A wireless and battery-free wound infection sensor based on DNA hydrogel

Ze Xiong1,2,3,‡, Sippanat Achavananthadith1,*, Sophie Lian4,†, Leigh Edward Madden5,6, Zi Xin Ong5,6,7, Wisely Chua8, Viveka Kalidasan1, Zhipeng Li1, Zhu Liu1, Priti Singh9, Haitao Yang10, Sascha P. Heussler11, S. M. P. Kalaiselvi12, Mark B. H. Breese12, Haicheng Yao13, Yuji Gao2, Kavitha Sanmugam3,†, Benjamin C. K. Tee1,2,3,13,15, Po-Yen Chen10, Weiqiang Loke9, Chwee Teck Lim3,4,15,16, Grace Shu Hui Chiang14, Boon Yeow Tan14, Hao Li7,17, David Laurence Becker5,6, John S. Ho1,2,3,15,*

The confluence of wireless technology and biosensors offers the possibility to detect and manage medical conditions outside of clinical settings. Wound infections represent a major clinical challenge in which timely detection is critical for effective interventions, but this is currently hindered by the lack of a monitoring technology that can interface with wounds, detect pathogenic bacteria, and wirelessly transmit data. Here, we report a flexible, wireless, and battery-free sensor that provides smartphone-based detection of wound infection using a bacteria-responsive DNA hydrogel. The engineered DNA hydrogels respond selectively to deoxyribonucleases associated with pathogenic bacteria through tunable dielectric changes, which can be wirelessly detected using near-field communication. In a mouse acute wound model, we demonstrate that the wireless sensor can detect physiologically relevant amounts of Staphylococcus aureus even before visible manifestation of infection. These results demonstrate strategies for continuous infection monitoring, which may facilitate improved management of surgical or chronic wounds.
dissemination from biofilms and bacteria evasion of neutrophil extracellular traps deployed by the host immune defense (Fig. 1A) (37). When exposed to extracellular Dnase, the DNAgel is degraded via nonspecific cleavage of DNA strands, resulting in dissolution of the hydrogel. This changes the dielectric permittivity of the region above an interdigitated electrode and therefore modulates its capacitance (Fig. 1B and note S1). By connecting the electrode to an embedded system, this electronic signal can be read out in a wireless and battery-free manner using near-field communication (NFC), a connectivity technology found on most modern smartphones for short-range communication and wireless power transfer (Fig. 1C and note S2) (4, 38, 39). WINDOW has a thin and flexible form factor that enables it to be conformally embedded into wound dressings to wirelessly track virulence factor activity on demand (Fig. 1D). We demonstrate the potential of WINDOW for real-time detection of clinically relevant amounts of S. aureus both in vitro and in a mouse wound model before visible manifestation of infection. This technology may facilitate timely detection of wound infections for improved management of surgical or chronic wounds.

RESULTS

DNAgel synthesis and processability

To convert Dnase activity into a smartphone-readable signal, we developed a DNAgel that meets processability requirements for integration into a bioelectronic sensor and exhibits a chemically tunable dielectric permittivity over the radio frequency spectrum. In contrast to previous work that used heating-cooling cycles to form physically crosslinked DNAgel (40), we used a chemical crosslinking strategy to provide increased stability in aqueous environments and capacity for functionalization (see Materials and Methods; Fig. 2, A and B; and fig. S1) (41). This strategy yields a DNAgel with several advantageous properties: (i) less steric hindrance to subsequent permittivity engineering, (ii) fewer DNA strands (~0.05 g of dehydrated DNA in 1 ml of DNAgel) for increased sensitivity to Dnase, and (iii) greater diffusion of reactive agents through the three-dimensional (3D) network for more rapid response time. The gelation reaction can be completed at room temperature without requiring heating or other harsh conditions. The ability of DNAgel to be gelated in situ enables it to be integrated into a wide range of bioelectronic interfaces. For instance, DNAgel precursor can be printed on either planar surface (Fig. 2, C and G) or curved contact lens (Fig. 2, D and H) or be molded into 3D macrostructures (Fig. 2, E and I) and microstructures (Fig. 2, F and J) with a spatial resolution as fine as 10 μm (movie S1).

Selective bioresponse and anti-dehydration capability of DNAgel

The selective degradation of DNAgel by DNases associated with pathogenic bacteria provides the reporting mechanism for detection of an active wound infection. We first validated that DNAgel is selectively degraded by the opportunistic pathogen S. aureus ATCC
DNAgel droplets were coincubated with live neonatal human dermal fibroblasts (NHDFs) or *S. aureus* culture with comparable total cellular volume (fig. S2). Confocal fluorescence imaging showed that coincubation with NHDFs (~2 × 10⁴ cells) for over 1 hour resulted in negligible change in the droplet morphology (Fig. 3A). In contrast, coincubation with *S. aureus* culture (~7 × 10⁷ colony-forming units (CFU)) for 1 hour resulted in complete degradation of the DNAgel droplet (Fig. 3B and movie S2). DNAgel degradation can be attributed to secretion of DNase (nuclease S7, also known as micrococcal nuclease) by *S. aureus*, as shown by immersing DNAgel (~2.3 × 10⁸ µm³ in volume) into a DNase solution (~1 U/µl) (Fig. 3C and fig. S3). 3D topography reconstructions show that DNAgel volume is reduced by 56% after 1 hour of immersion (Fig. 3D and E) and 68.3% after 1.5 hours (movie S3). We further assessed the selective degradation of DNAgel by *S. aureus* compared to a panel of commensal skin-associated bacteria by incubating DNAgel with the respective sterile-filtered bacteria culture supernatant. Whereas DNAgel incubated with *S. aureus* over 24 hours exhibited about 70% decrease in fluorescence intensity, DNAgel incubated with other skin commensal bacteria prevalent on the epidermis were not substantially degraded (fig. S4).

We further evaluated the ability of the DNAgel to detect *S. aureus* infection in wound swabs collected from diabetic foot ulcer (DFU) patients. From a cohort of 18 DFU patients with available clinical microbiology reports, 3 patients were positive for *S. aureus*. We determined the total CFU for these samples and compared the *S. aureus*-positive samples (*n* = 3 patients) with patients harboring low bacteria colonization (<10⁴ CFU/cm²) as controls (*n* = 5 patients). Figure 3F and table S1 show the change in fluorescence intensity of stained DNAgel coincubated with the wound culture supernatant after 24 hours. Whereas DNAgel exposed to the *S. aureus*-positive samples exhibited more than 52% decrease in fluorescence intensity in the test group, the fluorescence intensity of DNAgel exposed to control samples decreased by no more than 27%. These results suggest that DNAgel is degraded in the presence of *S. aureus* and the hydrolysis of DNAgel by other wound-colonizing microbes is minimal.
Incorporating DNAgel with conductive dopants can increase sensitivity of the radio frequency response to biological stimuli. We also characterized the dehydration properties of DNAgel by placing 0.5 g of hydrogel in an opened centrifuge tube exposed to an environment with constant temperature (37°C) and controlled relative humidity. The hydrogel maintains more than 80% weight at 70% relative humidity after 24 hours (Fig. 3A), which demonstrates a substantially longer dehydration time compared to common hydrogels such as κ-carrageenan/polyacrylamide hydrogel that retain only 30% weight after exposure to similar conditions (42). This anti-dehydration property of DNAgel can be partially attributed to the hydrophilic poly(ethylene glycol) in the crosslinker. The dehydration time represents a lower bound for the lifetime of DNAgel because moisture is typically maintained in the wound environment by a wide range of wound dressings (fig. S5). However, additional studies will be needed to establish functional lifetime across the range of wound conditions, dressing types, and surrounding environment relevant to clinical applications.

Permittivity tunability and biocompatibility of DNAgel
Incorporating DNAgel with conductive dopants can increase sensitivity of the radio frequency response to biological stimuli. We evaluated the tunability of dielectric permittivity of DNAgel by incorporating five different conductive dopants in the hydrogel network: poly(3,4-ethylenedioxythiophene)–poly(styrene sulfonate) (PEDOT:PSS), Ti₃C₂Tₓ MXene, graphene oxide (GOₓ), single-walled carbon nanotube (SWCNT), and silver nanowire (AgNW) (Fig. 4, A and B). The biocompatibility of dopants was first examined by coincubating DNAgels with NHDFs for 48 hours (Fig. 4C). Pristine DNAgel exhibited excellent biocompatibility with cell viability, assessed using trypan blue staining, similar to controls (Fig. 4D). The presence of conductive nanoparticles or polymers resulted in a reduction of the cell viability, although dopant cytotoxicity was reduced after incorporation into DNAgel network (fig. S6), which highlights the biocompatibility of the pristine DNAgel. We characterized the permittivity of doped DNAgels and found that AgNW can render a permittivity 1.47 times higher than that of pristine DNAgel within the tested bandwidth (1 to 200 MHz) (Fig. 4E). Figure 4F shows a radar plot of the achievable range of biocompatibility and permittivity for the different dopants. For a viability threshold of 80% (fig. S6), GOₓ and Ti₃C₂Tₓ MXene can achieve 29.5 and 28.6% increase in average dielectric permittivity, respectively. Nevertheless, we chose to use pristine DNAgel for this study due to its nontoxicity and sufficient permittivity for robust infection detection.

Design and optimization of the capacitive sensing structure
To establish an interface for signal transduction, we designed a capacitive sensing structure consisting of an interdigitated electrode pattern on a flexible polyimide coated with DNAgel (Fig. 5A). Finite element simulations show the effect of the gap between electrodes d, the thickness of the DNAgel layer t₁, and the thickness of the SU-8 layer t₂ on the sensor capacitance. As d is reduced from 350 to 50 μm, the intensity of the electric displacement field D fringing above the electrodes increases (Fig. 5B), which results in higher...
capacitance and sensitivity to the presence of the DNAgel layer. Figure 5C displays an overview of these parameters by showing a stacked contour plot of sensor capacitance, as a function of $d$, $t_1$, and $t_2$. With a specific DNAgel thickness, a smaller electrode gap and thinner SU-8 can boost the capacitance induced by hydrogel. On the basis of the optimization result, we choose $d = 250 \mu m$, $t_1 = 1 mm$, and $t_2 = 2 \mu m$ as the sensor parameters, which yield a capacitance of $\sim 0.15 pF/mm^2$.

We numerically analyzed three electrode configurations (Fig. 5D and fig. S7) and selected the design with $\sim 15 mm^2$ active area for subsequent use, unless otherwise stated. The interdigitated structures were fabricated through a printing-and-etching process on a flexible printed circuit board (see Materials and Methods). Cross-sectional images of the electrodes showed conformal coating of SU-8 over the copper (Cu) surface (Fig. 5E), which protects Cu electrodes and prevents potential cytotoxicity. A circuit comprising an $LC$ tank and a half-wave rectifier was used to convert the capacitance signal into a voltage output $V_{out}$ (Fig. 5F). The resonant frequency of the $LC$ tank was set as 13.56 MHz, in alignment with the frequency for NFC communication. We characterized the response of the sensing circuit by gradually decreasing the coverage area of DNAgel from $\sim 15$ to 0 mm$^2$ to mimic digestion by DNase. The circuit exhibited a change in the input impedance from $Z_{in} = 8.5 + 30.3 j$ to $Z_{in} = 42.5 + 42.6 j$, resulting in a $\sim 0.7$-MHz shift in the resonant frequency (Fig. 5, G and H) and an $\sim 0.25$-V increase in $V_{out}$ (Fig. 5I and fig. S8) ($43–45$).

In vitro and in vivo detection of $S. aureus$
WINDOW integrates the DNAgel capacitive sensor and front-end circuit with an NFC module (fig. S9) to enable battery-free and wireless data transmission through wound dressings. The wireless design uses two coils, the first for the $LC$ biosensing module and the second for the NFC module, which have an optimized spacing between the coils of 3.5 mm (fig. S10). Figure S11 shows that the coil design achieves stable transmission to an external reader except under extreme misalignments. WINDOW can be mounted on curved body surfaces by wound dressings, exhibiting negligible (±0.01 V) fluctuation in the readout signal when the bending angle is changed from 180° to 60° (Fig. 6A). The wireless readout of the sensor is highly reproducible, as DNAgel coverage is varied from 0 to 100% (Fig. 6B).

We evaluated the response of WINDOW to $S. aureus$ using the sterile-filtered culture supernatant. For in vitro experiments, WINDOW was attached to gauze ($1 \times 1 cm^2$) permeated with filter-sterilized (0.22-µm filter) $S. aureus$ culture supernatant at various concentrations at room temperature. The sensor was fixed by a Tegaderm film (3M) and wirelessly recorded via a smartphone over 48 hours. Whereas control samples exposed to culture medium resulted in no signal change after 24 hours, samples containing $S. aureus$ culture supernatant produced a signal increase of 0.15 V for the $10^5$ CFU group and 0.38 V for the $10^6$ CFU group (Fig. 6C). Figure S12 shows the dose-response curve of the sensor after 24 hours.
WINDOW produces a detectable signal when the amount of *S. aureus* exceeds $10^5$ CFU, which is at the lower end of clinical thresholds ($10^5$ to $10^6$ CFU) widely used for laboratory diagnosis of infection (46). For amounts of *S. aureus* greater than $10^5$ CFU, the sensor response saturates at about 0.45 V due to complete degradation of DNAgel, thereby providing binary detection of infection. These results indicate that the sensor can detect secretory DNase activity when the amount of *S. aureus* approaches or exceeds thresholds for clinical infection.

We next demonstrated the ability of WINDOW to detect wound infection in vivo using an acute wound model in mice (47). Full-thickness excisional bilateral wounds (~6 mm in diameter) through the panniculus carnosus were created on the dorsum with randomly assigned control and test sites (Fig. 6D). A piece of gauze with live *S. aureus* suspension ($10^5$ or $10^6$ CFU) or sterile tryptic soy broth (TSB) was attached to the wound followed by placement and fixation of the sensor using adhesive dressings. Infected wounds with WINDOW showed similar wound conditions as the uninfected control wounds after 24 hours (Fig. 6E). All sensors remained attached to the wound, and mice with the sensors did not show observable behavioral differences compared to controls (Fig. 6F and movie S4). No obvious infection-related clinical signs, such as erythema, supputation, and friable granulation tissue, were identified in both wounds. Wound culture at the experimental end point confirmed that *S. aureus* infection was established in the test groups ($>10^6$ CFU/ml for both the $10^5$ and $10^6$ CFU groups) (Fig. 6G). Trace amounts of *S. aureus* ($~10^7$ CFU/ml) were also measured in the control (TSB) group, which can be attributed to *S. aureus* normally present on the skin and variations due to handling. Using a custom app, signals from WINDOW were conveniently extracted by placing a smartphone in close proximity to the wound dressing (movie S5). Wounds with live *S. aureus* ($10^5$ and $10^6$ CFU) exhibited a 0.4-V change in signal after 24 hours (Fig. 6H), triggering an infection alert on the smartphone (Fig. 6I).
DISCUSSION

We have proposed and demonstrated WINDOW, a flexible, wireless, and battery-free sensor based on DNAgel that can interface with wounds and detect infection. WINDOW exploits material formulations, fabrication approaches, circuit layouts, and wireless techniques that collectively enable DNase activity associated with *S. aureus* virulence activity to be transduced into a wireless signal detectable by a smartphone. In vitro experiments establish that the sensor responds selectively to amounts of *S. aureus* near to thresholds for clinical infection (10^5 CFU or more per gram of viable tissue) in both culture supernatant and clinical wound exudates from DFUs (46). In vivo studies in a mouse wound model further demonstrate the utility of WINDOW to detect clinically relevant amounts of *S. aureus* when interfaced with wounds for 24 hours.

Beyond detecting *S. aureus*, we postulate that WINDOW can be used to detect secreted DNases from other wound-associated pathogens such as *P. aeruginosa* and *S. pyogenes* (36). Although *S. aureus* and other bacteria commonly implicated in wound infections can be isolated from unaffected skin in many patients, they have markedly lower abundance and expression of virulence factors on sites where the epidermis is not breached (48, 49). Consequently, the presence of these and other skin commensal bacteria is not expected to substantially affect the signal reported by the sensor. Nevertheless, further clinical investigations of the wound microbial composition...
together with WINDOW’s performance on human wounds are needed to determine whether secreted DNase activity can serve as a general biomarker for infections. Possible effects of other virulence factors on the response of DNAgel should also be carefully investigated.

Future work may expand the functionality of DNAgel for wound monitoring. Depending on the wetness of the wound environment, dehydration of DNAgel can limit time duration over which the sensor is effective. Material engineering strategies may improve the anti-dehydration properties of DNAgel. For example, bonding a thin elastomer film to hydrogel has been shown to greatly increase anti-dehydration (50), and adding microfluidic structures can constrain dehydration while helping to dissipate liquified gel after enzymatic degradation (39, 51). Using CRISPR-associated nucleases, DNAgel degradation actuated by specific RNA inputs have also been recently demonstrated, which could be exploited as a detection mechanism for pathogens not associated with DNase (52). The sensitivity of DNAgel is presently limited by the cytotoxicity of conductive dopants (53–55). Systematic exploration of biocompatible dopants, such as coated inert gold particles (56), could also yield approaches to increase sensitivity.

Clinically, the sensor could be embedded in wound dressing to enable patients to monitor their wounds between clinical assessments and seek appropriate intervention in the event that infection is detected. Future work will focus on technological developments to provide additional capabilities for wound care. For example, in addition to detecting infection, quantitative assessment of infection severity could be valuable in helping to determine the appropriate treatment at the point of care. In this aspect, existing sensors for measuring wound temperature, moisture, and pH (20, 57) as well as specific biomarkers could be integrated with the device to provide multiplexed analysis. Alternative wireless technologies could also be used to enable passive streaming of data from the sensor without requiring patients to bring a smartphone in proximity to the wound. This mode of operation requires the sensor to have a power supply, which may be addressed using a combination of solutions for energy storage, wireless charging, and energy harvesting (58–60).

**MATERIALS AND METHODS**

**DNAgel synthesis**

DNA strands were covalently crosslinked using poly(ethylene glycol) diglycidyl ether (PEGDE) to form a 3D hydrogel network. The hydrophilic poly(ethylene glycol) component of PEGDE confers increased anti-dehydration and biocompatibility, while the two epoxide groups in PEGDE react with primary amine groups on the adenosome, guanine, and cytosine nucleotide bases and bonds adjacent DNA strands (61, 62). Specifically, DNAgel precursor was prepared by dissolving 10 weight % (wt %) deoxyribonucleic acid sodium salt (smDNA) in 4.0 mM NaBr solution at room temperature. Crosslinker (2.5 wt %), PEGDE (M<sub>n</sub> = 500), was uniformly mixed with the precursor. N,N,N',N'-Tetramethylthelyenediamine (TMEDA; 0.5 wt %), as the catalyst, was further mixed with the hydrogel precursor. The precursor can be printed onto planar/curved surface or casted into macro/micromold and kept under 90% relative humidity for 48 hours to complete the crosslinking reaction. To speed up the reaction, the precursor can be transferred into a sealed centrifuge tube and immersed in a water bath at 85°C for 2 hours to complete the gelation. After gelation, the prepared DNAgel was thoroughly rinsed by deionized (DI) water to remove unreacted chemicals. All DI water used in the experiment was from a Barnstead Nanopure ultrapure water system (Thermo Fisher Scientific).

**Bioreponse and anti-dehydration of DNAgel**

Wound isolate S. aureus ATCC 29213 (SA29213) from a streak plate was inoculated into 10 ml of sterilized TSB (Sigma-Aldrich) and allowed to grow overnight at 37°C at 200 rpm. The CFU of S. aureus was characterized by optical density (OD) using a spectrometer. NHDFs were incubated in the medium composed of Dulbecco’s modified Eagle’s medium (Thermo Fisher Scientific) + 10% fetal bovine serum + 1% penicillin-streptomycin at 37°C in a humidified atmosphere (5% CO₂). DNAse (3000 U/ml; Nuclease S7, Sigma-Aldrich) stock was prepared using 0.5 mM CaCl<sub>2</sub> solution (sterilized by 0.22-μm filter). All stocks were further diluted by corresponding medium before imaging. To make a fair evaluation of selectivity, the total cellular numbers of NHDFs and S. aureus were determined on the basis of an assumption that the effective metabolites produced by NHDFs and S. aureus, for instance, DNase, are equivalent per unit cell volume. Given the volume of fibroblast (~2000 μm³) and S. aureus (~0.52 μm³) (63), the total cellular volume of NHDFs (~2 × 10⁴ cells, 4 × 10⁷ μm³) and S. aureus (~7 × 10⁶ CFU, 3.7 × 10⁷ μm³) are comparable, therefore confirming the selective response of DNAgel.

For the imaging, DNAgel samples were transferred into a chambered borosilicate coverglass system (Lab-Tek, Thermo Fisher Scientific). Fluorescent images were acquired with a confocal microscope (Zeiss LSM 710) in Z-stack mode with controlled ambient by Zeiss incubation system. 3D topography of fluorescent images was reconstructed by Imaris package (Oxford Instruments). A bacterial viability stain (LIVE/DEAD BacLight Bacterial Viability Kit, Invitrogen, Thermo Fisher Scientific) was used for S. aureus, a plasma membrane stain (CellMask, Invitrogen, Thermo Fisher Scientific) for NHDFs, and a fluorescent stain (NucBlue, Invitrogen, Thermo Fisher Scientific) for DNAgel and NHDF nuclei. The dehydration tests were performed in a chamber (SH-262, ESPEC) with controlled temperature and relative humidity.

**In vitro degradation of DNAgel by S. aureus and skin commensal bacteria strains**

DNAgel was prepared as described above. SYBR Gold Nucleic Acid Stain (Invitrogen, Thermo Fisher Scientific) was added to the gel precursor immediately after the addition of TMEDA and mixed uniformly. Twenty-five microliters of precursor drops was placed onto the lids of 150-mm tissue culture–treated dishes (Corning), sealed with parafilm, and kept away from light for 48 hours for complete crosslinking. The crosslinked DNAgel was then washed thoroughly with ultrapure water (Merck Millipore).

Cultures of ATCC bacterial strains were grown for 24 hours in TSB at 37°C. The OD at 600 nm (OD₆₀₀) of each culture was noted. The cultures were then centrifuged (5000g, 10 min, 4°C), sterile-filtered (0.22 μm filter), and stored at −20°C until needed. Twenty-five microliters of sterile-filtered culture supernatant was added to each DNAgel drop and incubated at 37°C for 24 hours. Positive controls (DNAse I, Zymo Research) and negative controls (sterile TSB) were also set up.

DNAgel drops were imaged immediately after addition of culture supernatants and after 24 hours of incubation, using the Gel Doc EZ Imager and UV Tray (Bio-Rad). The change in size and fluorescence intensity of each DNAgel drop was quantified with ImageJ. Experiments were performed in technical triplicates.
DNAgel response to patient wound samples

Wound sampling from DFU patients was approved by St Luke’s Hospital Institutional Review Board (IRB-02-2019-08-28), and all subjects provided written consent before participation. The inclusion criteria for this study comprised male/female individuals >21 years old who (i) have received a clinical diagnosis of diabetes, (ii) are able to provide consent, and (iii) have one or more DFUs present on the lower limb. Patients who were involved in other interventional clinical trials were excluded from this study. The wounds were cleansed with sterile water before sampling, and one sterile Levine swab was used to collect the wound fluid and microbes from each patient before debridement. Five hundred microliters of 50 mM tris (pH 6) with 5 mM CaCl$_2$ was added to each swab on the same day the swab was collected and vortexed for 30 s. One hundred microliters of the sample was mixed with 400 µl of TSB containing 15% glycerol and stored at −80°C in aliquots until further processing. For 18 patient samples with available clinical microbiology reports, we determined the CFU count. Ten microliters of the sample was thawed, diluted, and plated onto TSB agar plates and incubated for 48 hours before a manual count was done (in triplicates). Samples with S. aureus (three patients, >10$^4$ CFU/cm$^2$) and low microbial colonization (five patients, without S. aureus, <10$^4$ CFU/cm$^2$) were selected for DNAgel test. To assess DNase hydrogel degradation, 10 µl of each sample was added to 4 ml of TSB and incubated for 24 hours at 37°C with shaking at 200 rpm. The culture supernatant was obtained by centrifuging the culture at 5000 rpm for 5 min and then filtered with 0.22-µm filters. The DNAgel degradation assay was performed as per the cultured bacteria strains above.

DNAgel doping and characterization

Ti$_3$C$_2$T$_x$ MXene nanosheets were prepared according to the literature. Lithium fluoride (LiF) (1.0 g; ≥99.0%; Sigma-Aldrich, BioUltra) was added to 6.0 M hydrochloric acid (HCl; 37%; Sigma-Aldrich, ACS reagent) solution (20 ml) under vigorous stirring. After the dissolution of LiF, 1.0 g of Ti$_3$AlC$_2$ powder (Tongrun Info Technology Co. Ltd.) was added slowly into the hydrogen fluoride (HF)-containing solution, and the mixture was then kept at 35°C for 24 hours. Thereafter, the solid residue was washed with DI water several times until the pH value increased to ca. 7.0. Subsequently, the washed residue was added into 100 ml of DI water (Millipore), ultrasonicated for 30 min, and centrifuged at 3000 rpm for 30 min. The supernatant was collected as the suspension of Ti$_3$C$_2$T$_x$ MXene nanosheets.

Ti$_3$C$_2$T$_x$ MXene (0.2 wt %), GO$_x$ (Timesnano), PEDOT:PSS (Clevios PH 1000, Heraeus), SWCNTs (Timesnano), and AgNWs (50 nm in diameter; Kechuang Advanced Materials) were doped into hydrogel precursor, respectively. The gelation was completed following the protocol of undoped hydrogel. After the gelation, the permittivity of hydrogels was obtained using a dielectric probe (8880, Fuji Xerox) after calibration by DI water.

To evaluate the cytotoxicity of dopants, ~1 µl of DNAgel and dopants (1 wt%) were spiked into 50 µl of NHDFs (~4 × 10$^4$ cells) and incubated for 48 hours. The NHDFs were then observed under a microscope (Nikon Eclipse Ti2 microscope) after treatment with the LIVE/DEAD Cell Imaging Kit (Invitrogen, Thermo Fisher Scientific). For viability results, 50 µl of NHDFs (~4 × 10$^4$ cells) was incubated with ~1 µl of DNAgel and dopants (1 wt %) for 48 hours and tested through trypan blue staining and standard 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

**WINDOW design and fabrication**

Interdigital capacitive sensing electrodes were modeled by using more than 2.48 × 10$^6$ tetrahedrons and simulated by the finite-difference time-domain method (CST Microwave Studio, Dassault Systems) to evaluate the capacitive sensing performance. The optimization was realized through systematically sweeping key geometrical parameters. Mutual coupling analysis was conducted by CST with circuit layout from Altium Designer.

The interdigitated electrodes were fabricated by printing (ColorQube 8880, Fuji Xerox) the traces (fig. S7) on a copper-polyimide substrate (18-µm-thick copper, 25-µm-thick polyimide layer; DuPont). After baking at 70°C for 10 min, the printed substrate was etched using H$_2$O$_2$ and HCl and cleaned by immersion in hexane and ethanol to yield the patterned traces. SU-8 was coated and ultraviolet (UV) crosslinked over the sensor surface as protection layer with a thickness of ~2 µm. A crescent silicone pillar (~1 mm in thickness) was added onto the capacitive sensor for mechanical support, followed by DNAgel functionalization (~1 mm in thickness) of the active region of the sensor. The circuit diagram for NFC module and the electronic components involved can be found in fig. S9.

**In vitro evaluation of WINDOW**

Culture supernatant of SA29213 was grown overnight on tryptic soy agar (Sigma-Aldrich). Single colony of SA29213 was inoculated in TSB (Sigma-Aldrich) and allowed to grow to OD$_{600}$ = 0.8 at 37°C. TSB was then inoculated (OD$_{600}$ = 0.01) and cultured at 37°C overnight. Overnight culture, with tested CFU number, was clarified via centrifugation (3000g, 30 min, 4°C), sterile-filtered (0.22-µm filter), and stored at −20°C until needed. For in vitro experiment, culture supernatant was diluted by TSB as an equivalent substitute for live S. aureus suspension with effective secretory DNase. The hydrogel coverage response was recorded with a mixed domain oscilloscope (MDO3012, Tektronix) and a vector network analyzer (N9923A FieldFox, Keysight).

**In vivo evaluation of WINDOW**

Male C57 black 6 inbred mice (C57BL/6) between 8 and 10 weeks of age and 25 and 30 g of weight were used. Mice were provided by in-house colony by the animal facility of Lee Kong Chian (LKC) School of Medicine. The skin on the back of the mice was prepared by shaving and applying depliatory cream (Nair). The injury site was then wiped three times with 70% ethanol. Surgery was performed under inhaled isoflurane (2 to 5%), and depth of anesthesia was checked by testing pedal reflex. Buprenorphine (1.5 mg/kg) was injected subcutaneously before wounding for sustained pain relief. Full-thickness excisional wounds through the panniculus carnosus were achieved by lifting the back skin of the mice from the dorsum and making an incision with a 6-mm punch biopsy (Acuderm Inc.). The two bilateral wounds equidistant from the midline and spaced either side of the dorsum were randomly assigned as the control wound or the test wound for WINDOW application.

Mice were divided into three groups, where test wounds were applied with either TSB or live bacteria suspension of SA29213 at 10$^5$ or 10$^6$ CFU (n = 2 mice per group). Overnight S. aureus culture was diluted with TSB to achieve target CFU numbers for the experiment. Gauze was overlaid onto the wound, and 20 µl of bacteria
supernatant/TSB was applied directly onto the gauze and wound. DNAGel-functionalized WINDOW was then placed onto the gauze and fixed by a small piece of Tegaderm film (3M). The whole back of the mice was then covered with an OPSITE dressing (Smith & Nephew) to ensure that both the WINDOW and gauze remained in place. A mobile phone with a custom app was used to record the signal 0, 1, 4, and 24 hours after WINDOW attachment. To quantify the amount of bacteria 24 hours after wounding, mice skin surrounding the wound was sampled (approximately 1 cm × 1 cm) and placed in pre-weighed 2-ml microcentrifuge tubes containing 1 ml of sterile phosphate-buffered saline. The mice skin sample was weighed and sonicated in a chilled sonicator (Elasonic S 30 H, Elma Schmidbauer GmbH, Germany) to dissociate adherent bacteria (37 kHz, 10 min per cycle, three cycles, 1-min vortex after each cycle). Bacteria in each sample were enumerated via CFU counting and normalized by sample weight. All experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, USA, and protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the Animal Research Facility of Nanyang Technological University, with consideration to ethical use and animal welfare.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.1617 View/request a protocol for this paper from Bio-protocol.

**REFERENCES AND NOTES**

1. M. Mehrali, S. Bagherfard, M. Akbari, A. Thakur, B. Mirani, M. Mehrali, M. Hasany, G. Orive, P. Das, J. Emneus, T. L. Andresen, A. Dolatshahi-Pirouz, Blending electronics with the human body: A pathway toward a cybernetic future. Adv. Sci. 5, 1700931 (2018).
2. C. Xu, Y. Yang, W. Gao, Skin-interfaced sensors in digital medicine: From materials to applications. Mater. 2, 1414–1445 (2020).
3. T. Stuart, L. Cai, A. Burton, P. Gutruf, Wireless and battery-free platforms for collection of biosignals. Biosens. Bioelectron. 178, 113007 (2020).
4. R. Lin, H.-I. Kim, S. Achavananthadith, S. A. Kurt, S. C. C. Tan, H. Yao, B. C. K. Tee, J. W. Lee, J. S. Ho, Wireless battery-free body sensor networks using near-field-enabled clothing. Nat. Commun. 11, 444 (2020).
5. S. R. Madhavpattrthy, H. Wang, J. Kong, M. Zhang, J. Y. Lee, J. B. Park, H. Jang, Z. Xie, J. Cao, R. Avila, C. Wei, V. D'Angeolo, Z. Hui, U. Chung, S. Couchlin, M. Patel, J. Winograd, J. Lim, A. Banks, S. Xu, Y. Huang, J. A. Rogers, Reliable, low-cost, fully integrated hydation sensors for monitoring and diagnosis of inflammatory skin diseases in any environment. Sci. Adv. 6, eaob7146 (2020).
6. S. Niu, N. Matsuhiha, L. Becker, J. Li, S. Wang, J. Wang, Y. Jiang, X. Yan, Y. Yun, W. Burnett, A. S. Y. Poon, J. B. H. Tok, X. Chen, Z. Bao, A wireless body area sensor network based on stretchable passive tags. Nat. Electron. 2, 361–368 (2019).
7. Y. Song, J. Min, Y. Yu, H. Wang, Y. Yang, H. Zhang, W. Gao, Wireless battery-free wearable sweat sensor powered by human motion. Sci. Adv. 6, eaay9842 (2020).
8. J. Kim, J. R. Sempionatto, S. Imanii, M. C. Hartel, A. Barfidiokht, G. Tang, A. S. Campbell, P. P. Mercier, J. Wang, Simultaneous monitoring of sweat and interstitial fluid using a single wearable biosensor platform. Adv. Sci. 5, 1800880 (2018).
9. C. K. Sen, G. M. Gordillo, S. Roy, R. Kirsner, L. Lambert, T. K. Hunt, F. Gottrup, G. C. Gurtner, M. T. Longaker, Human skin wounds: A major and snowballing threat to public health and the economy. Wound Repair Regen. 17, 763–771 (2009).
10. P. G. Bowler, B. J. Duerden, D. G. Armstrong, Wound microbiology and associated approaches to wound management. Clin. Microbiol. Rev. 14, 244–269 (2001).
11. V. Falanga, Wound healing and its impairment in the diabetic foot. Lancet 366, 1736–1743 (2005).
12. P. Salvo, V. Dini, F. Di Francesco, M. Romanelli, The role of biomedical sensors in wound healing. Wound Med. 8, 15–18 (2015).
13. S. E. Gardner, R. A. Frantz, B. N. Doebbeling, The validity of the clinical signs and symptoms used to identify localized chronic wound infection. Wound Repair Regen. 9, 178–186 (2001).
14. A. R. Siddiqui, J. M. Bernstein, Chronic wound infection: Facts and controversies. Clin. Dermatol. 28, 519–526 (2010).
15. N. Mehmoord, A. Hariz, R. Fitridge, N. H. Voelcker, Applications of modern sensors and wireless technology in effective wound management. J. Biomed. Mater. Res. B 102, 885–895 (2014).
16. W.-J. Deng, L.-F. Wang, L. Dong, Q.-A. Huang, LC wireless sensitive pressure sensors with microstructured PDMs dielectric layers for wound monitoring. IEEE Sensors J. 18, 4866–4892 (2018).
17. O. Pang, D. Lou, S. Li, G. Wang, B. Qiao, S. Dong, L. Ma, C. Gao, Z. Wu, Smart flexible electronics-integrated wound dressing for real-time monitoring and on-demand treatment of infected wounds. Adv. Sci. 7, 1902673 (2020).
18. D. Sharp, Printed composite electrodes for in-situ wound pH monitoring. Biosens. Bioelectron. 50, 399–405 (2013).
19. T. Guinovart, G. Valdés-Ramírez, J. R. Windmiller, F. J. Andrade, J. Wang, Bandage-based wearable potentiometric sensor for monitoring wound pH. Electroanalysis 26, 1335–1339 (2014).
20. P. Mostafalu, A. Tamayol, R. Rahimi, M. Ochoa, A. Khalipour, G. Kiae, I. K. Yazdi, S. Bagherfard, M. R. Dokmeci, B. Ziaie, S. R. Sonkusale, A. Khademhosseini, Smart bandage for monitoring and treatment of chronic wounds. Small 14, 1703509 (2018).
21. R. Rahimi, U. Brener, S. Chittiboyina, T. Soleimani, D. A. Detwiler, S. A. Lelièvre, B. Ziaie, Laser-enabled fabrication of flexible and transparent pH sensor with near-field communication for in-situ monitoring of wound infection. Sensors Actuators B Chem. 267, 198–207 (2018).
22. S. D. Milne, J. Seoudi, H. Al Hamad, T. K. Talal, A. A. Anoop, N. Allahverdi, Z. Zakaria, R. Menzies, P. Connolly, A wearable wound moisture sensor as an indicator for wound dressing change: An observational study of wound moisture and status. Int. Wound J. 13, 1309–1314 (2016).
23. T. R. Dargaville, B. L. Farrugia, A. J. Broadbent, S. Pace, Z. Upton, N. H. Voelcker, Sensors and imaging for wound healing: A review. Biosens. Bioelectron. 41, 30–42 (2013).
24. H. J. Smitaan, A. Banerjee, S. McIntosh, M. Tarralba, S. Lucas, A. Chan, V. K. Shamgammad, E. D. Goluch, Electrochemical detection of pseudomonas in wound exudate samples from patients with chronic wounds. Wound Repair Regen. 24, 366–372 (2016).
25. G. A. Zelada-Guillen, J. L. Sebastian-Avila, P. Blondeau, J. Riu, F. X. Rius, Label-free detection of staphylococcus aureus in skin using real-time potentiometric biosensors based on carbon nanotubes and aptamers. Biosens. Bioelectron. 31, 226–232 (2012).
26. M. A. C. Stuart, W. T. S. Huck, J. Genzer, M. Müller, C. Ober, M. Stamm, G. S. Sukhorukov, I. Szleifer, V. V. Tsukruk, M. Urbán, F. Winink, S. Zauscher, I. Luzinov, S. Minko, Emerging applications of stimuli-responsive polymer materials. Nat. Mater. 9, 101–113 (2010).
27. G. Orive, P. Das, J. Emneus, T. L. Andresen, A. Dolatshahi-Pirouz, Blending electronics with the human body: A pathway toward a cybernetic future. Adv. Sci. 5, 1700931 (2018).
40. P. Karacan, H. Cakmak, O. Okay, Swelling behavior of physical and chemical DNA hydrogels. J. Appl. Polym. Sci. 128, 3330–3337 (2013).

41. T. Amiya, T. Tanaka, Phase transitions in crosslinked gels of natural polymers. Macromolecules 20, 1162–1164 (1987).

42. J. Wu, Z. Wu, H. Xu, Q. Wu, C. Liu, B.-R. Yang, X. Gui, X. Xie, K. Tao, Y. Shen, J. Miao, L. K. Norford, An intrinsically stretchable humidity sensor based on anti-drying, self-healing and transparent organogelohydrs. Mater. Horiz. 6, 595–603 (2019).

43. C. Gong, D. Liu, Z. Miao, M. Li, A magnetic-balanced inductive link for the simultaneous uplink data and power telemetry. Sensors 17, 1768 (2017).

44. A. K. RamRakhyani, G. Lazzi, On the design of efficient multi-coil telemetry systems for biomedical implants. IEEE Trans. Biomed. Circuits Syst. 7, 11–23 (2013).

45. S. S. Ivkovic, M. Z. Markovic, D. Z. Ivkovic, N. Cvetaovic, LCR circuit: New simple methods for measuring the equivalent series resistance of a capacitor and inductance of a coil. Eur. J. Phys. 48, 55705 (2017).

46. S. E. Gardner, R. A. Frantz, C. L. Saltzman, H. Park, M. Scherubel, Diagnostic validity of three swab techniques for identifying chronic wound infection. Wound Repair Regen. 14, 548–557 (2006).

47. K. K. L. Chong, W. H. Tay, B. Janela, A. M. H. Yong, T. H. Liew, L. Madden, D. Keogh, T. M. S. Barkham, F. Ghinoux, D. L. Becker, A. K. Aline, Enterococcus faecalis modulates immune activation and slows healing during wound infection. J. Infect. Dis. 216, 1644–1654 (2017).

48. C. P. Parlet, M. M. Brown, A. R. Horswill, Commensal staphylococci influence staphylococcus aureus skin colonization and disease. Trends Microbiol. 27, 497–507 (2019).

49. C. Hauser, B. Wuethrich, L. Matter, J. A. Wilhelm, S. Donnabend, K. Schofer, Staphylococcus aureus skin colonization in atopic dermatitis patients. Dermatology 170, 35–39 (1985).

50. H. Yuk, T. Zhang, G. A. Parada, X. Liu, X. Zhao, Skin-inspired hydrogel–Elastomer hybrids with robust interfaces and functional microstructures. Nat. Commun. 7, 12038 (2016).

51. J. T. Reeder, J. Choi, Y. Xue, P. G. Gutruf, J. Hanson, M. Liu, T. Ray, A. J. Bandodkar, R. Avila, W. Xia, S. Krishnan, S. Xu, K. Barnes, M. Pahnke, R. Gaffarri, Y. Huang, J. A. Rogers, Waterproof, electronics-enabled, epidermal microfluidic devices for sweat collection, biomarker analysis, and thermography in aquatic settings. Sci. Adv. 5, eaau6356 (2019).

52. M. A. English, L. R. Soenksen, R. V. Gayet, H. de Puig, N. M. Angenent-Mari, A. S. Mao, P.-Q. Nguyen, J. J. Collins, Programmable CRISPR-responsive smart materials. Science 365, 780–785 (2019).

53. E. Hidalgo, C. Dominguez, Study of cytotoxicity mechanisms of silver nitrate in human dermal fibroblasts. Toxicol. Lett. 98, 169–179 (1998).

54. Y. Liu, Y. Zhao, B. Sun, C. Chen, Understanding the toxicity of carbon nanotubes. Acc. Chem. Res. 46, 702–713 (2013).

55. A. B. Seabara, A. J. Paula, R. de Lima, O. L. Alves, N. Durán, Nanotoxicity of graphene and graphene oxide. Chem. Res. Toxicol. 27, 159–168 (2014).

56. J. Zhang, L. Mou, X. Jiang, Surface chemistry of gold nanoparticles for health-related applications. Chem. Sci. 11, 923–936 (2020).

57. S. Lin, H. Yue, T. Zhang, G. A. Parada, H. Koo, C. Yu, X. Zhao, Stretchable hydrogel electronics and devices. Adv. Mater. 28, 4497–4505 (2016).

58. J. Ren, L. Li, C. Chen, X. Chen, Z. Cai, L. Qiu, Y. Wang, X. Zhu, H. Peng, Twisting carbon nanotube fibers for both wire-shaped micro-supercapacitor and micro-battery. Adv. Mater. 25, 1155–1159 (2013).

59. X. Lu, P. Wang, D. Niyato, D. I. Kim, Z. Han, Wireless charging technologies: Fundamentals, standards, and network applications. IEEE Commun. Surv. Tutor. 18, 1413–1452 (2016).

60. Y. Yu, J. Nassar, C. Xu, J. Min, Y. Yang, A. Dai, R. Doshi, A. Huang, Y. Song, R. Gehlhar, A. D. Ames, W. Gao, Biofuel-powered soft electronic skin with multiplexed and wireless sensing for human-machine interfaces. Sci. Robot. 5, eaaz7946 (2020).

61. M. Eriksson, S. K. Kim, S. Sen, A. Graslund, B. Jernstroom, B. Norden, Location of excimer-forming adducts of (+)-anti-benzo[a]pyrene diol epoxide in DNA. J. Am. Chem. Soc. 115, 1639–1644 (1993).

62. M. Koskinen, K. Pímaker, Specific DNA adducts induced by some mono-substituted epoxides in vitro and in vivo. Chem. Biol. Interact. 129, 209–229 (2000).

63. R. Milo, R. Phillips, Cell Biology by the Numbers (Garland Science, Taylor & Francis Group, 2015).

Acknowledgments: We thank the National University of Singapore Medicine Confocal Microscopy Unit for supporting confocal imaging and Y. X. Guo for facilitating the dielectric measurements. Funding: J.S.H. acknowledges support from grants from the National Research Foundation Singapore (NRF2017-07 and A15SG-GC-2019-002), Ministry of Education Singapore (MOE2016-T3-1-004), and Institute for Health Innovation and Technology. D.L.B. acknowledges support from the Agency for Science, Technology and Research (A*STAR) under its Industry Alignment Fund–Pre-Positioning Programme (IAF-PP) grant (H17/01/a0/0C9) as part of the Wound Care Innovation for the Tropics Programme, IAF-PP grant (H17/01/a0/004), and Skin Research Institute of Singapore, Phase 2: SRI@Novena. H.L. acknowledges support from the Wound Care Innovation for the Tropics Programme, A*STAR IAF-PP Grant (H19/01/a0/GG09), Skin Innovation grant (SIG18005), MOE AcRF Tier 1 Grant (R-143-000-097-114), and Singapore Ministry of Health’s National Medical Research Council OF-IRG (MOH-000612-00), W.L. acknowledges support from MOE AcRF Tier 1 grant (R-221-000-093-133). B.C.K.T. acknowledges support from National University of Singapore Startup Grant (NUS-2017-01) and Agency of Science, Technology and Research Singapore (A18A1B0045). H. Yao acknowledges Research Scholarship from NUS Materials Science and Engineering. Y.G. acknowledges support from the EMULSION Programme H18/01/A0/017 (IAF-PP, A*STAR), S.M.P.K. acknowledges support from the National Research Foundation Singapore, under its NRF Large Equipment Grants–Grant Addendum 3: Operations of the Singapore Synchrotron Light Source (SSL). Author contributions: Z.X. and J.S.H. conceived and guided the research. Z.X. and S.A. designed and tested the wireless sensor. Z.X., S.L., W.C., V.K., P.S., W.L., and Z.X. and J.S.H. conceived and guided the research. Source (SSLS). The paper is present in the paper and/or the Supplementary Materials. Submitted 27 April 2021 Accepted 1 October 2021 Published 19 November 2021 10.1126/sciadv.abc1617

Xiong et al., Sci. Adv. 7, eabj1617 (2021) | 19 November 2021