ADVANCED MATERIALS INTERFACES

Supporting Information

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Dosage-Dependent Antimicrobial Activity of DNA-Histone Microwebs Against *Staphylococcus Aureus*

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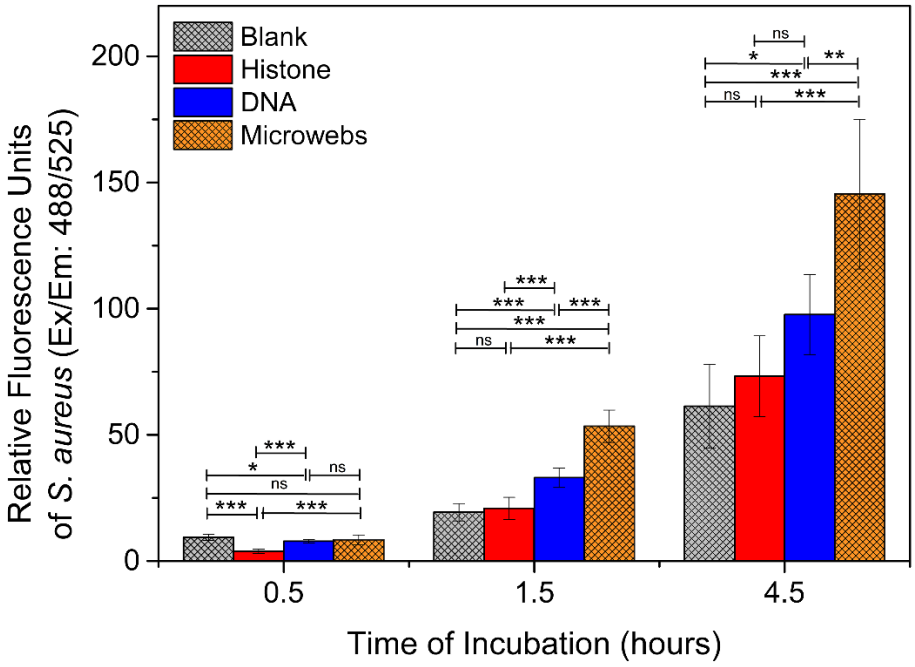

Figure S1. Statistics of the relative fluorescence intensity of *S. aureus* (RN4220-sfGFP) attached to blank, DNA, histone, or μwebs supplemented microplates, with condition carried out six times. The statistics were performed by ANOVA followed by post-hoc Tukey test: ns, not significant; *p<0.05, **p < 0.01 and ***p < 0.001.Two-way ANOVA was applied for statistical analysis of different component at different time on bacteria adhesion and the result showed all data had significant influence.

Experimental Section for Figure S2

Preparation of DNA-histone mesostructures (DHMs) and suspended microwebs (μwebs): To mimic DNA-histone complex in suspension, methylated lambda phage DNA (λ-phage DNA, Sigma, D3779) were crosslinked with calf thymus histone (calf thymus histone, Sigma, H9250). The suspended μwebs were produced using a probe sonicator (Qsonic 125, intensity set: 20%, 15 seconds) to homogenized different concentrations and ratios of DNA and histone mixed together directly in Hank's Balanced Salt Solution (HBSS) solutions.

Neutrophil isolation: Whole blood was drawn from healthy donors, with informed consent, and kept in EDTA-coated hematology tubes (Becton Dickinson). All protocols for blood collection and processing were approved by the Institutional Review Boards of Georgia Institute of Technology and Emory University. Neutrophils were purified from blood using the MACSxpress® Whole Blood Neutrophil Isolation Kit (Miltenyi Biotec), following the manufacturer's protocol. Briefly, whole blood was mixed with a bead solution that negatively selects for neutrophils, and the resulting mixture underwent magnetic separation. The supernatant was collected and centrifuged at 300 xg for 10 min to remove plasma, and the resulting pellet was reconstituted with 1X Red Blood Cell Lysis Solution (Miltenyi), at most twice, to clear of residual red blood cells. The neutrophils were again centrifuged and reconstituted in neutrophil media, RPMI 1640 (Gibco) with 3% FBS (Gemini Bio). This procedure typically results in 3-5 million live neutrophils per mL of whole blood.

NET production: A mixture of approximately 10 million neutrophils in media was

prepared with 500 nM phorbol 12-myristate 13-acetate (PMA) (Sigma Aldrich) before immediately seeding onto 60 mm Petri dishes (ThermoFisher). The dishes were kept at 37˚C for at least 4 hours in order induce NETosis. NETs were collected by washing with cold PBS and purified by serial centrifugation as described by Najmeh1. To quantify the amount of NET DNA, NETs were first homogenized with probe-sonication, and the DNA in the NET suspension was purified by a phenol-chloroform extraction approach. The released free DNA was quantified with NanoDrop 2000c.

Bacteria culture: A frozen glycerol stock of GFP-*S. aureus* (USA300, USA) was streaked on a tryptic soy agar (TSA, Sigma 22091) plate and incubated at 37 °C. After overnight culture, one bacterial colony was scratched from TSA plate and suspended in 1 mL tryptic soy broth (TSB, Sigma 22092) supplemented with 1wt% glucose (TSBg). The bacteria culture was further incubated under a rotation speed of 220 rpm at 37 °C for 3-4 hours until their optical density OD600 reaches 0.3~0.6. Then, the culture is diluted using TSBg until OD_{600}=0.02 (*S. aureus* density: ~10^7 CFU mL^{-1}) for bacterial adhesion and growth curve measurement.

Bacterial killing test: 10 µL of *S. aureus* (USA300, USA) culture (OD=0.02) were separately mixed with 100 µL HBSS, HBSS supplemented with DNA (0.2 mg mL^{-1}), HBSS supplemented with histone (0.2 mg mL^{-1}) and HBSS supplemented with suspended µwebs (0.4 mg mL^{-1}). After 1 h incubation at 37°C, the bacterial culture was diluted with deionized water at a ratio of 1:1000. Subsequently, 10 µL of the diluted bacterial culture was extracted and spotted on a tryptic soy agar plate. Each

sample was serially diluted 6 times to obtain a minimum of 50 CFU counts in the blank control group. After further incubation overnight, the CFU counts in each bacterial sample were enumerated. The CFU data were presented as mean values ± S.D. The percent of killed *S. aureus*, P, was calculated from the reduced CFU counts relative to that in the blank control group in HBSS. ANOVA followed by post-hoc Tukey test was applied to quantify statistical significance.

Bacterial proliferation test: Suspended μwebs were pelleted by centrifugation (10^4 xg for 10 min) and resuspended in HBSS to concentrations of 80, 200, 400, and 800 μg mL^{-1}. Next, 100 μL of the suspended μwebs at different concentrations were separately mixed with 100 μL of the diluted bacterial culture (OD=0.02) for bacterial growth curve measurement. As comparison groups, 100 μL of HBSS supplemented with DNA (40, 100, 200, 400 μg mL^{-1}), or histone (40, 100, 200, 400 μg mL^{-1}) were separately mixed with 100 μl of the diluted bacterial culture and reallocated into 96-well plates (200 μL total in each well). Proliferation of *S. aureus* was monitored at 37°C in a plate reader for 16-24 hours, and the fluorescence intensity of GFP-*S. aureus* (Ex/Em = 488/525 nm) were measured.

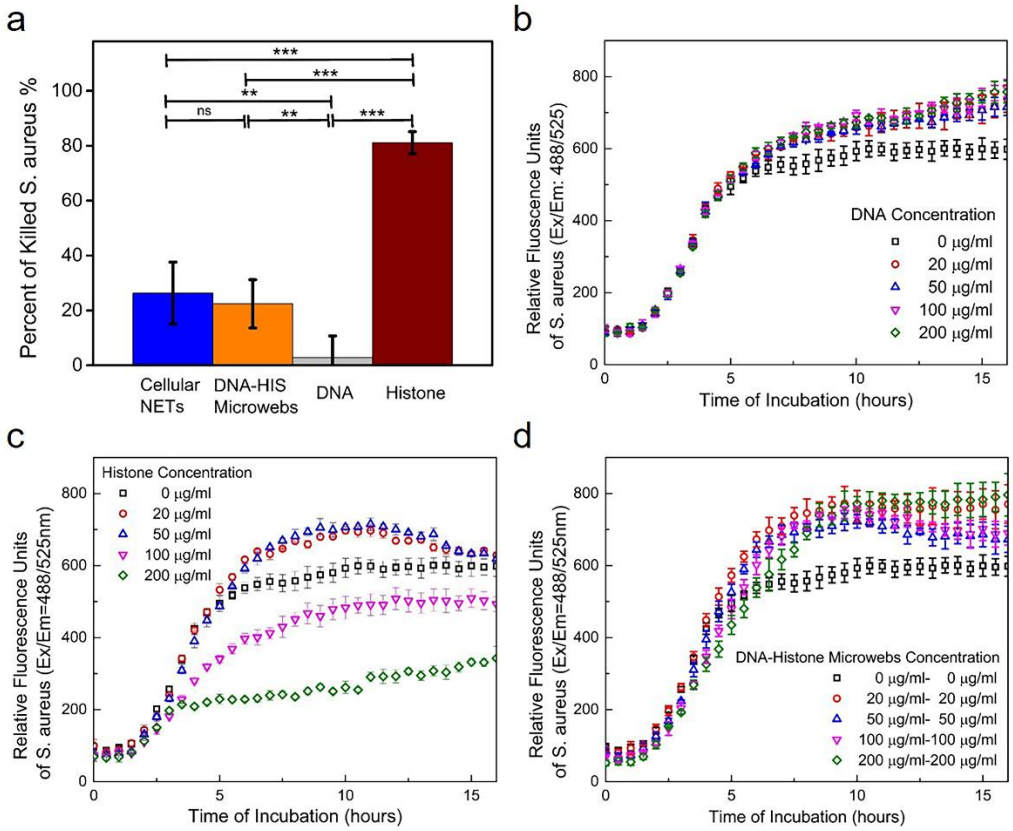

Figure S2. Evaluation of the bacterial killing potency of DNA, histone and DNA-histone µweb suspensions against *S. aureus* (USA300, USA). a) Enumeration of colony forming units of *S. aureus* (seeding density: 10^6 CFU mL^{-1}) after culture in nutrient-poor HBSS media containing neutrophil-derived NETs (DNA is concentrated to 200 µg mL^{-1}), DNA-histone µwebs suspensions (200 µg mL^{-1} DNA + 200 µg mL^{-1} histone), DNA solution (200 µg mL^{-1}) and histone solution (200 µg mL^{-1}). ANOVA followed by Tukey's test was used for statistical analysis: ns, not significant; *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$, N=6 for each condition. b-d) Growth curve of *S. aureus* in nutrient-rich TSBg medium containing (b) DNA, (c) histone, or (d) µwebs at physiologically relevant concentrations.

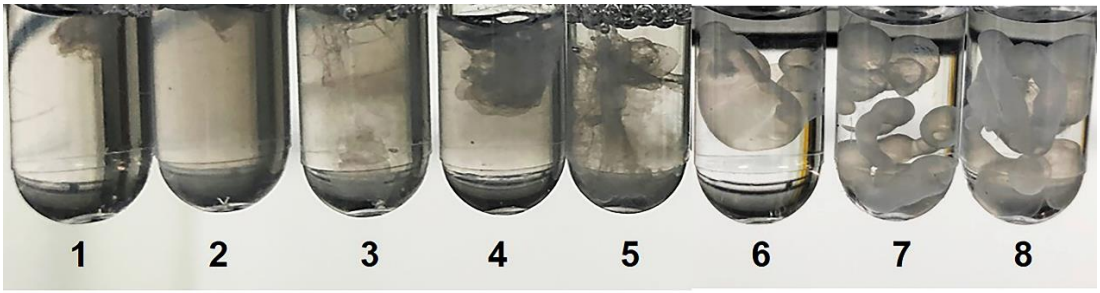

Figure S3. The images of different concentration of microwebs when the ratio of DNA and histone was 1:1. 1) 50 μg mL^{-1} (it means the final concentration of DNA and histone), 2) 75 μg mL^{-1}, 3) 100μg mL^{-1}, 4) 200 μg mL^{-1}, 5) 300 μg mL^{-1}, 6) 500 μg mL^{-1}, 7) 750 μg mL^{-1}, 8) 1000 μg mL^{-1}. The hydrogels was formed when the concentration of microwebs was higher than the critical concentration of 500 μg mL^{-1}.

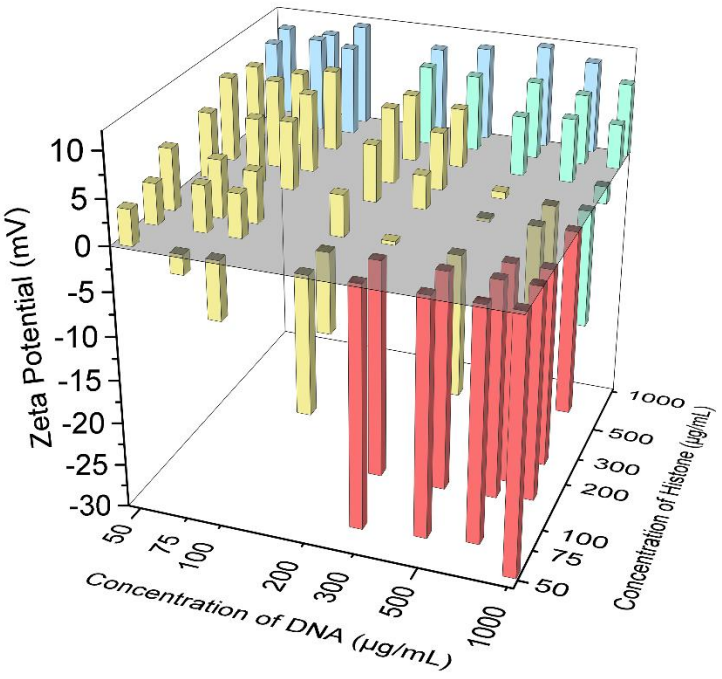

Figure S4. Zeta potentials of microwebs with histone and DNA at different concentrations in HBSS. The different colors are correspondent to the four region in Figure 5c.

Table S1. Zeta potentials of *S. aureus* (RN4220-sfGFP and USA300) in HBSS supplemented with histone at different concentrations.

Histone Conc. (μg mL^{-1})	0	50	100	200	400
Zeta Potential of *S. aureus* (RN4220-sfGFP, mV)	-5.4±0.5	-3.3±0.2	2.6±0.1	5.2±0.1	7.7±0.3
Zeta Potential of *S. aureus* (USA300, mV)	-8.4±0.1	1.2±2.3	6.8±0.7	7.9±0.1	8.1±0.2

REFERENCES

1. Najmeh, S.; Cools-Lartigue, J.; Giannias, B.; Spicer, J.; Ferri, L. E., Simplified Human Neutrophil Extracellular Traps (NETs) Isolation and Handling. *Journal of visualized experiments : JoVE* **2015,** (98).