Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

The study was approved by The Committees of Health Research Ethics in the Capital Region of Denmark (H-15020632).

Establishment of bacterial biofilms and procurement of supernatants

Bacterial biofilm supernatants were produced from one laboratory strain of *Staphylococcus aureus* (8325-4) in 50 ml BD Bacto™ Tryptic Soy Broth (TSB) (Becton Dickinson Diagnostic Systems, Heidelberg, Germany) and a pyocyanin-producing *Pseudomonas aeruginosa* PAO1 strain (MH340) in 50 ml agrobacterium (AB) medium containing 0.5% glucose and 0.5% casamino acids in a biofilm mode for 72 h in 250-ml Erlenmeyer flasks without agitation. The number of bacteria was estimated by plate count.

Isolation of human PMNs from whole blood

PMNs were isolated from 2 male and 2 female healthy donors (28–29 years).

Venous whole blood was collected in citrate containing evacuated Vacuette® tubes (Greiner Bio-One, Frickenhausen, Germany) and mixed with 5% T500 dextran in 0.9% NaCl and allowed to sediment. The leukocyte-enriched plasma was applied to Lymphoprep™ (Stemcell Technologies, Vancouver, Canada), and PMNs were isolated after centrifugation at 855×g. The contaminating erythrocytes were lysed with 0.2% NaCl. The PMNs were resuspended in Krebs-Ringer balanced salt solution (S1) with 10 mmol/l glucose and counted (NucleoCounter®, ChemoMetec, Allerød, Denmark).

Exposure of PMNs to biofilm supernatants

The PMNs (10×10^6 cells/ml) were incubated for 60 min at 37°C with an equal volume of the sterile-filtered (0.22 μm) biofilm supernatants adjusted with the respective medium to 50×10^6 CFU/ml (final concentration) and 250×10^6 CFU/ml in 0.3–0.4 ml (S2, S3). The control comprised equal volumes of Krebs-Ringer balanced salt solution with 10 mmol/l glucose and TSB/AB medium. The PMN viability was evaluated by flow cytometry sorting and propidium iodide before and after incubation. Samples were centrifuged at 1,000×g for 5 min, and conditioned media and pellets in equivalent volumes of Krebs-Ringer balanced salt solution with 10 mmol/l glucose were stored at –80°C.

Wound fluid collection

Wound fluid was collected from VLUs and STSG donor sites for 24 h by applying our standardised method using a hydrophobic polyurethane foam (S4, S5).

MMP-8 analyses

MMP-8 total levels were determined by enzyme-linked immunosorbent assay (RayBiotech, Norcross, GA, USA).

MMP-8 molecular species were examined by Western immunoblotting. Samples (non-reduced) were separated on Criterion XT Precast 26-well 4–12% SDS-PAGE gradient gels and electrotransferred onto a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA), which was blocked with an Odyssey buffer, incubated for 18 h at 4°C with monoclonal rabbit antibody against human MMP-8 (ab81286; Abcam Cambridge, UK) diluted 1:1,000, and then with goat anti-rabbit DyLight® (Thermo Fisher Scientific, Waltham, MA, USA) 800-conjugated secondary antibody diluted 1:5,000 for 1 h at ambient temperature. The immunoreactions were visualized using infrared imaging (S6).

Gelatin and casein zymography

Gelatin (4 h incubation at 37°C) zymography was carried out according to Trøstrup et al. (S5) including rhMMP-9 (RayBiotech) standard. Casein (72 h incubation at 37°C) zymography was carried out as described by Ågren et al. (S6) with rhMMP-1 (420-01; PeproTech, Rocky Hill, NJ, USA) and APMA (4-aminophenylmercuric acetate)-activated rhMMP-8 (908-MP; R&D Systems, Minneapolis, MN, USA) standards.

Statistical analyses

One-way analysis of variance (ANOVA) was applied to the MMP-8 levels determined by ELISA, followed by the Tukey HSD post-hoc test (IBM SPSS® Statistics 26.0, Armonk, NY, USA). The level of statistical significance was $p < 0.05$.

SUPPLEMENTARY REFERENCES

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