



Healing cascades and infections in wounds monitored using a wearable sensor of gaseous flux

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Contributed by John A. Rogers; received April 21, 2026; accepted August 27, 2026; reviewed by Milica Radisic and Bozhi Tian

Capabilities for quantitative monitoring of chronic wounds remain an unmet clinical need, as existing diagnostic approaches rely on semiquantitative evaluation of symptoms that lack sensitivity especially during early stages of infection. Here, we present a scheme for tracking wound physiology that leverages a miniature, wireless skin-interfaced device for noncontact, transient measurements of the flux of volatile organic compounds (VOCs) and water vapor from the wound microenvironment. Unlike emerging smart bandage platforms that rely on physical contact with the fragile wound bed to interrogate liquid-phase biomarkers, this strategy uses an engineered microclimate and suspended suite of sensors to measure the diffusive transport of wound-derived gases across the wound surface but separated from it. The result enables quantitative evaluation of metabolic activity and healing progression without perturbing the healing tissues. In biofilm growth models of *Staphylococcus aureus*, measurements demonstrate that trends in VOC flux correlate strongly with bacterial growth kinetics and precede any visible biofilm formation. Longitudinal monitoring of infected and noninfected wounds on diabetic mice shows that concurrent measurements of water vapor and VOC flux provide complementary physiological insights, capturing both the trajectory of barrier restoration and the dynamics of bacterial burden. The findings establish this noncontact sensing scheme as a distinct and clinically translatable paradigm for wound monitoring, with broad implications for noninvasive surveillance of disease states in which tissue metabolic activity and skin barrier integrity serve as actionable physiological readouts.

wounds | bioelectronics | sensors

Chronic wounds remain a major clinical challenge and economic burden (1, 2), partly due to the lack of technologies for identifying subtle signs of emerging complications (1, 3, 4). Early detection of infection is especially critical because bacterial proliferation can progress rapidly to cause persistent inflammation and impede re-epithelialization and extracellular matrix remodeling (5). In medical practice, diagnosis of infection relies largely on visual inspection and assessment of clinical symptoms, including appearance of redness, exudate, and signs of delayed healing (6–8). These signatures, however, only appear after bacteria colonization, at which point the infection has increased resistance to treatment, thereby limiting opportunities for effective intervention. Likewise, microbiological cultures and susceptibility testing provide accurate analysis, but their turnaround times are often long relative to the rapid progression of infections (3, 4, 9). Detection of complications at the earliest stages can significantly improve the outcomes of treatment strategies, which rely largely on antibiotics, despite concerns that their widespread and indiscriminate use may contribute to antimicrobial resistance (10, 11). Accurate evaluation of the efficacy of a selected therapeutic strategy can help prevent antibiotic overuse (11). Episodic clinical follow-ups, often separated by days or weeks, provide only fragmented insights into the wound healing process, with potential delays in important adjustments to care procedures. These challenges underscore the additional need for rapid, frequent, and noninvasive monitoring strategies for assessing wound status, especially those that can be used by the patients themselves in the home.

The concept of electronically instrumented wound dressings—so-called “smart bandages”—is the subject of considerable research attention as a means of continuous in situ wound monitoring. Early demonstrations show that flexible electrochemical sensors integrated into dressing substrates can wirelessly track parameters that shift measurably during infection and inflammation (12, 13), from pH and temperature to uric acid, lactate, hydrogen peroxide, and glucose, as signatures of metabolic activity (14–16). These platforms (12, 14–18), however, require complex engineered systems and direct physical contact with the

Significance

Limited capabilities in continuous, quantitative assessment of a wound site create challenges in early diagnosis and effective management, particularly in cases of infection that rapidly progress before symptoms appear. Noncontact approaches for wound monitoring using biophysical and/or biochemical sensors that preserve fragile tissue have the potential to transform methods in wound care. In this context, gaseous flux from the wound bed provides an integrative measure of microbial activity and barrier restoration. This study establishes a wearable sensing platform that quantifies these fluxes in real time, enabling early infection detection and temporal tracking of wound healing. These results highlight a path toward precision wound care management strategies with reduced reliance on episodic clinical evaluation.

Author contributions: S.C., A.T., K.R.P., Y.H., G.A.A., and J.A.R. designed research; S.C., A.T., Z.C., K.R.P., A.Z., N.F., E.J.N., A.D.M., and J.E.Y. performed research; S.L., F.Y., J.T., and Y.H. contributed new reagents/analytic tools; S.C., A.T., Z.C., S.L., F.Y., J.S., and J.W.S. analyzed data; and S.C., A.T., Z.C., K.R.P., S.L., G.A.A., and J.A.R. wrote the paper.

Reviewers: M.R., University of Toronto; and B.T., University of Chicago.

The authors declare no competing interest.

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This article contains supporting information online at <https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2613824123/-/DCSupplemental>.

Published September 29, 2026.

fragile wound bed and/or with exudate. Disadvantages range from the potential for physical disruptions to healing tissues, to restrictions in wound care approaches, biofouling of the sensors and difficulties in sterilization for cost-effective reuse.

An alternative is in assessments that rely on volatile organic compounds (VOCs), including organic acids, small alcohols, carbonyls, ammonia, and CO₂, that diffusively contribute to the microclimate above the wound site (19, 20). Quantitative evaluation of these molecules and the rate of their emergence from the wound can yield important insights into the physiology of the healing tissues for quantitative evaluation of the wound status. Previous studies based on gas sensing demonstrate detection of the concentration of microbial VOCs, but many of these methods involve direct contact of the sensor to the wound tissue and/or require complex instrumentation and time-consuming purging systems (20–23). Means for noncontact, quantitative analysis of gaseous biomarkers in situ with a small, convenient device would overcome these challenges. Additional clinical value could follow from simultaneous measurements of the flux of water vapor from the wound, as a basis for tracking the phases of tissue repair that reestablish the structural and functional integrity of regenerated skin at the wound site (1, 24). Because water vapor flux (f_w) often remains elevated even after clinical closure of the wound relative to that of intact skin, such measurements could also define the integrity of the healed site (25, 26).

Here, we report the design and use of a miniature skin-interfaced device capable of noncontact monitoring of molecular gaseous flux for evaluation of wound status and correlation to physiological analysis, without constraining care procedures or occluding diffusive exchange between the wound site and the surrounding ambient. Studies on in vitro biofilm growth (168 h), supplemented by rigorous analytical models and microbiological characterization, demonstrate that sharp increases of VOC flux (f_{voc}) correlate strongly with bacterial growth trends and appear prior to any visible biofilm formation. Through long-term monitoring (up to day 30) of infected wounds (*Staphylococcus aureus*) in mouse models, the data reveal correlations between f_{voc} , bacterial concentration, and biomarkers associated with the inflammatory phase of wound healing, strongly suggesting its potential for early detection of infection, differentiation of infection severity, and evaluation of therapeutic response. Water vapor flux density emerges as a dynamic indicator of skin barrier restoration, capturing progressive changes otherwise quantified through image-based assessments of wound closure and histological analysis.

Results

The key findings described herein rely on the use of a miniature (Fig. 1 A and B and SI Appendix, Fig. S1) skin-interfaced device capable of quantifying molecular gaseous flux from wound beds in a noncontacting fashion. As reported in detail elsewhere (27), the device features a chamber that seals against healthy skin (HS) around the perimeter of the site of the wound. A valve on the opposite side allows the chamber to diffusively equilibrate with the surroundings when in its open state. In the closed state, water vapor and VOCs from the wound bed accumulate at a rate determined by their characteristic flux. Miniaturized digital sensors within the chamber quantify the time dependence of the corresponding increases and decreases in concentration as the valve closes and opens, respectively, in a cyclic fashion during the monitoring period (Fig. 1C). Interactions between a hard magnet and current passing through an electromagnetic coil produce the forces for actuation, while a pair of soft magnets located at the top and bottom of the chamber fix the valve in open and closed states,

respectively, to allow for power-efficient, bistable switching. Supporting electronics, including a Bluetooth system-on-chip for wireless communication, enable remote operation powered with a lithium polymer battery (28). The chamber unit includes the suspended sensors, the bistable magnetic valve system, and a disposable adaptor (Fig. 1D) that attaches to the bottom of the sensor housing. Disposal of the adaptor after each measurement prevents contamination of the chamber unit through direct contact with the wound area (SI Appendix, Fig. S2).

Fig. 1E illustrates changes in molecular concentration inside the chamber over time during repeated cycles of valve actuation. When closed, the concentration rapidly increases due to accumulation of molecular vapor released from the wound area and then saturates to an equilibrium value (Fig. 1F). Flux corresponds to the change in concentration per unit area per unit time, defined by the slope of the tangent to the concentration plot immediately after the valve closes. This analysis assumes diffusion-dominated transport within the enclosed chamber immediately following valve closure. Owing to the small chamber dimensions and the rapid valve actuation (~10 ms), convective contributions are negligible relative to molecular diffusion during the measurement interval. Although transient spatial concentration gradients develop immediately after chamber isolation, these effects are accounted for through a simulation-derived correction factor relating the measured concentration slope to the average molecular flux (SI Appendix). After the valve opens, the chamber concentration rapidly decreases to a value defined by an equilibrium with the surrounding environment. Benchtop experiments using water and organic solvents as controlled sources of emission demonstrate that f_w correlates inversely with the diffusion barrier thickness and that the f_{voc} correlates directly with the source concentration, both quantifiable (SI Appendix, Figs. S3 and S4). Minimal variation across f_{voc} and f_w obtained over five independent days also indicates high reproducibility of the valve actuation processes and flux measurements (SI Appendix, Fig. S5). As considered in the discussion section, this simple analysis method neglects effects of gradual accumulation of gaseous species within the chamber continuously over time across multiple cycles of valve operation which can be significant in certain circumstances. Nevertheless, approximate values of flux determined according to the procedures outlined above provide valuable data in tracking changes of interest in the following.

The reproducibility of the chamber concentration over repeated valve cycles can be further evaluated under both steady and dynamically changing VOC emission conditions. Constant VOC emission produces stable baseline concentrations over successive measurement cycles, whereas an increase in source emission results in a transient baseline shift before a new steady state was established (SI Appendix, Fig. S6). Simulations reproduce this behavior and demonstrate that the chamber returns to a reproducible initial condition under steady emission, while transient source changes temporarily alter the initial concentration for subsequent measurements (SI Appendix, Fig. S7).

The studies summarized here leverage this technology in in vitro and in vivo models, the latter with control (noninfected wound) and infected wounds in groups of small animals with single circular excision wounds formed each on their posterior dorsal region to examine the relationship between flux measurements and the wound healing process (Fig. 1G). Here, the chamber of the device aligns with the wound area through attachment to an annulus splint held by sutures to the surrounding skin of the wound, providing stability during measurements without contacting or otherwise perturbing the wound bed (Fig. 1H).

For the in vitro model, the sensor monitors emissions from bacteria cultured on an agar plate. Specifically, the measurements track the behavior of low (10³ CFU) and high (10⁷ CFU)

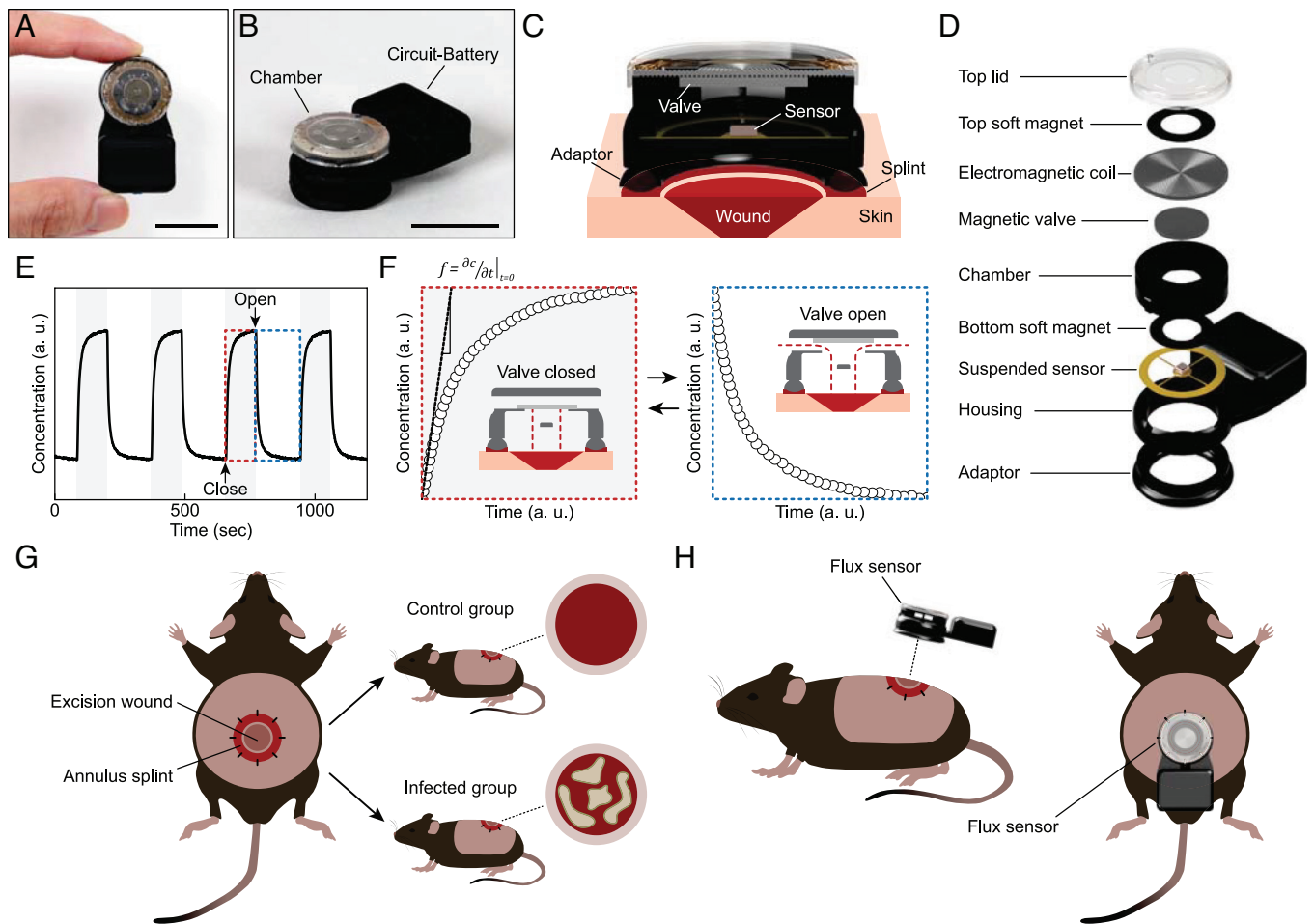


Fig. 1. Flux sensor for wound monitoring. (A and B) Top view of a flux sensor held between fingertips (A) and perspective view featuring the chamber and circuit-battery unit (B). (Scale bars, 2 cm.) (C) Cross-sectional view of the analytical chamber of the flux sensor mounted on the wound of the mouse model. (D) Exploded view illustration of the sensor featuring the major components. (E) Representative data collected through multiple cycles of valve actuation. Shaded regions correspond to periods when the valve is closed. (F) Accumulation of gaseous species in the chamber while the valve is closed (Left) and ventilation while the valve is open (Right), corresponding to regions outlined by red and blue dashed lines in (E), respectively. The slope of the tangent (black dashed line in graph) to the curve at times immediately after the valve closes corresponds to the flux. Inset images describe the position of the valve and gaseous flow during each phase. (G) Use of the flux sensor in mice studies for wound healing monitoring of control and infected wounds. (H) Side- and top-view illustrations of the flux sensor placement on the mouse model.

concentration bacterial suspensions (*S. aureus*) introduced onto the plates and then cultured in controlled environments, with the sensor fixed on the plate lid aligned with an aperture (Fig. 2A). Bacterial load, defined as the total quantity of bacteria present within the biofilm at a given time, exhibits significantly different trends between the two concentrations. 10^3 CFU shows a continuous increase in bacterial load while 10^7 CFU shows an initial increase followed by a decrease after 120 h (Fig. 2B). Analytical models of bacterial growth kinetics indicate that initial seeding density is a critical parameter because colony geometries can determine nutrient accessibility and spatial constraints. For high inoculation concentrations, closely spaced microcolonies merge into a quasi-homogeneous film that can be described by Monod kinetics with an added decay term, inducing a later decrease in bacterial load. For low inoculation concentrations, bacteria form large discrete colonies and growth is confined to the colony edge, resulting in the monotonic increase in bacterial load. These models show excellent agreement with the experimentally quantified trends. f_{voc} measurements involve three consecutive cycles of opening and closing the valve, performed once every three hours. The valve otherwise remains in an open state to minimize effects of occlusion. f_{voc} plotted by bacterial load shows a steep increase at low bacterial load and saturates at distinct levels depending on the

inoculation concentration (Fig. 2C). Analytical models of VOC emission predict a saturation-type relationship due to diffusion-limited release and feedback inhibition by accumulated VOCs and thus verify the experimental results. Further details on analytical models are described in SI Appendix.

The use of Congo red agar allows for visualization of the biofilm through color changes from red to black due to reactions with extracellular polymeric substances produced during proliferation of bacteria cells (SI Appendix, Fig. S8) (29). Analysis of plate images and plots of biofilm growth, bacterial load, and f_{voc} for cultures inoculated with 10^3 CFU over 7 d show continuous increases in all parameters, although with different onset times (Fig. 2D). Increases in bacterial load begin immediately after the start of the experiments. f_{voc} shows a sharp increase beginning at 39 h, roughly 33 h before the biofilm starts to form at 72 h. Cultures inoculated with 10^7 CFU exhibit similar trends. Specifically, bacterial load increases immediately, followed by a pronounced increase in f_{voc} somewhat later, in this case 18 h, which is 24 h before the biofilm appears at 42 h (Fig. 2E). For the 10^7 CFU inoculation, biofilm entirely fills the plate area at 96 h, leading to accelerated cell death and corresponding decreases in bacterial load and f_{voc} after 120 h and 129 h, respectively. Live/dead stain results reveal that both live cell count and total bacterial load

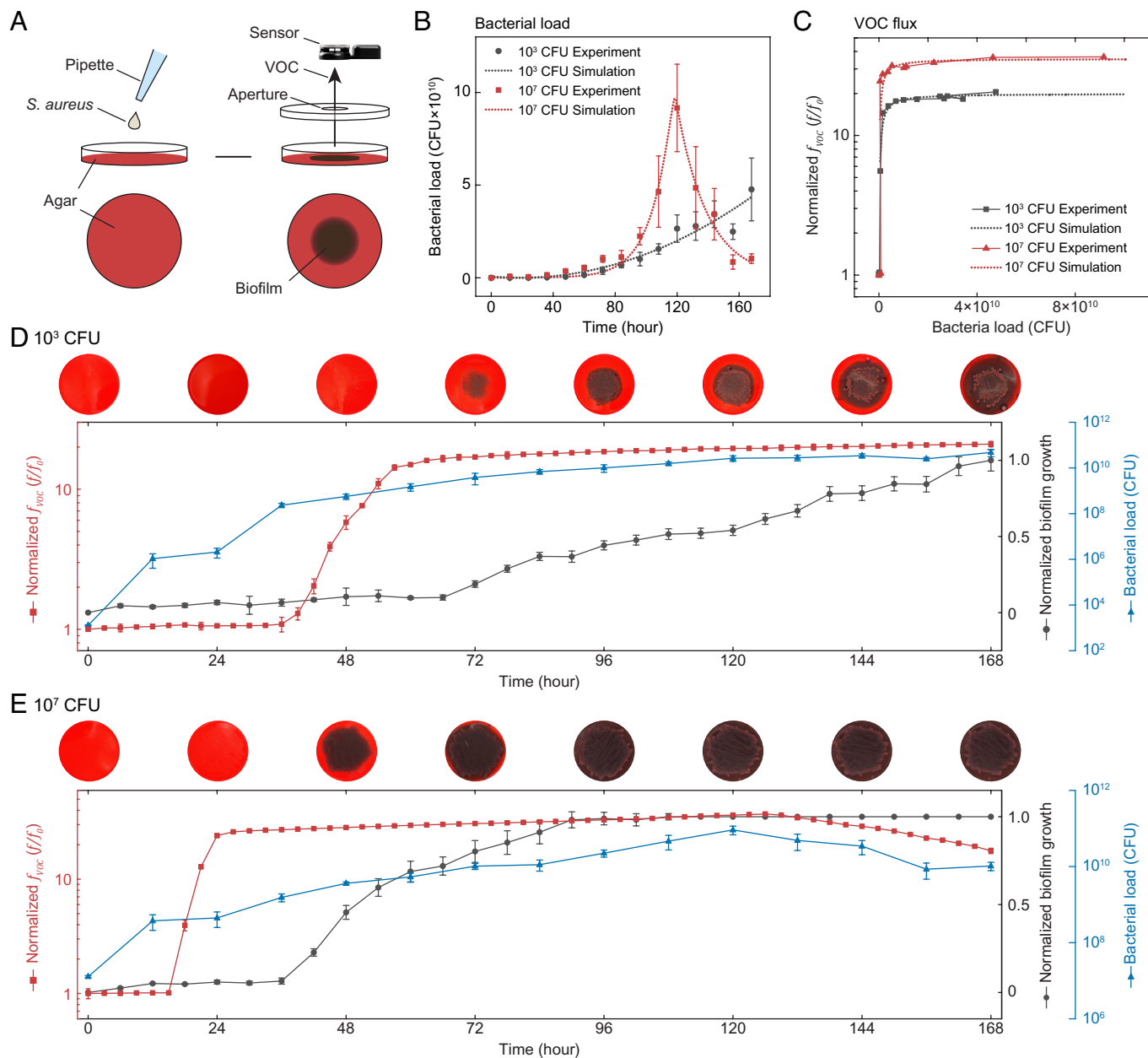


Fig. 2. Monitoring VOC flux from an in vitro biofilm model. (A) Schematic illustration of the experimental setup for studies of *S. aureus* biofilms grown in Congo red agar plates with the sensor placed on the lid with an aperture. (B and C) Experimental (solid line with symbol) and analytical model results (dotted line) for changes in bacterial load as a function of time (B) and normalized f_{voc} with increasing bacterial load for inoculation doses (C) of 10^3 CFU (black) and 10^7 CFU (red). (D and E) Images (Top) of biofilm plates inoculated with 10^3 CFU (D) and 10^7 CFU (E) taken at 0, 24, 48, 72, 96, 120, 144, 168 h time points and plots (Bottom) for normalized f_{voc} (red; $n = 3$), normalized biofilm growth (black; $n = 4$), and bacterial load (blue; $n = 3$) as a function of time. f_{voc} : VOC flux. Data are mean $\pm$ SD.

for the 10^7 CFU inoculation are larger than those of the 10^3 CFU inoculation at 84 h but smaller at 156 h (SI Appendix, Fig. S9).

These in vitro results suggest the potential for this sensing approach to detect early signs of infection in wounds, as examined here in diabetic mouse models with skin excision wounds (Fig. 3A) infected by inoculation with various doses of *S. aureus* (10 , 10^3 , and 10^5 CFU). The experiments involve alignment of the sensor onto the wound (SI Appendix, Fig. S10) and determination of f_{voc} and f_w through three consecutive cycles of opening and closing the valve for each measurement session. Additional assessments of the animals include routine determination of wound area, bacteria concentration in the wound, body weight, blood glucose, and wound temperature (SI Appendix, Fig. S11). These collective measurements occur on HS before surgery and immediately after surgery (day 0) to establish a baseline and every 3 days thereafter,

with subsequent application of wound care including passive cleaning without antimicrobial effects.

Weight loss in control ($\sim 18\%$ by day 30) and infected mice ($\sim 29\%$ by day 30) reflects the degree of systemic distress and metabolic burden (SI Appendix, Fig. S11A). Throughout the 30-d observation period, blood glucose levels remain within 400 to 600 mg·dL⁻¹ (SI Appendix, Fig. S11B) and wound temperatures range from 24 to 37 °C for both control and infected wounds (SI Appendix, Fig. S11C). Images of the wounds and quantified values for the extent of wound closure highlight differences between the healing processes of control and infected cases (Fig. 3 B and C). On day 3, both control and infected wounds display negative wound closure values (-0.1 for control and -0.05 for infected), indicating an enlargement of the wound, consistent with previous reports on the initial inflammatory phase of wound healing (24, 30, 31). Control

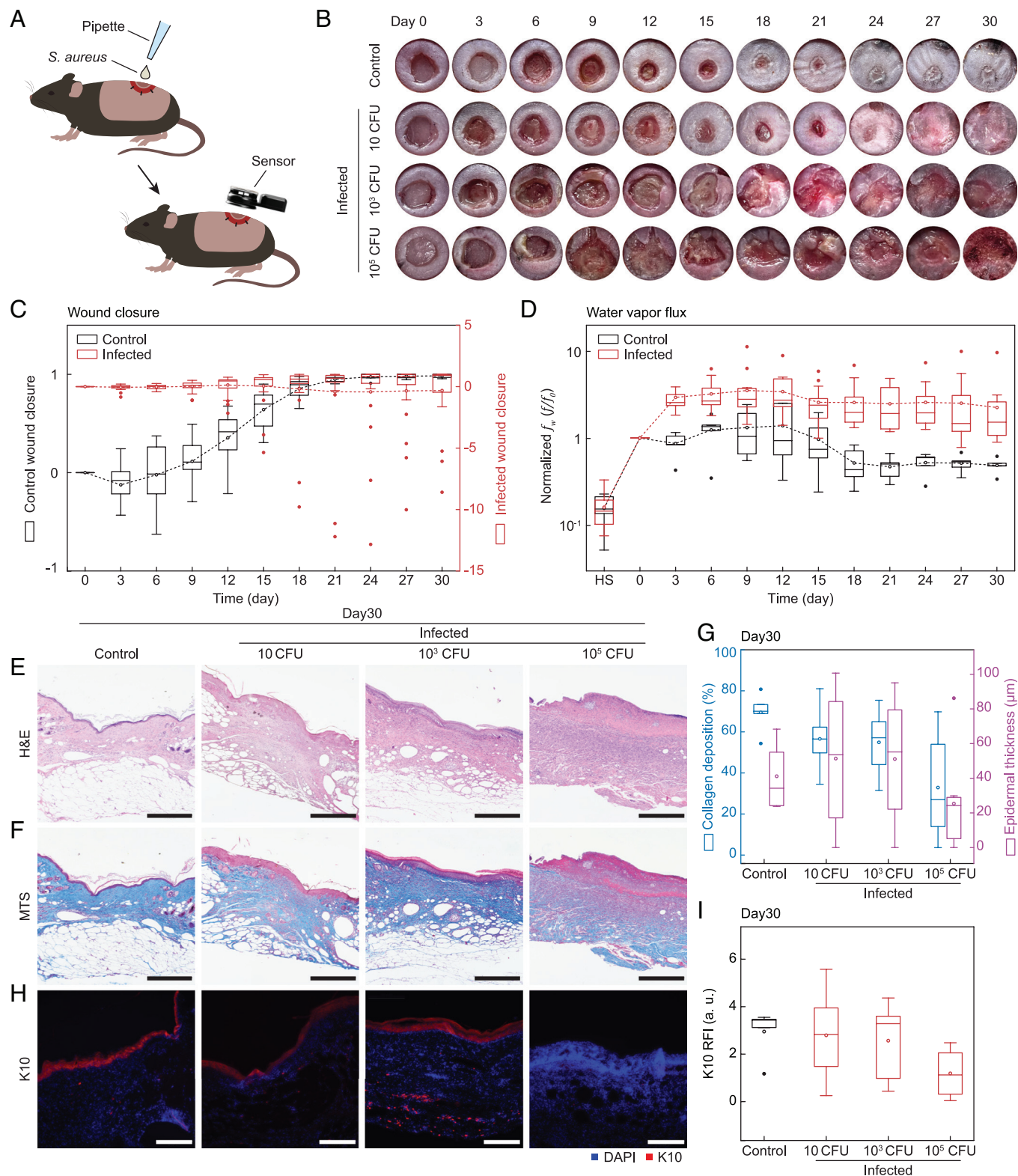


Fig. 3. Characteristics of wound healing and re-epithelialization in infected mouse wounds. (A) Schematic illustration of the infected mouse wound model. (B) Representative images of control (noninfected) and infected wounds (bacteria inoculation doses of 10^3 , 10^4 , and 10^5 CFU) recorded every 3 d. (C and D) Wound closure (C) and normalized f_w (D) of control (black) and infected (red; 10^3 , 10^4 , and 10^5 CFU inoculation). $n = 11$ control and $n = 30$ infected for days 3 to 9, $n = 8$ control and $n = 27$ infected for days 12 to 18, $n = 5$ control and $n = 24$ infected for days 21 to 30 in (C). $n = 5$ control and $n = 15$ infected in (D). (E and F) Representative Hematoxylin and eosin (H&E) staining (E) and Masson's trichrome staining (MTS) (F) images of wound areas on day 30 from control and infected wounds with inoculation doses of 10^3 , 10^4 , and 10^5 CFU. (Scale bars, 500 μm .) (G) Quantitative histological evaluation of collagen deposition (blue) and epidermal thickness (purple) for control ($n = 5$) and infected ($n = 8$ per group) mice. (H) Representative immunofluorescent staining images for K10 of wound areas on day 30. (Scale bars, 200 μm .) (I) Quantitative histological evaluation of K10 expression on day 30 for control (black; $n = 5$) and infected (red; $n = 8$ per group) mice. For each box plot, the horizontal line within the box represents the sample median and the ends of the box correspond to the interquartile range (first to third quartiles). The whiskers extend beyond the ends of the box by 1.5 $\times$ (interquartile range). The open and filled circles represent the mean and outliers, respectively. f_w : water vapor flux. HS: healthy skin. RFI: relative fluorescence intensity.

wounds approach nearly complete closure by day 21, with an average wound closure of 0.95 and full wound closure occurred in 60% of all mice with wound closure values of 1. By contrast, infected wounds exhibit delayed wound closure, with an average value of -0.32 even on day 30. Infected wounds include cases of full wound closure (36% of all mice) and extreme expansion (average wound closure values of <-5.0). Further comparison between different bacteria inoculation doses reveals that wounds with mild infections (low-inoculation) are more likely to close by day 30 (6 fully closed out of 8 for 10 CFU), whereas severely infected wounds (high inoculation) are more likely to expand (2 expanded out of 8 for 10^3 CFU and 4 expanded out of 8 for 10^5 CFU) (SI Appendix, Fig. S12).

Values of f_w recorded from wounds reflect the extent of barrier restoration and thus decreases reflect wound closure (Fig. 3D) (32, 33). f_w for control wounds shows an increase (days 0 to 12) to 1.4 times their initial values on day 0 followed by a decrease (days 12 to 21) and stabilization to half of their initial values. Infected wounds also display similar increases (days 0 to 12) followed by decreases (days 12 to 15) and stabilization, but at values that are 2.5 times higher than that on day 0. More specifically, higher inoculation doses show larger increases and stabilization to higher values in f_w , reaching a maximum of 2.4, 3.2, and 4.6 times their initial values for 10, 10^3 , and 10^5 CFU, respectively, followed by stabilization to 1.3, 1.6, and 3.8 times their initial values (SI Appendix, Fig. S13).

Histological analysis of the wound tissue from day 30 reveals delayed re-epithelialization in infected wounds (Fig. 3E and F). H&E staining images of mice inoculated with 10^5 CFU show damaged epidermis layers and excessive formation of scar tissue (Fig. 3E), along with decreasing epidermal thickness with increasing inoculation dose for infected wounds ($51.3\text{ }\mu\text{m}$ for 10 CFU, $51.1\text{ }\mu\text{m}$ for 10^3 CFU, and $25.3\text{ }\mu\text{m}$ for 10^5 CFU) (Fig. 3G). Control wounds appear closed with an average epidermal thickness of $41\text{ }\mu\text{m}$. Masson's trichrome staining (MTS) images show that collagen deposition is most pronounced in control mice and significantly reduced in mice with higher inoculation doses (Fig. 3F). Quantitatively, collagen deposition on day 30 is greatest for control wounds (70%) and decreases with increasing inoculation dose in infected wounds (56.6% for 10 CFU, 54.9% for 10^3 CFU, and 32.9% for 10^5 CFU) (Fig. 3G). Cytokeratin-10 (K10) expression in wounds reflects epidermal maturation and functional restoration of the skin barrier (34). Histological images show notably stronger K10 expression in control mice and almost absent K10 expression in mice inoculated with 10^5 CFU (Fig. 3H). Control wounds show the highest K10 expression (2.9%), with expression levels decreasing with increasing bacterial inoculation of infected wounds (2.7% for 10 CFU, 2.6% for 10^3 CFU, 1.2% for 10^5 CFU) (Fig. 3I).

f_{voc} measured in control and infected mice wounds shows significantly different trends (Fig. 4A). Control wounds exhibit a monotonic decrease in f_{voc} , eventually reaching a value on day 30 that is one tenth of that on day 0. In infected wounds, this parameter rapidly increases during days 0 to 12, achieving a value on day 12 that is more than fifty times that on day 0, and decreases until day 30 to a value that is twice that of day 0. The bacteria concentration in infected wounds displays a temporal profile similar to that of f_{voc} , with a maximum on day 12 and a decrease until day 30 (Fig. 4B and SI Appendix, Fig. S14). While the temporal profiles vary by mouse due to heterogeneity in host response, the trends of f_{voc} and bacteria concentration show excellent agreement over the 30-d monitoring period for all mice (SI Appendix, Fig. S15). Temporal changes in f_{voc} of infected mice with higher inoculation show a prolonged increase and a higher maximum value relative to that on day 0, further supporting that f_{voc} can assist in evaluating the initial infection severity. For inoculation doses of 10 CFU, 10^3 CFU, and 10^5 CFU, values continuously

increase until days 9, 12, and 15, reaching values 21, 86, and 80 times that of their values on day 0, respectively (SI Appendix, Fig. S16).

Extremely high bacteria inoculation doses of 10^7 CFU show outlying trends, where f_{voc} increases reaching at day 12 a value 168 times that on day 0 and then decreases to 19 times the day 0 value, suggesting persistent infection (SI Appendix, Fig. S17). This behavior is evident through measurements of bacteria concentration, which show elevated values of $1.84 \times 10^8\text{ CFU}\cdot\text{mL}^{-1}$ on day 30 (SI Appendix, Fig. S18). The temporal profiles for other parameters, however, resemble those of less severe infections, where wound closure is similar to that of 10^5 CFU and water vapor flux is similar to that of 10 CFU. This observation can be attributed to the rapid loss of structural integrity in the surrounding skin due to severe infection, which leads to splint detachment and results in more advanced wound closure and restoration of skin barrier.

On day 3 since inoculation, infected wounds show significantly different values of f_w ($P = 0.00069$ for 10 CFU, $P = 0.0079$ for 10^3 CFU, $P = 0.051$ for 10^5 CFU) (SI Appendix, Fig. S19) and f_{voc} ($P = 0.00031$ for 10 CFU, $P = 0.0058$ for 10^3 CFU, $P = 0.031$ for 10^5 CFU) from those in control wounds, but the differences for f_{voc} exhibit stronger statistical significance (Fig. 4C). These differences can be associated with the rapid growth in bacteria concentration during the first 3 d of infection, where inoculations of 10 CFU, 10^3 CFU, and 10^5 CFU on day 0 result in $2.6 \times 10^6\text{ CFU}\cdot\text{mL}^{-1}$, $1.5 \times 10^7\text{ CFU}\cdot\text{mL}^{-1}$, and $2.5 \times 10^8\text{ CFU}\cdot\text{mL}^{-1}$, respectively, on day 3 (Fig. 4D). Evaluation of wound images through visual inspection on day 0 and 3 by medical professionals yielded an accurate assessment in only 64% of instances (Fig. 4E). Differences in bacterial growth rate by inoculation dose are most prominent during early stages of infection, but these differences reduce over time, with bacteria concentrations converging to the same order of magnitude by day 30, where inoculations of 10 CFU, 10^3 CFU, and 10^5 CFU on day 0 result in $3.0 \times 10^7\text{ CFU}\cdot\text{mL}^{-1}$, $5.0 \times 10^7\text{ CFU}\cdot\text{mL}^{-1}$, and $6.7 \times 10^7\text{ CFU}\cdot\text{mL}^{-1}$, respectively (SI Appendix, Fig. S20). This finding suggests that even initially mild infections can intensify to levels comparable to more severely infected wounds, without appropriate intervention.

Immunofluorescence analysis of tissue on day 30 allows for further investigation of the effects of bacterial infection on the wound healing status (Fig. 4F and G). Ly6G and F4/80 are each markers for neutrophils and mature macrophages which are predominant during early inflammatory phases and active immune response phases of wound healing, respectively (35, 36). Staining images indicate that both Ly6G and F4/80 expressions are negligible for control wounds and markedly elevated in severely infected wounds. Quantitatively, infected wounds with high inoculation (10^5 CFU) show significantly higher Ly6G expressions compared to those of controls ($P = 0.048$) (Fig. 4H). Similarly, F4/80 expression for the 10^5 CFU inoculation case is substantially higher than those of the control ($P = 0.00050$) (Fig. 4I). Results for inoculation doses 10 and 10^3 CFU exhibit weaker statistical separation from control than 10^5 CFU (SI Appendix, Fig. S21). Blood analysis results also indicate prolonged inflammation in animals with bacterial infections as per higher total white blood cells ($3.1 \times 10^4\text{ }\mu\text{L}^{-1}$) and increased proportions of neutrophils (51%) compared to those of the control ($1.7 \times 10^4\text{ }\mu\text{L}^{-1}$ white blood cells and 23% neutrophils) (SI Appendix, Fig. S22).

Further studies that compare infected wounds with an inoculation dose of 10^5 CFU [standard wound care (SWC) group] to those with the same inoculation dose treated with hypochlorous acid [antimicrobial wound care (AWC) group] demonstrate the potential utility of f_{voc} in the assessment of inflammation (Fig. 5A). f_{voc} in the SWC group shows a continuous increase over days 0 to

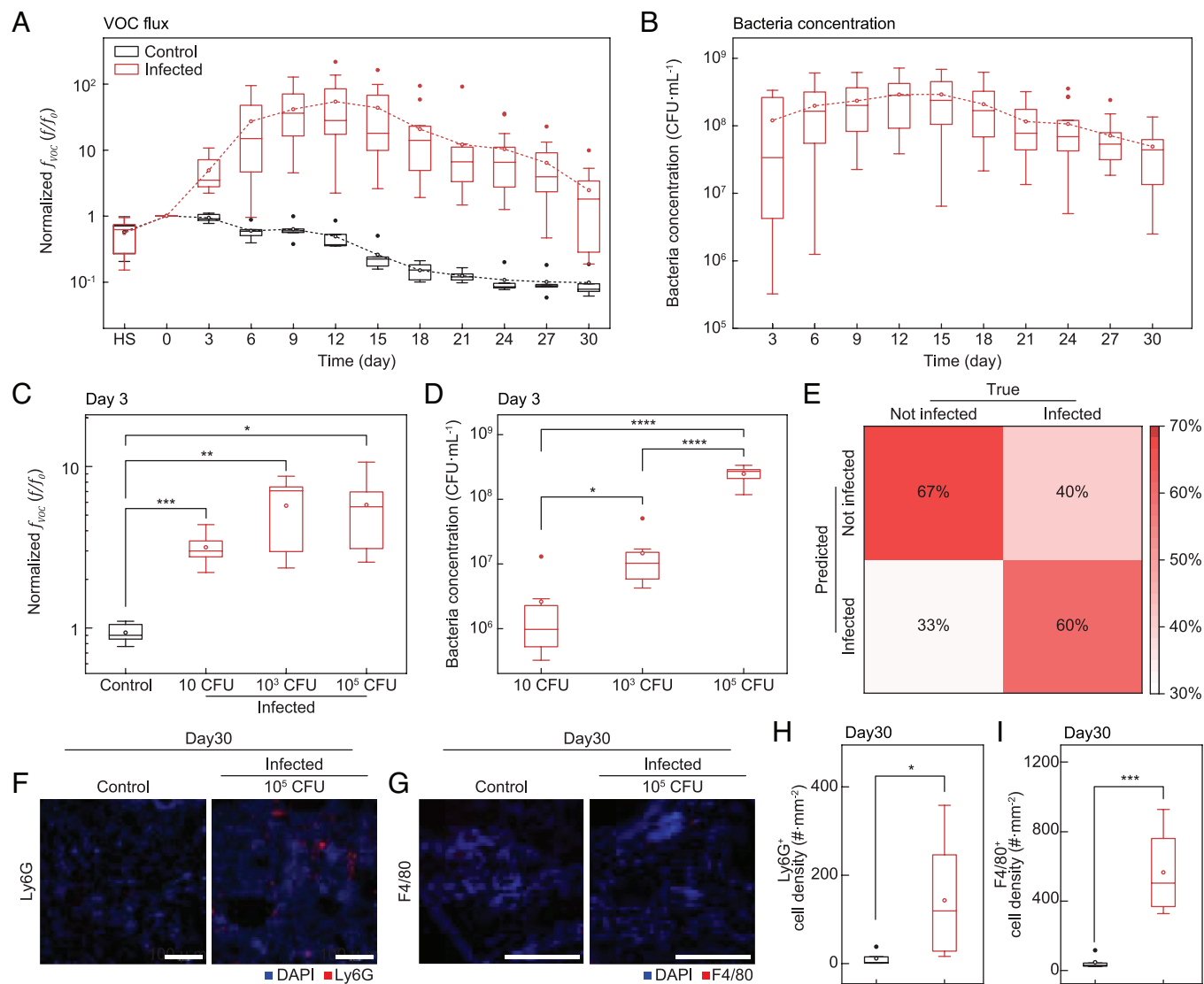


Fig. 4. Trends in VOC flux and inflammation in infected mouse wounds. (A) Normalized f_{voc} of control (black, $n = 5$) and infected (red, $n = 15$) mice. (B) Bacteria concentration of infected mice. $n = 30$ for days 3 to 6, $n = 27$ for day 9, $n = 18$ for days 12 to 18, $n = 15$ for days 21 to 30. (C) Normalized f_{voc} from the wound on day 3 for control (black) and infected (red) groups. $n = 5$ per group. (D) Bacteria concentration in wounds of mice with inoculation doses of 10, 10³, and 10⁵ CFU on day 3. $n = 8$ for 10 CFU, $n = 8$ for 10³ CFU, and $n = 14$ for 10⁵ CFU. (E) Confusion matrix summarizing the prediction performance of medical professionals for diagnosing infection in wounds on day 3 through visual assessment only. (F and G) Representative immunofluorescent staining images for Ly6G (F) and F4/80 (G) of wound areas on day 30. [Scale bars, 100 μm (F) and 200 μm (G).] (H and I) Quantitative histological evaluation of Ly6G⁺ (H) and F4/80⁺ (I) cell density for control ($n = 5$) and infected mice with the inoculation dose of 10⁵ CFU ($n = 8$). For each box plot, the horizontal line within the box represents the sample median and the ends of the box correspond to the interquartile range (first to third quartiles). The whiskers extend beyond the ends of the box by 1.5× (interquartile range). The open and filled circles represent the mean and outliers, respectively. *****, ***, **, and * indicate $P < 0.0001$, $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively. f_{voc} : VOC flux. HS: healthy skin.

15 reaching a maximum of 80 times that on day 0 and decreases to a value that is 1.5 times that on day 0. In contrast, the AWC group exhibits a rapid increase in f_{voc} with a higher maximum on day 6 (87 times that on day 0) followed by a continuous decrease to a lower value that is 0.41 times that on day 0. Bacteria concentration also differs between the two groups. The SWC group displays a maximum on day 15 followed by an overall decrease, while the AWC group shows a monotonic decrease until day 30 (Fig. 5B).

Antimicrobial treatment of the wounds significantly enhances wound healing as per quantification of blood vessel and macrophage markers via immunofluorescence (Fig. 5C and D). Cells positive for α SMA and CD31 visualize blood vessel density at the wound site (37, 38). Qualitatively, both standard and AWC groups show the strongest α SMA/CD31 expression on day 18 compared to days 9 and 30 (Fig. 5C). Quantitatively, blood vessel densities of the AWC group on day 18 and 30 are significantly higher ($P =$

0.015) and lower ($P = 0.033$), respectively, than those of the SWC group (Fig. 5E). Additionally, CD86 and CD163 each stain for polarized phenotypes of macrophages that are proinflammatory and proregenerative, respectively (39, 40). Staining images exhibit a higher expression of CD86 in the SWC group and an increased expression of CD163 in the AWC group across days 9, 18, and 30 (Fig. 5D). Analysis of the CD86⁺/CD163⁺ cell ratio reveals significant differences between the two groups ($P = 0.0012$ for day 18 and $P = 0.00042$ for day 30), where macrophages present in the SWC group are predominantly proinflammatory (CD86⁺/CD163⁺ > 1) while those in the AWC group are primarily proregenerative (CD86⁺/CD163⁺ < 1) (Fig. 5F). Temporal analysis of Ly6G⁺ cells indicates that the AWC group exhibits expression levels comparable to those of control wounds, whereas the SWC group shows a significantly higher expression ($P = 0.0053$ for day 18 and $P = 0.0090$ for day 30) (Fig. 5G). Blood analysis further supports these

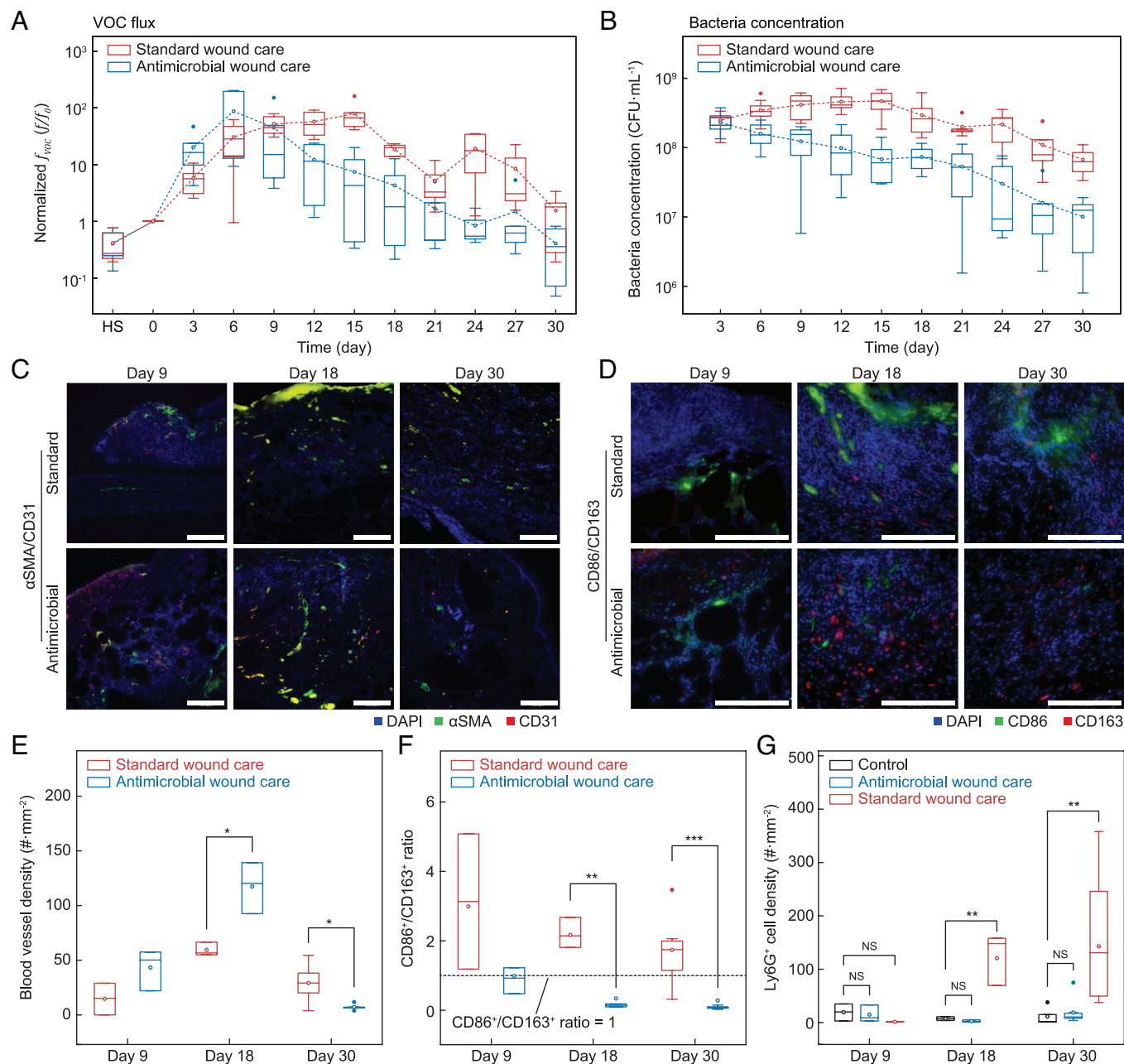


Fig. 5. Influence of antimicrobial treatment of infected wounds on healing and VOC flux. (A and B) Normalized f_{voc} (A) and bacteria concentration (B) from infected wounds with an inoculation dose of 10^5 CFU with SWC (black; $n = 5$) and antimicrobial treatment (blue; $n = 5$). (C and D) Representative immunofluorescent staining images for α SMA/CD31 (C) and CD86/CD163 (D) of wound areas at time points of day 9, 18, and 30. (Scale bars, 200 μ m.) (E–G) Quantitative histological evaluation of blood vessel density (E), CD86⁺/CD163⁺ ratio (F), and Ly6G⁺ cell density (G) on days 9, 18, and 30. $n = 3$ control and $n = 3$ infected mice per wound care group for days 9 and 18. $n = 5$ control and $n = 8$ infected mice per wound care group for day 30. For each box plot, the horizontal line within the box represents the sample median and the ends of the box correspond to the interquartile range (first to third quartiles). The whiskers extend beyond the ends of the box by 1.5 $\times$ (interquartile range). The open and filled circles represent the mean and outliers, respectively. ***, **, *, and NS indicate $P < 0.001$, $P < 0.01$, $P < 0.05$, and $P > 0.05$, respectively. f_{voc} : VOC flux. HS: healthy skin.

findings, with control and AWC groups maintaining similar levels below $2.0 \times 10^4 \mu\text{L}^{-1}$ across days 9, 18, and 30, while the SWC group displays a continuous increase up to $4.0 \times 10^4 \mu\text{L}^{-1}$ (SI Appendix, Fig. S23). Consistent trends are observed in wound closure and f_w measurements, where the AWC group closely resembles control wounds (SI Appendix, Figs. S10, S11, and S24).

Discussion

The results presented here demonstrate that continuous monitoring of gaseous flux from wound beds yields data with strong relationships to epidermal barrier integrity and biological processes

that govern infection and wound healing progression. A noncontact, wireless wearable platform for quantitating gaseous flux yields measurements of f_{voc} that indicate a divergence between behaviors of infected and noninfected wounds at early time-points. These findings demonstrate that molecular emissions from wounds contain clinically relevant information before the appearance of more prominent clinical signs of infection (22, 41). As complementary information, temporal variations in f_w provide insight into the recovery of skin barrier function during wound healing. Skin excision disrupts the stratum corneum and increases water loss through loss of lipid barrier integrity (32, 42). As wound closure progresses, keratinocyte migration and proliferation drive re-epithelialization,

while subsequent maturation of keratinocytes restores the epidermal structure (33, 34). Decreasing f_w therefore reflects maturation of the regenerated epidermis and restoration of barrier properties. Infected wounds, however, exhibit persistently elevated flux, consistent with delayed epithelialization and altered epidermal architecture associated with sustained inflammatory stress (43, 44). Histological features further support these functional observations, including differences in epidermal thickness and delayed epithelial maturation in more severely infected wounds (K10). Together, these findings suggest that molecular flux—in the form of both f_{voc} and f_w —from the wound bed represents a previously underexplored class of physiological biomarkers that integrate microbial metabolism, immune activity, and structural recovery within the wound microenvironment (27, 45–47).

The mechanistic basis of these flux signatures arises from interactions between bacterial metabolism, immune response, and structural remodeling of the skin barrier. VOCs emerge during early stages of bacterial proliferation as metabolic by-products generated during active growth and enable detection of microbial activity before macroscopic biofilm formation (48, 49). As infection progresses, host immune responses further influence the chemistry of the wound. Recruitment of innate immune cells introduces oxidative and inflammatory pathways that generate additional VOCs through lipid peroxidation and reactive oxygen species-mediated reactions, reflecting sustained inflammatory activity within the wound bed (Ly6G, F4/80) (50, 51). In parallel, f_w reflects the structural progression of epidermal repair, as mentioned above. Persistent infection disrupts these processes through inflammatory signaling and bacterial toxins that impair keratinocyte proliferation and matrix remodeling (43, 44). Elevated f_w in these cases therefore reflects prolonged exposure of hydrated dermal tissue and incomplete maturation of the stratum corneum. Simultaneous monitoring of f_{voc} and f_w thus provides powerful insights into the physiological status of the wound, capturing both microbial activity and the structural restoration of tissue. Unlike imaging-based wound assessment methods, including smartphone-enabled platforms that infer wound status primarily from morphological features such as wound size, tissue appearance, color, and exudate, the present approach directly quantifies physiological processes associated with microbial metabolism (f_{voc}) and epidermal barrier restoration (f_w). This distinction may be particularly valuable during the earliest stages of infection, when visual manifestations remain subtle or ambiguous. Consistent with this interpretation, f_{voc} increases approximately 24 to 33 h before visible biofilm formation *in vitro*, and significant differences between infected and noninfected wounds are evident by day 3 *in vivo*, when visual assessment by medical professionals correctly classify wound status in only 64% of cases. Molecular flux sensing therefore provides objective physiological information that complements image-based evaluation and may improve confidence in early wound assessment.

The same principles allow evaluation of treatment efficacy. Suppression of bacterial proliferation through antimicrobial intervention reduces microbial metabolism and leads to rapid decreases in f_{voc} . Concurrently, histological analysis of immunofluorescent markers indicates a transition toward a regenerative wound environment, accompanied by normalization of inflammatory markers (Ly6G expression and total white blood cell counts) and convergence of f_w and wound closure toward control levels. Resolution of inflammation accompanies improved vascularization and tissue remodeling, processes that enhance oxygen and nutrient delivery to the regenerating tissue and facilitate progression toward the remodeling phase of healing (α SMA, CD31) (37, 38). Because transitions between inflammatory and regenerative phases are

critical indicators of whether wounds progress toward healing or chronicity, the correspondence between these biological processes and flux measurements serves a physiologically meaningful role in wound status assessment during therapy.

The enabling technology is uniquely well suited for this application because it largely decouples measurements of flux from absolute concentrations, thereby significantly improving the robustness against baseline drift and environmental variability. The present implementation extracts flux from the initial transient concentration change following valve closure, where transport is well approximated by molecular diffusion. The short valve actuation time relative to the concentration evolution inside the chamber minimizes perturbations associated with switching, while simulation-derived corrections account for transient concentration nonuniformities immediately after chamber isolation. These assumptions are expected to remain valid under the chamber dimensions and operating conditions used throughout this study. Under conditions in which molecular emissions change rapidly, the simplified flux extraction based on the initial tangential slope may not fully account for transient accumulation of gaseous species within the chamber before complete diffusive equilibration is achieved. Consequently, the chamber concentration immediately prior to valve closure may no longer represent the same quasi-steady initial condition over successive measurement cycles, resulting in transient changes in both the baseline concentration and the extracted molecular flux. As demonstrated experimentally and through numerical simulations (SI Appendix, Figs. S6 and S7), these deviations are governed primarily by the temporal dynamics of molecular emission relative to the valve cycling period, rather than by the absolute molecular flux alone. Although limiting the number of measurement cycles (*in vivo*) and extending ventilation times (*in vitro*) can mitigate these effects, further studies incorporating full transient analysis of concentration–time profiles may improve the quantitative accuracy of flux values determined from the recorded data. The strong temporal correspondence between f_{voc} and bacterial load together suggests that infection-related processes dominate the measured signals under the conditions explored here.

Future introduction of chemical selectivity through integration of high-dimensional sensor arrays with multivariate models may enable deconvolution of overlapping transport phenomena, allowing differentiation of bacterial species and polymicrobial compositions based on characteristic emission patterns. Addition of collection reservoirs can extend the capabilities of this platform further for *ex vivo* analysis (e.g., GC–MS), linking real-time flux measurements with molecular identification. Translation of this sensing technology to clinical settings may involve integration into wound dressings or wearable bandage platforms (46, 47, 52). Wireless transmission of data to clinicians from use in outpatient settings may reduce the need for frequent clinical visits (16, 53). Further studies in large animal models and clinical trials will be necessary to determine how this technology and associated measurements translate to human wounds, which exhibit greater variability in size, microbiome composition, and physiology than those examined here.

Materials and Methods

Flux Sensor Fabrication. The sensors consist of a flexible printed circuit board (f-PCB), surface mount electronic components, magnetic elements for the valve system, a lithium polymer battery, and a structural frame. The electronic components bonded to the f-PCB include a Bluetooth low energy system-on-chip (BLE SoC, ISP1807, Insight SIP, Sophia Antipolis, France), a sensor that measures humidity and VOC (BME680, Bosch, Reutlingen, Germany), and a planar electromagnetic coil. For VOC, the measured concentration represents the combined sensor response to a mixture of VOCs and should therefore be interpreted as an aggregate measure of the detected vapor rather than individual VOC species.

The magnetic elements include a neodymium magnet (diameter 11.3 mm; height 0.9 mm) as the valve and two soft magnets. The soft magnets use 20 wt% magnetite nanoparticles (50 to 100 nm, Merck, Burlington, MA) mixed with polydimethylsiloxane (PDMS; Sylgard 184, Dow, Midland, MI; 10:1 mixing ratio) and spin-cast at 400 rpm, thermally cured at 50 °C for 12 h and laser cut to define annulus shapes (inner diameter 8.4 mm; outer diameter 14.2 mm). 3D printing (Form3+, Formlabs, Somerville, MA) defined the structural components including the chamber and encapsulation. For the disposable adaptors, PDMS (10:1 mixing ratio) was poured into molds defined by 3D printing, degassed, and cured. The adaptors were sterilized by spraying 70% isopropyl alcohol and air dried before use. Attachment to the bottom side of the sensor chamber unit was achieved through an interference fit.

Flux Calculation. Flux, defined as the change in concentration per unit area per unit time, is calculated based on the slope of the tangent to the concentration curve immediately after valve closing as

$$f = V/S \times \partial c / \partial t|_{t=0}, \quad [1]$$

where V is the chamber volume, S is the area of the analytical region, c is the molecular concentration of the chamber, and t is time.

Biofilm Model. Agar plates were prepared with 10 mL of brain heart infusion agar (Merck, Burlington, MA), which consists of agar (15.0 g/L), brain extract (7.8 g/L), dextrose (2.0 g/L), disodium phosphate (2.5 g/L), heart extract (9.7 g/L), proteose peptone (10.0 g/L), sodium chloride (5.0 g/L), Congo red dye (0.8 g/L), and deionized water (1 L). *S. aureus* (ATCC 23235, Manassas, VA) was cultured overnight in Luria-Bertani (LB) broth at 37 °C and 225 rpm. Inoculation involved introduction of 10 μ L suspensions containing approximately 10^3 and 10^7 CFU of *S. aureus* (SI Appendix, Table S1). Plates were kept upside down, in the dark, at temperatures of 18 to 23 °C. Plate images were taken every 6 h using a custom light box setup and a digital SLR camera (Canon EOS 6D, Oita, Japan). For quantification of the bacterial load of biofilm, the entire agar and biofilm were removed from the plate, dissolved in phosphate-buffered saline, and vortexed gently for 1 to 2 min to allow for homogenization. Concentrations were calculated using a Grant bio Den-1B densitometer, and a LIVE/DEAD™ BacLight™ kit (Thermo Fisher Scientific, Waltham, MA) was used to fluorescently stain for bacterial viability. Plates for imaging and bacteria quantification were separate batches from those for VOC measurement to minimize influence from intervention.

Mice. Male diabetic mice (BKS.Cg-m+/+Lepr^{db}, 000642; homozygous for Lepr^{db}) aged 8 to 12 wk were obtained from Jackson Laboratory (Bar Harbor, ME). The Institutional Animal Care and Use Committee at Northwestern University's Center for Comparative Medicine approved all animals and procedures before experimentation (IS00018748).

Infected Wound Model. To anesthetize the mice, a portable gas induction and inhalation system (RC² Rodent Circuit Controller, VetEquip, Livermore, CA, USA) delivered 2.5% isoflurane in oxygen. The fur on the dorsal side was then removed. Once asleep, injections of meloxicam (20 mg/kg) and bupivacaine (2 mg/kg) were administered subcutaneously, followed by addition of 6–0 nylon sutures (Ethicon, Raritan, NJ) to attach an annulus acrylate splint (inner diameter, 10 mm; outer diameter, 12 mm) to prevent skin contraction. A full-thickness dermal excision was then created to define the wound using a 6 mm diameter punch biopsy (Acuderm, Fort Lauderdale, FL). For the infected wounds, a 10 μ L suspension of *S. aureus* (10^3 , 10^5 , and 10^7 CFU) was added to the wound bed (SI Appendix, Table S2). *S. aureus* inocula were prepared as described previously. Occlusive dressings (Tegaderm, 3M, Maplewood, MN) were used to cover the wound area.

Postoperative Care. Mice were housed individually with access to standard laboratory chow and water ad libitum. The animal facility maintained a 14 h:10 h light/dark cycle, ambient temperatures of 18 to 23 °C, and humidity levels between 40 and 60%. Approximately 24 h following initial wound creation, a second dose of meloxicam (20 mg/kg) was delivered subcutaneously. Wound care was performed every 3 d utilizing a dry swab to remove and collect exudate, followed by cleansing using a gauze pad soaked with hypochlorous acid (Vashe, Urgo Medical North America, Fort Worth, TX) for the AWC group and with saline for all other infected wounds (SWC). Wound area, weight loss, blood glucose concentration, and wound temperature were monitored prior to wound care. The

wound area was characterized by three blinded observers using ImageJ (version 2.16.0/1.54p). Blood glucose concentrations were measured from whole venous blood using a blood glucose monitor (Contour next, Ascensia, Basel, Switzerland) with a measurement upper limit of 600 mg·dL⁻¹. Wound temperatures were determined using a handheld infrared camera (Flir Edge Pro, FLIR, Goleta, CA) and the FLIR Thermal Studio software (v.2.0.58).

Blood Tests. Once the mice were euthanized following standards/guidelines provided by the Institutional Animal Care and Use committee at Northwestern University's Center for Comparative Medicine (IS00018748), blood samples were collected by making an incision at the nape of the neck and stored in EDTA-coated tubes to prevent coagulation. Samples were transferred to the Northwestern University Center for Comparative Medicine for complete blood count (CBC) analysis with leukocyte differential. For hematological analysis, targeted traits included hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell (RBC) count, RBC distribution width (RDW), and total white blood cell (WBC) count. The concentration of WBC subtypes (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) was also measured.

Bacteria Quantification. Before each flux measurement, wounds were gently swabbed with sterile dry cotton tipped applicators (Puritan®, Guilford, ME) three times in a circular motion ensuring coverage of all sides of the applicator with wound exudate. The collected swab samples were then stored briefly in 1 mL phosphate-buffered saline (PBS) solution. Immediately following wound care, swab samples were vortexed at 3200 rpm for 30 s (Analog Vortex Mixer, Fisherbrand™, Waltham, MA) to allow for thorough homogenization of the wound exudate. Serial dilutions of the exudate solution were performed at a 1:10 ratio in PBS. Dilutions were cultured onto nutrient agar (Difco™, BD, Franklin Lakes, NJ) using disposable inoculating loops (Fisherbrand™, Waltham, MA) and incubated overnight at 37 °C. Agar plates containing a countable range (30 to 300 CFU) of colonies were used for quantifying bacterial concentration using the following equation:

$$\frac{\text{CFU}}{\text{mL}} = \frac{\# \text{ of Colonies (CFU)} \times \text{Dilution Factor}}{\text{Volume Cultured onto Agar Plate (mL)}}. \quad [2]$$

Tissue Processing and Sampling. The mice were euthanized by carbon dioxide inhalation followed by cervical dislocation. The skin tissue was excised and fixed with 4% paraformaldehyde for 24 h. The fixed tissues were then washed with PBS for 48 h and then gradually dehydrated to be embedded in paraffin. The embedded tissues were sectioned into slides with 5 μ m thickness for histological analysis. Brightfield histology, including H&E (Sigma-Aldrich, Burlington, MA, USA; GHS332 and HT110116) and Masson's trichrome (Abcam, Cambridge, UK; ab150686) staining, was performed to assess epidermal thickness, granulation tissue formation, and collagen deposition. Immunofluorescence staining was conducted to evaluate vasculature using rabbit anti-CD31 antibody (Abcam, Cambridge, UK; ab182981) and mouse anti- α SMA antibody (Abcam, Cambridge, UK; ab7817), keratinocyte differentiation using mouse anti-cytokeratin-10 antibody (Invitrogen, Waltham, MA; MA5-13705), macrophage presence using rat anti-F4/80 antibody (Invitrogen, Waltham, MA; 14-4801-82), macrophage polarization using rat anti-CD86 antibody (Invitrogen, Waltham, MA; 14-0862-82) and rabbit anti-CD163 antibody (Abcam, Cambridge, UK; ab182422), and neutrophil presence using rat anti-Ly6G antibody (BD, Franklin Lakes, NJ; 551459). ImageJ (version 2.16.0/1.54p) was used for quantitative analysis.

Histological Analysis. Images of wound tissues were acquired using an inverted fluorescence microscope (Eclipse Ti2-E, Nikon, Tokyo, Japan) equipped with NIS-Elements software. For each tissue, two fields were imaged at 10 $\times$ for H&E staining, one field at 10 $\times$ for Masson's trichrome staining, three fields at 20 $\times$ for α SMA/CD31 and K10 immunofluorescence staining, and four fields at 40 $\times$ for CD86/CD163, F4/80, and Ly6G immunofluorescence staining. Epidermal thickness was quantified from H&E-stained images by averaging measurements taken at six locations distributed along the wound site, with an average spacing of approximately 500 μ m. Collagen deposition was quantified from Masson's trichrome-stained images by color deconvolution of the blue channel and measurement of the nonbackground area fraction using a fixed threshold. Blood vessel density was quantified by manually counting α SMA⁺/CD31⁺ lumen-containing structures normalized to the area of granulation tissue in each image. K10 fluorescence was quantified by measuring the mean gray value of the K10 channel

within the epidermal layer and subtracting the mean gray value measured from a negative control region. CD86/CD163, F4/80, and Ly6G positive cell densities were quantified by manually counting DAPI-positive nuclei that exhibited overlapping fluorescence signals for the corresponding marker, followed by normalization to tissue area. ImageJ (version 2.16.0/1.54p) was used for all the quantitative histological analysis.

Clinical Survey for Visual Inspection of Wound Infection. Medical professionals ($n = 8$) were recruited to complete a single-blinded survey administered via an online link. The survey consisted of 15 questions, each presenting a pair of wound images from mouse models, including one image obtained immediately after surgery (day 0) and a corresponding image from day 3. For each pair, participants were asked to determine whether the wound on day 3 appeared infected. The dataset included a mixture of control and infected wounds, and participants were not informed of the proportion or identity of infected cases, ensuring blinding to ground truth labels. Responses were aggregated and evaluated using a confusion matrix to quantify accuracy of classification.

Data Processing. Calculation of flux from VOC concentration and humidity data involved regression fitting based on the exponential form of

$$c = A(1 - \exp(-t/\tau^*)), \quad [3]$$

where c is the VOC concentration or humidity, t is time, and A and τ^* are fitting parameters.

For normalized flux in *in vitro* and *in vivo* studies, each value (f) was divided by its baseline value (f_0) which is from 0 h immediately after inoculation and day 0 immediately after surgery, for *in vitro* and *in vivo* studies, respectively, and therefore presented as normalized quantities (f/f_0) to express the relative change with respect to the baseline value.

Biofilm growth was quantified as Euclidean distance in RGB space calculated as

$$d = \sqrt{(R - R_0)^2 + (G - G_0)^2 + (B - B_0)^2}, \quad [4]$$

where (R, G, B) and (R_0, G_0, B_0) are the average plate color at each time point and at 0 h, respectively. Wound closure corresponds to the fraction healed compared to day 0 calculated as

$$(1 - A/A_0), \quad [5]$$

where A and A_0 are the wound area at each time point and on day 0, respectively.

Statistical Analysis. One-way ANOVA tests (SPSS) were used to derive two-sided P values. Minimum sample sizes for experiments were not predetermined.

Finite Element Analysis (FEA). FEA simulations of VOC transport were performed with COMSOL Multiphysics 6.2. The two-dimensional axisymmetric diffusion process within the device was modeled using the Transport of Diluted Species module. A prescribed time-dependent VOC flux was applied at the bottom boundary to represent VOC release from the tissue surface, while the concentration at the vent boundary was fixed at the ambient value. An internal boundary at the valve location was switched between continuity in the open state and zero normal flux in the closed state. The sensor was represented by a domain enclosed by thin impermeable barriers, except for a small opening that allowed diffusive exchange with the surrounding chamber. The transient VOC concentration within the sensor region was extracted. Mesh convergence was verified to ensure that the predicted concentration fields and transient sensor responses were independent of mesh size.

Data, Materials, and Software Availability. Data presented in this study are available at Arch (<https://doi.org/10.21985/n2-3k1c-3s43>) (54).

ACKNOWLEDGMENTS. We thank Prof. Hartman for providing laboratory space for *in vitro* experiments; Dr. Steve Xu for useful discussions. This work is supported by the Querrey-Simpson Institute for Bioelectronics, the Querrey Simpson Institute for Regenerative Engineering at Northwestern University, the NIH Grant on wound healing: R01DK131302, and the CZ Biohub Spoke Project-Agreement 2/4/2025. A.T. was supported by the NIH-funded Regenerative Engineering Training Program Grant 5 T32 EB 031527-05.

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