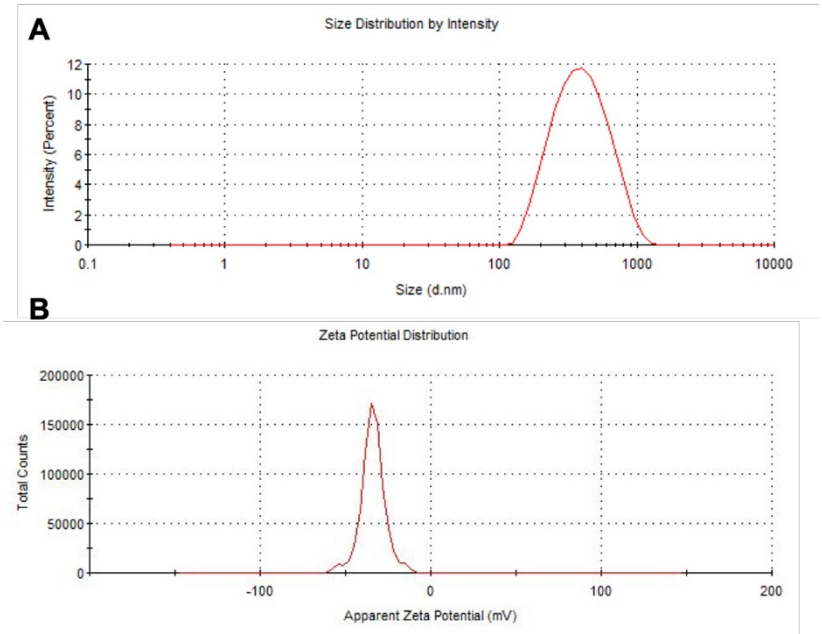

Figure S2. (A) Size distribution and **(B)** zeta potential distribution of BSA nanogels in 1 mM KCl solution.

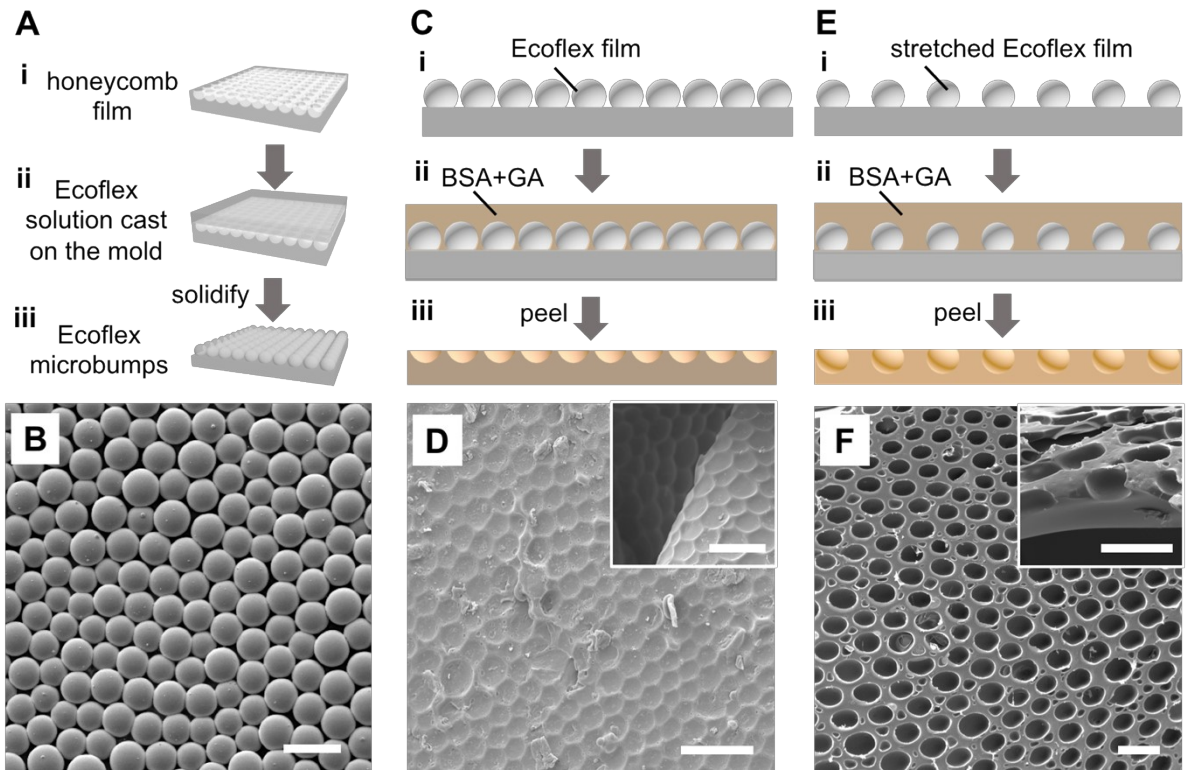

Figure S3. The preparation of BSA honeycomb hydrogel films: **(A)** Schematic diagram of the process of casting Ecoflex secondary template with microbumps; **(i)** a polystyrene honeycomb film is prepared; **(ii)** Ecoflex solution is cast on polystyrene honeycomb film under vacuum; **(iii)** After Ecoflex solution turns solid, the Ecoflex film can be peeled off from the template. Then the Ecoflex film with microbumps can be peeled from the honeycomb film. **(B)** SEM image of Ecoflex film with microbumps (scale bar 50 μm). **(C)** Schematic diagram of the process of preparing BSA honeycomb film with shallow pores; **(i, ii)** BSA solution mixed with glutaraldehyde is added to the top of Ecoflex microbumps, **(iii)** and the Ecoflex template is subsequently peeled off. **(D)** SEM image of BSA honeycomb film with shallow pores (scale bar 20 μm). **(E)** Schematic diagram of the process of preparing BSA honeycomb film with deep pores; **(i)** the Ecoflex template is first stretched to 1.5 times its original length and then **(ii)** BSA solution mixed with glutaraldehyde is added on top. **(iii)** Independent BSA film is obtained by simply peeling off from the template. **(F)** SEM image of BSA honeycomb film with deep pores (scale bar 20 μm). Insert in D and F show cross-sectional SEM image of the same hydrogel (scale bar 50 μm).

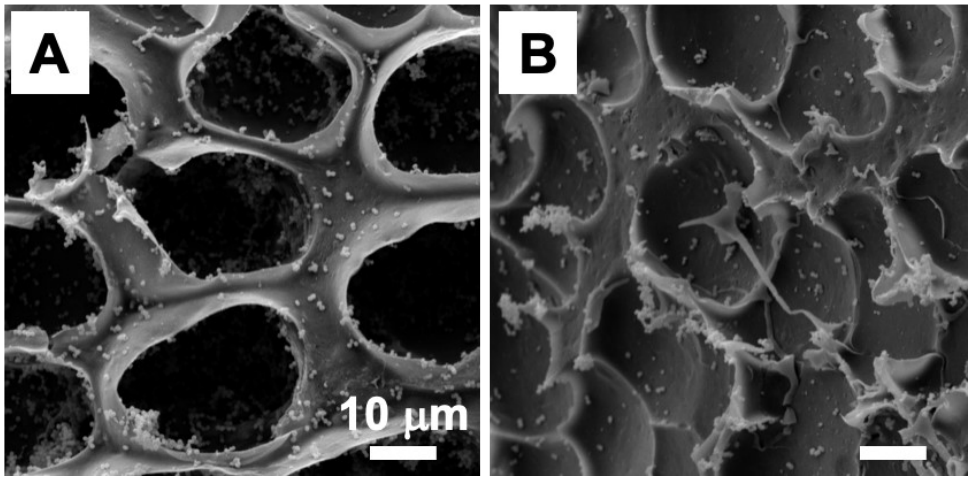

Figure S4. SEM images of BSA honeycomb hydrogel films with different microstructural surface incubated with *Staph. aureus* for 2 days in LB media: **(A)** deep pores; **(B)** shallow pores.

Reference:

1 C. Weber, C. Coester, J. Kreuter and K. Langer, *Int. J. Pharm.*, 2000, **194**, 91–102.