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Supplementary information

Biomimetic Microcavity Interface for Label-free Capture of Pathogens in Fluid

Bloodstream by Vortical Crossflow Filtration

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Experimental Section

Materials. Single-crystal silicon wafers (N-type phosphorus-doped, (100)-orientation, resistivity 1-10 Ω cm, 100 mm diameter, thickness 625 μ m) were purchased from KAYEX (Rochester, MN, USA). Lotion, acetone (98%), ethanol (99.8%), nitric acid (69 wt.%), hydrofluoric acid (48 wt.%), glycerol, sodium chloride, sodium carbonate and sodium carboxymethyl cellulose (600 mpa.s) were purchased from Mecox Lane (Shanghai, China) without further purification. The photoresist S1813 was purchased from Micro Chem Corp (Santa Clara, CA, USA). Silver nitrate (AgNO_3), isopropyl- β -D-thiogalactopyranoside (IPTG), NHS-rhodamine B and ampicillin (AMP) were purchased from Sigma-Aldrich. Glutaraldehyde (25 wt.%) and heparin were purchased from Alfa-Aesar. The yeast extract and tryptone of Luria-Bertani medium (LB) were obtained from Beijing Shuangxuan Microbe Culture Medium Products Factory (Beijing, China). Phosphate buffered saline (PBS), the LIVE/DEAD BacLight Bacterial Viability kit, Calcein Acetoxymethyl ester (AM)/propidium Iodide (PI) Viability Dye kit and human C5a&C4d ELISA kit were purchased from Thermo Fisher Scientific. The Rhodamine phalloidin was purchased from Invitrogen. The Human PF4 ELISA kit were obtained from Abcam. The *Staphylococcus aureus* bacteria strain was purchased from Invitrogen (Karlsruhe, Germany). The biovector of PET 6XH7S/EFGP and BL21 *E. coli* strain were purchased from NTCC (Beijing, China). Deionized (DI) water was obtained from Merck Millipore water purification system and used for all experiments. All reagents were used as received. Blood was obtained from anonymized healthy volunteers from Wenzhou Medical University and used under protocols approved by the Ethics Committee at Wenzhou Medical University (serial number: 2021-22-055).

Methods

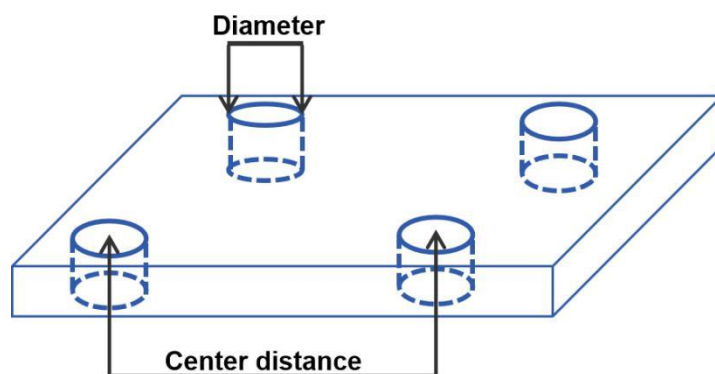
Bacteria Culture and Staining. Plasmid DNA of PET 6XH7S/eGFP was transferred into *Escherichia coli* BL21 (DE3) competent cells, and then positive colons were selected by cultivation on LB plates with 100 μ g mL^{-1} ampicillin. Z Subsequently, the single eGFP-

expressing *E. coli* colony was cultured in 20 mL of LB media containing 1 mM L-1 IPTG and 100 $\mu\text{g mL}^{-1}$ AMP. After 12 h growing at 26 °C while being agitated at 100 rpm min⁻¹ in an orbital shaker incubator, bacteria were harvested by centrifugation at 1200 \times g for 5 min, and then the pellet was resuspended in PBS buffer for further experiments. To prepare *S. aureus* suspension, 100 μL of *S. aureus* stock solution was defrosted and inculcated into 25 mL of LB media overnight at 37 °C with shaking at 110 rpm min⁻¹. Afterwards, 100 μL of bacteria suspension was transferred into another tube containing 50 mL of LB media for further culture in the shaking incubator until cell densities (OD600) reached 0.6~0.8, which corresponded to the concentration of $\sim 10^6$ - 10^8 CFU mL⁻¹. Subsequently, the bacteria were collected by centrifugation at 1200 \times g for 5 min, and followed by gently washing in PBS solution for 2 times. Next, the bacteria were stained in PBS containing 0.15% (vol/vol) component A and 0.15% (vol/vol) component B of LIVE/DEAD BacLight Bacterial Viability kit for 10 min under dark condition. Finally, bacteria were harvested for further use.

Bacteria Morphology. The microcavity interfaces (SNMA_{30/10}, SNMA_{30/7}, SNMA_{50/10}, SNMA_{50/7} and PSN) which treated with cross-flowing filtration were fixed with 2.5% (V/V) glutaraldehyde solution in 0.01 M PBS and stored at 4 °C. After 12 h, microcavity interfaces were washed with PBS and dehydrated through ethanol with concentration gradients (25%, 50%, 75%, 90%, 95% and 100%) for 15 min with each step and last step twice. Then the samples were lyophilized overnight. For high contrast imaging, all samples were sputter-coated with platinum in a sputtering device (Leica EM ACE200) and observed using SEM.

Statistical Analysis. All data were presented as means \pm s.d. from three or more than three replicates. Two group comparisons were made using two-tailed Student's T-tests. Statistical significances were analyzed by using the SPSS (Statistical Product and Service Solutions Software, version 22.0). *P values* < 0.05 were considered statistically significant with **P* < 0.05 and ***P* < 0.01. *P values* > 0.05 were considered non-significant (ns).

Supporting Figures



Scheme S1 Schematic diagram of microstructure of photomask. Diameter = 7 μm or 10 μm and center distance = 30 μm or 50 μm .

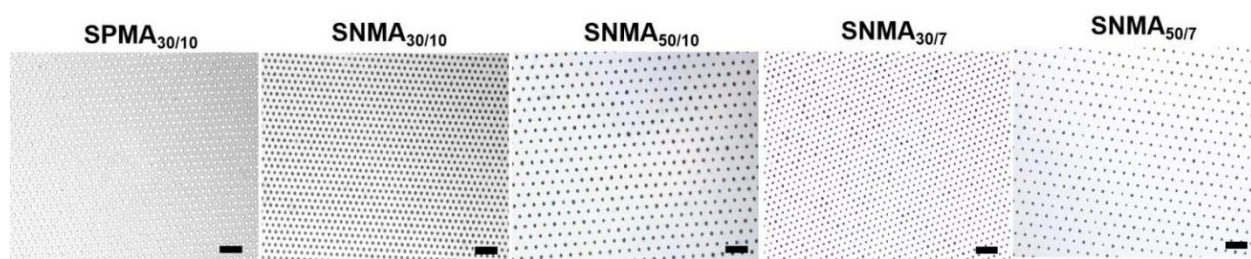


Figure S1 The microscopy images of SPMA_{30/10}, SNMA_{30/10}, SNMA_{50/10}, SNMA_{30/7} and SNMA_{50/7}, scale bar: 100 μm .

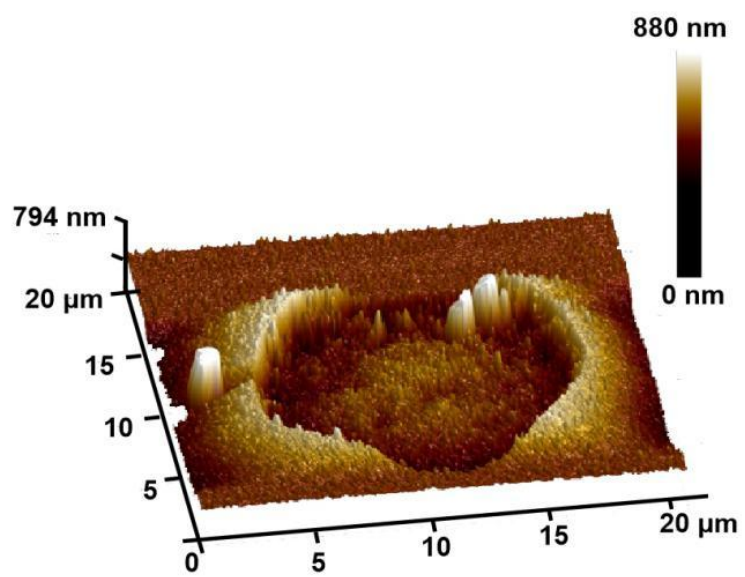


Figure S2 The 3D AFM image of SNMA_{50/10}.

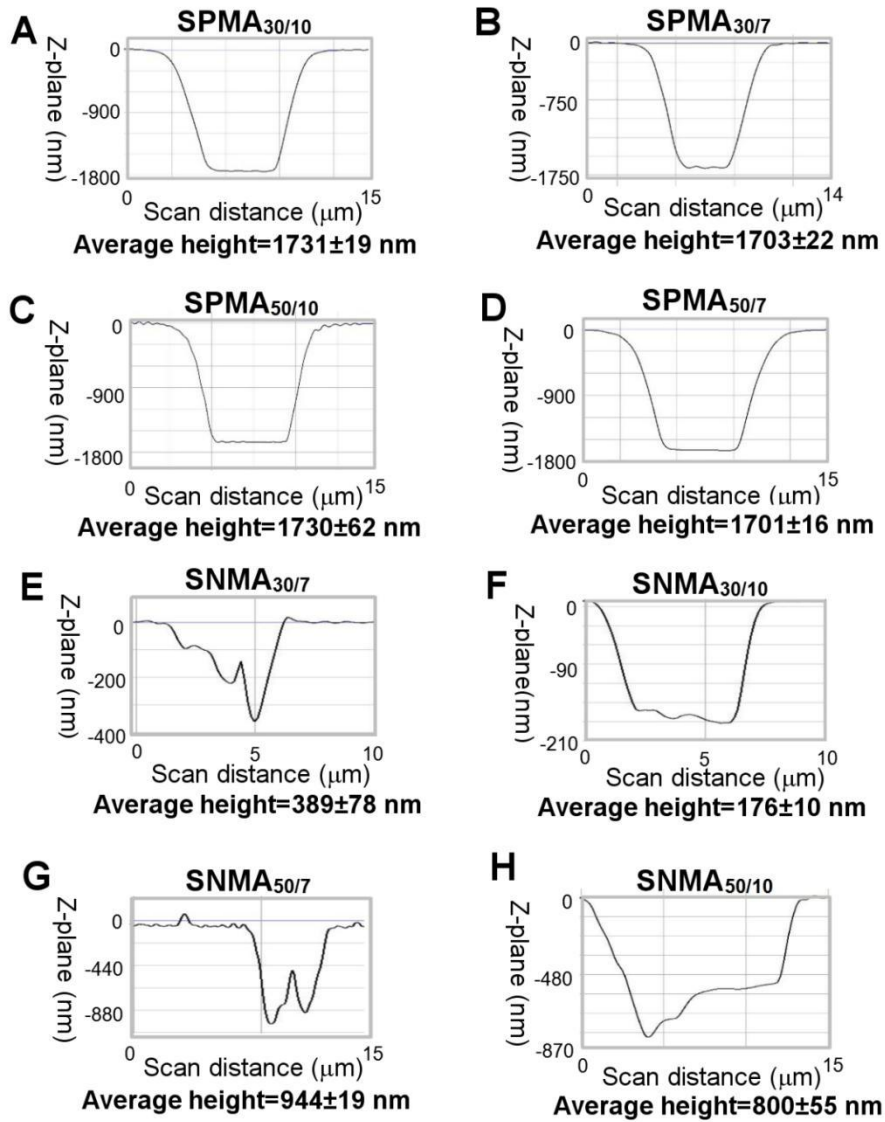


Figure S3 The average microcavity depth of SPMA_{30/10} (A), SPMA_{30/7} (B), SPMA_{50/10} (C), SPMA_{50/7} (D), SNMA_{30/7} (E), SNMA_{30/10} (F), SNMA_{50/7} (G) and SNMA_{50/10} (H) by stylus profiler measurement. Data were presented as means ± s.d. (n=5 per group).

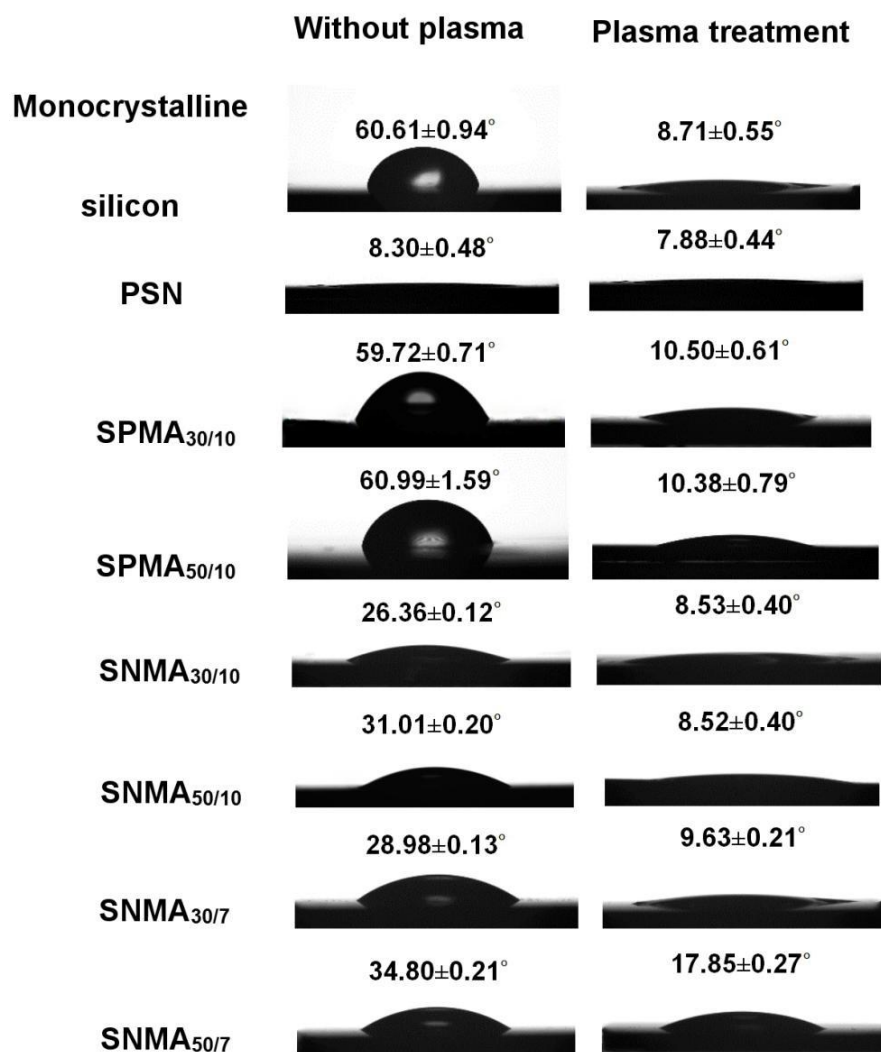


Figure S4 Morphologies of water droplets on the surfaces of monocrystalline silicon, PSN, SPMA and SNMA without plasma treatment or with plasma treatment. The Eigen contact angles were analyzed using contact angle meter. Data were presented as means \pm s.d. (n=3 per group).

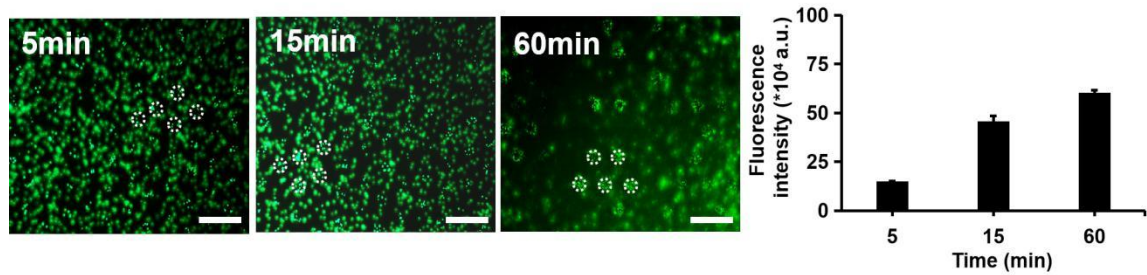


Figure S5 The real-time fluorescence images of SNMA_{30/10} under cross-flowing treatment of *E. coli* solution at a concentration of 6×10^6 CFU mL⁻¹. Scale bar: 50 μ m.

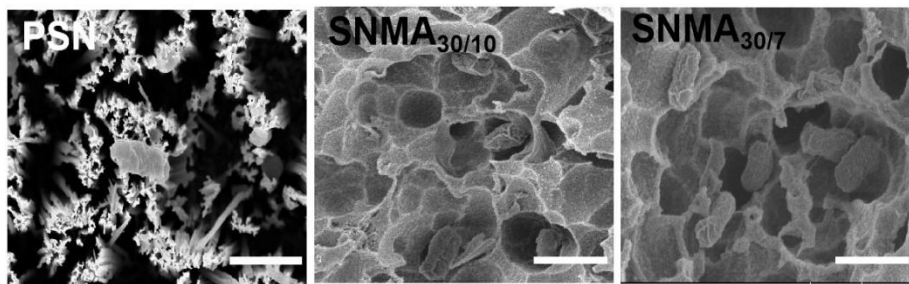


Figure S6 SEM images of plasma-treated PSN, SNMA_{50/10} and SNMA_{50/7} after cross-flowing treatment of *E. coli* solution. Scale bar: 2 μm.

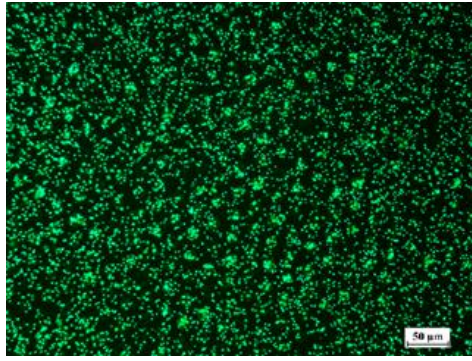


Figure S7 Representative fluorescence images of suspended bacteria treated with cross-flowing treatment with eGFP-expressing *E. coli* following with static culture treatment.

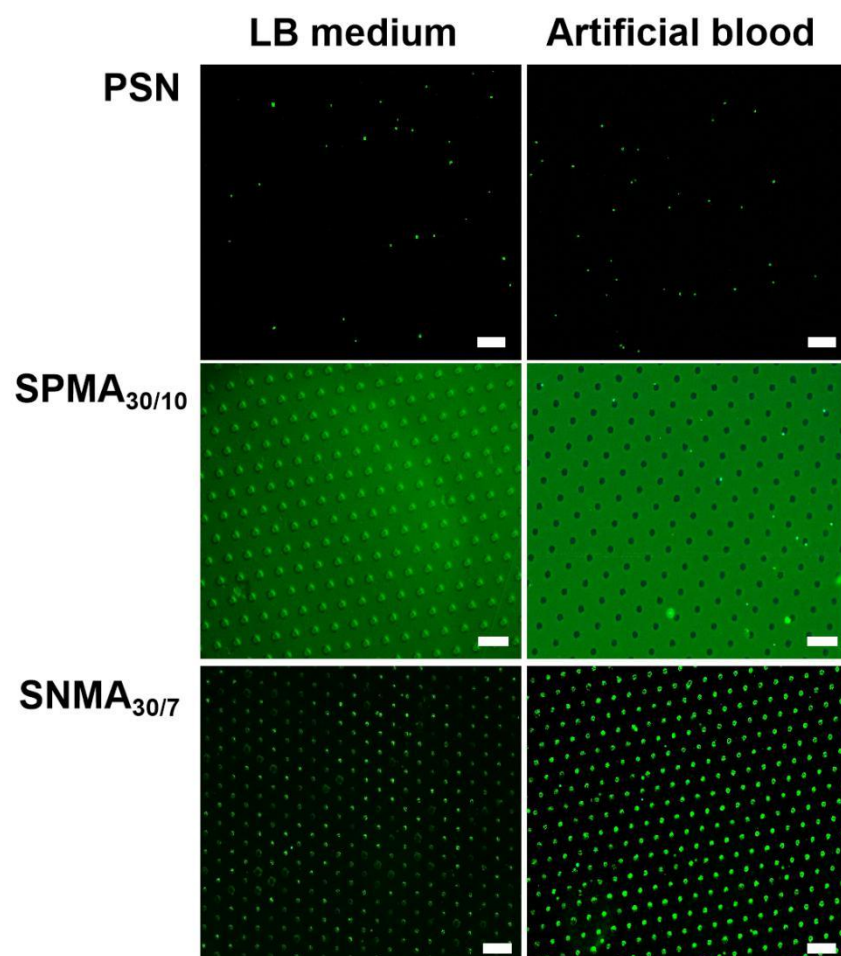


Figure S8 Representative fluorescence images of PSN, SPMA_{30/10} and SNMA_{30/7} after cross-flowing treatment of *E. coli* in the LB and artificial blood medium. The concentration of *E. coli* was 6×10^6 CFU mL⁻¹, the flow velocity was 8.5 cm s⁻¹ and the flow entering angle was 30°. The Scale bar: 50 μm.

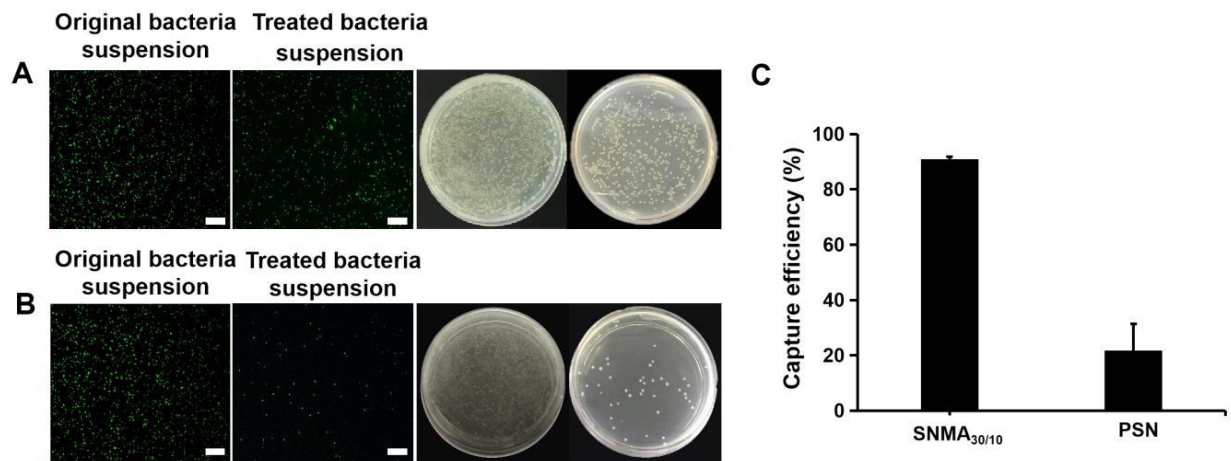


Figure S9 (A) Representative fluorescence images of *E. coli* in human blood before or after cross-flowing treatment from PSN. Scale bar: 50 μm . A representative photograph of agar plates with colonies growing from *E. coli* in the original bacteria suspension or the bacteria suspension after cross-flowing treatment from PSN. (B) Representative fluorescence images of *E. coli* in human blood before or after cross-flowing treatment from SNMA_{30/10}. Scale bar: 50 μm . A representative photograph of agar plates with colonies growing from *E. coli* in the original bacteria suspension or the bacteria suspension after cross-flowing treatment from SNMA_{30/10}. (C) The bacterial capture efficiency of PSN and SNMA_{30/10} after human blood cross-flowing treatment. The concentration of *E. coli* in human blood was 6×10^6 CFU mL⁻¹. The flowing velocity was 8.5 cm s⁻¹ and the flowing entering angle was 30°. Data were presented as means \pm s.d. (n=10 per group).

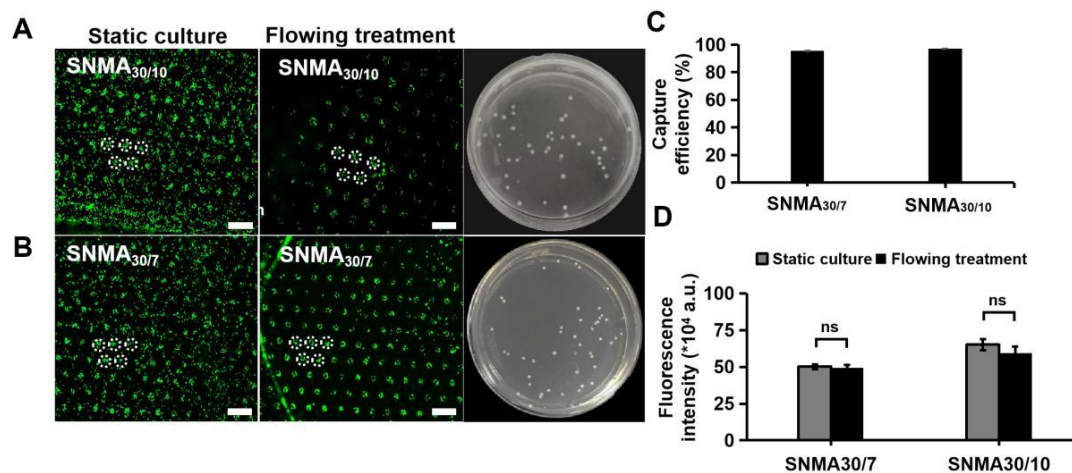


Figure S10 Representative fluorescence images of (A) SNMA_{30/10} and (B) SNMA_{30/7} cultured with *s. aureus* solution at a concentration of 0.6×10^6 CFU mL⁻¹ under static culture and cross-flowing treatment, respectively. Scale bar: 30 μ m. Photos of agar plates with colonies growing from the residual *s. aureus* in human blood after cross-flowing treatment from SNMA_{30/10} and SNMA_{30/7}. (C) The bacterial capture efficiency of SNMA_{30/10} and SNMA_{30/7} after human blood cross-flowing treatment. The concentration of *s. aureus* in human blood was 0.6×10^6 CFU mL⁻¹. The flowing velocity was 8.5 cm s⁻¹ and the flowing entering angle was 30°. (D) Fluorescence intensities of *s. aureus* inside microcavities of SNMA_{30/10} and SNMA_{30/7} after different treatments. Fluorescence intensities were measured by microscopy using Image J software. Data were presented as means \pm s.d. (n=10 per group). ns = no significance, ** $p < 0.01$.

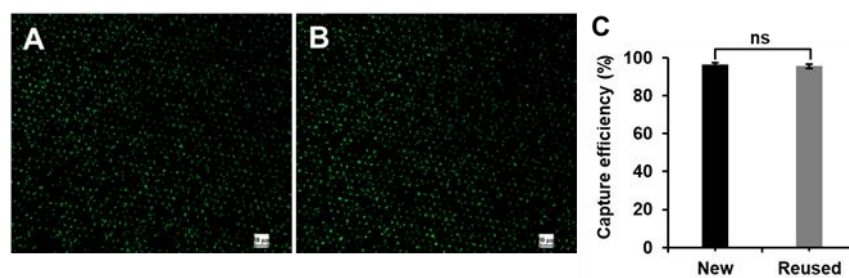


Figure S11 Fluid blood cleaning using biomimetic microcavity interface. Representative fluorescence images of newly synthesized SNMA_{30/7} (A) and recycled materials SNMA_{30/7} (B) after cross-flowing treatment of human blood containing *E.coli* at 6×10^6 CFU mL⁻¹. (C) The capture efficiencies of newly synthesized SNMA_{30/7} and recycled materials SNMA_{30/7} after cross-flowing treatment of *E. coli* in human blood.

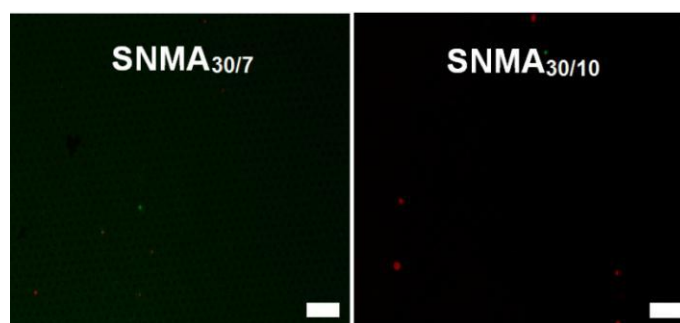


Figure S12 The fluorescence images of blood cells trapped in SNMA_{30/7} and SNMA_{30/10} after human blood cross-flowing treatment. Green fluorescence indicated live cells; red fluorescence indicated dead cells. Scale bar: 100 μm .

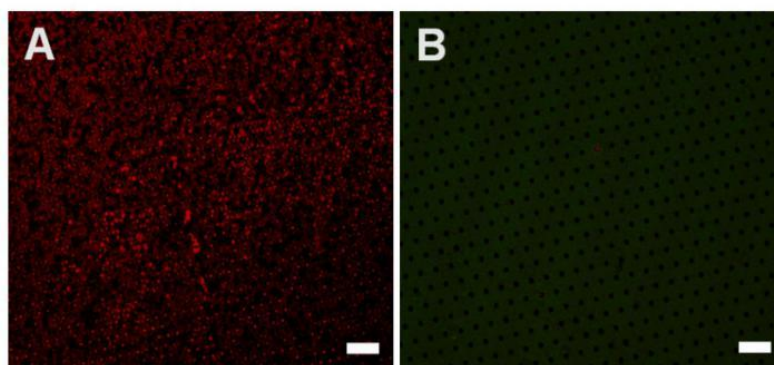


Figure S13 (A) A representative fluorescence image of SNMA_{30/10} when 50 μL of blood containing fluorescent BSA was deposited onto the microcavity interface before cross-flowing treatment. (B) A representative fluorescence image of SNMA_{30/10} after cross-flowing treatment of blood containing fluorescent BSA. The flow velocity was 8.5 cm s^{-1} and the flow entering angle was 30° . BSA was labeled with rhodamine-B in PBS solution at a concentration of 2 mg mL^{-1} . Scale bar: $100 \mu\text{m}$.

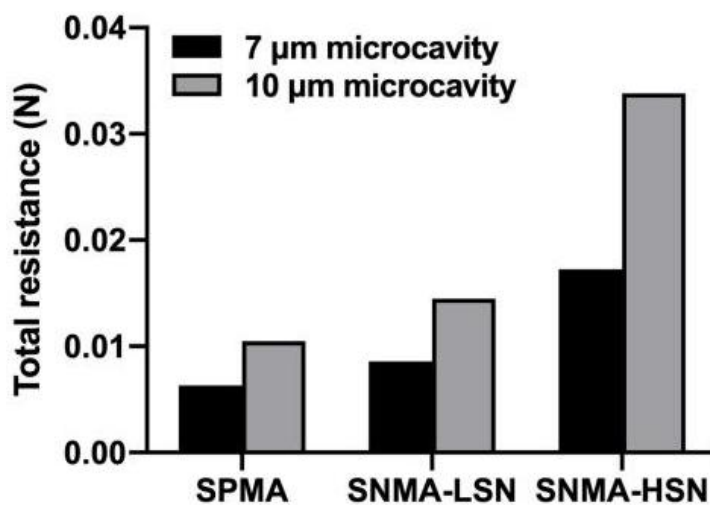


Figure S14 The simulated total resistance of fluid in microcavities with an opening diameter of 10 μm or 7 μm in SPMA, SNMA-LSN and SNMA-HSN. The slopes of nanowires in SNMA-LSN and SNMA-HSN were 0.48 and 5.33, respectively, which were calculated as the nanowire height divided by half of the bottom width. The flow entering velocity was simulated as 8.5 $\text{cm}\cdot\text{s}^{-1}$.

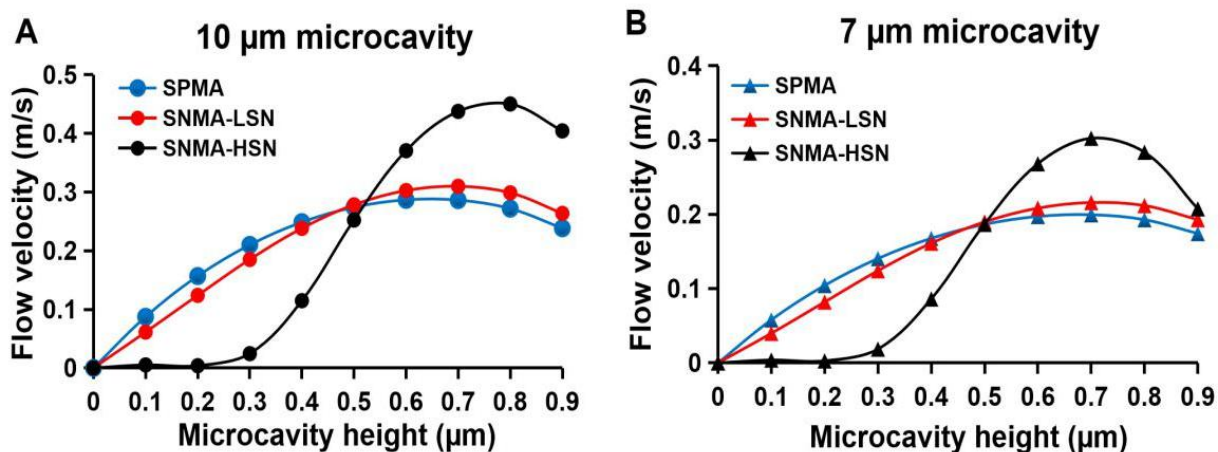


Figure S15 The simulated flow velocity of the solution in microcavities with a diameter of 10 μm (A) or 7 μm (B) as a function of the microcavity height. The points were collected from the geometric center of the bottom circle to the geometric center of the top circle. SNMA-LSN meant SNMA with low-slope nanowires. SNMA-HSN meant SNMA with high-slope nanowires. The flow entering velocity was 8.5 cm s^{-1} and the flow entering angle was 30° .

Table S1 Microcavity depth of microcavity interface

Sample	Microcavity depth (nm)*	Microcavity depth (nm)**
SPN	115±25	-
SPMA_{30/10}	1731±19	-
SPMA_{30/7}	1703±22	-
SPMA_{50/10}	1730±62	-
SPMA_{50/7}	1701±16	-
SNMA_{50/10}	800±55	1046±104
SNMA_{50/7}	944±78	1134±101
SNMA_{30/10}	176±10	246±57
SNMA_{30/7}	389±19	439±65

* Microcavity depth was detected using stylus profiler;

** Microcavity depth was detected using atomic force microscopy (AFM);