A NOVEL BIO-PATCH FOR DETECTION AND TREATMENT OF BACTERIAL WOUND INFECTIONS

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Introduction

Wearable, patch-like devices that monitor wound conditions and deliver targeted interventions offer a powerful platform for the timely detection and treatment of pathogenic outbreaks, leading to highly efficient wound management. Here, we present a novel wearable bio-patch with a closed-loop, sense-and-respond mechanism for the treatment of wound-related complications. This platform comprises transdermal microneedles (MNs) fabricated from a biocompatible hydrogel matrix loaded with engineered cells. These cells are genetically programmed to sense lactate – a prominent bacterial infection biomarker – and autonomously produce antimicrobial compounds to combat pathogens directly at the site of injury.

***Figure 1**. Battlefield triage using a smart hydrogel bandage for wound care.* *Image generated by Google Gemini.*

Methods

The engineered sense-and-respond mechanism was mediated by the lactate-responsive, allosteric transcription factor LldR, which regulates bacterial gene expression related to lactate utilization. Specifically, a probiotic *E. coli* Nissle 1917 strain was engineered with optimized LldPRD promoter, which is regulated by the LldR repressor. In our model system, the presence of lactate induced the production of the E7 lysis protein, mediating host cell lysis and the subsequent release of a constitutively expressed fluorescent reporter (Figure 2). In the final therapeutic application, this reporter will be replaced with an antimicrobial peptide to facilitate the targeted eradication of wound infections.

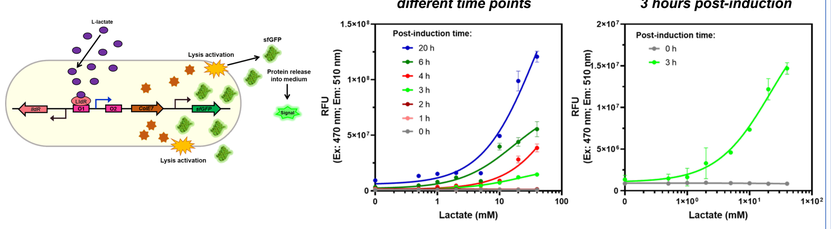

***Figure 2**. Characterization of sense-and-release mechanism in engineered E. coli Nissle 1917 cells in response to L-lactate.*

Hydrogel-based MNs encapsulating engineered probiotic sensors were fabricated *via* a micro-molding method using hyaluronic acid (HA) and a bifunctional polyethylene glycol crosslinker (PEGDE, M_n = 500 Da) (Figure 3). While optimizing the chemical crosslinking yielded robust, tunable structures, the encapsulated cells themselves act as physical crosslinks, further improving mechanical stability and reducing hydrogel swelling. In addition to providing bio-adhesion and tissue biocompatibility, this versatile biopolymer network serves as a protective matrix that maintains cell viability, functional performance, and long-term stability during storage and sterilization.

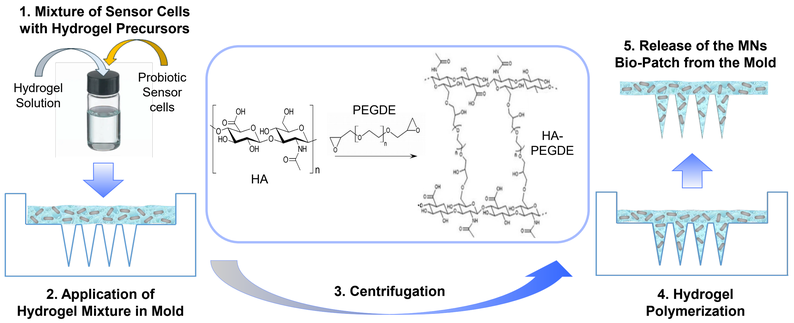

***Figure 3**. Generalized protocol for preparing hydrogel-based MN patch containing probiotic sensors.*

Characterization of Hydrogel-Based Microneedles

Following fabrication, the MN bio-patches were characterized and tested for porosity, swelling degree, and mechanical stability in both dry and swollen states. Hydrogel MNs exhibited a uniform structure and retained their shape in the swollen state (Figure 3). The combination of different concentrations of HA and the PEG crosslinker resulted in robust MN structures with tunable permeability, swelling degrees, and pore sizes. The degree of crosslinking was varied to obtain a matrix with optimal swelling and pore sizes (Figure 4). An optimal swelling degree of 600–800% w/w was achieved while maintaining an elastic behavior strong enough to penetrate the stratum corneum (Young's modulus, E' = 4.5–5 kPa) (Figure 5).

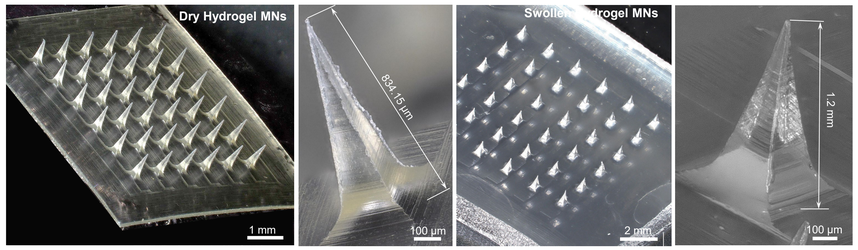

***Figure 3**. Optical images of the HA-based MNs prepared via micro-molding method from 1000 µm x 250 µm x 1000 µm templates (Micropoint™).*

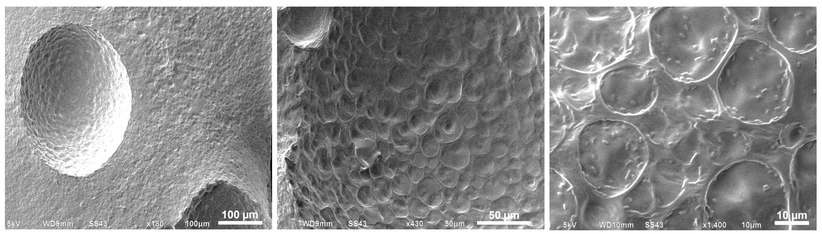

***Figure 4**. Microstructure of HA-PEG hydrogels in swollen state.*

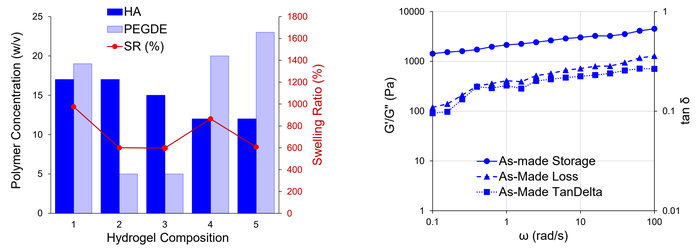

***Figure 5.** Swelling and rheological properties of the HA-PEG hydrogels.*

Conclusions

- We have successfully developed a novel, transdermal microneedle bio-patch that achieves a closed-loop "sense-and-respond" system for smart wound management. By optimizing the crosslinking density and composition of a biocompatible hyaluronic acid (HA) and PEG hydrogel matrix, we fabricated mechanically robust microneedles.
- We demonstrated that incorporating living, genetically engineered probiotic *E. coli* into this hydrogel network does not compromise cell viability or function. The integrated bio-patch actively consumes the bacterial infection biomarker lactate within two hours and triggers a highly tunable, dose-dependent therapeutic response.
- Ultimately, this platform combines the natural anti-inflammatory, healing-promotive benefits of hyaluronic acid with living therapeutics, offering a highly customizable, autonomous, point-of-care platform to detect and eliminate wound infections in real time.

Acknowledgements

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In Vitro Characterization of Bio-Patches

To evaluate protein release, we monitored the bio-patch's sfGFP response to L-lactate within simulated interstitial fluid (sISF) (Figure 6). Peak response occurred four hours post-stimulation, yielding a tunable half-maximal effective concentration (EC_{50}) between 0.7 and 8.3 mM depending on cell loading density and hydrogel crosslinking degree (Figure 7). This wide tunable range allows the platform to be precisely calibrated to detect elevated clinical lactate thresholds associated with inflammation or sepsis (> 2.5 mM).

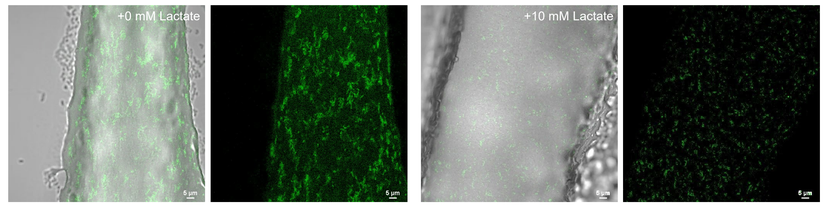

***Figure 6.** Activation of release mechanism in bio-patch (HA(17%)-PEG(5%)-Nissle(14%)) during stimulation with L-lactate in sISF.*

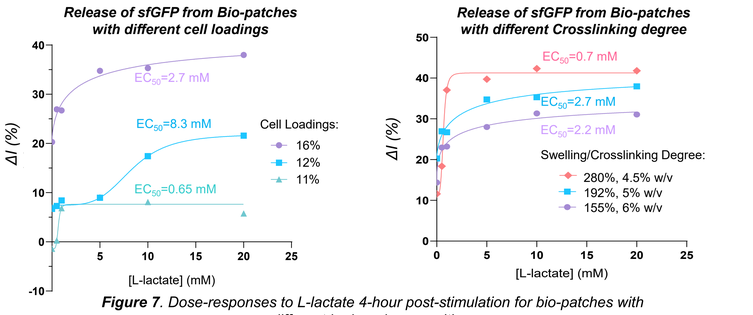

***Figure 7**. Dose-responses to L-lactate 4-hour post-stimulation for bio-patches with different hydrogel composition.*

To monitor therapeutic kinetics, *ex vivo* studies were performed by applying bio-patches to SynDaver tissue models infused with varying L-lactate concentrations (Figure 8). At designated time points, bio-patches were removed to extract the absorbed analyte via centrifugation for enzymatic assay analysis. Bio-patches containing engineered cells exhibited rapid lactate uptake within 2 hours of application, followed by a steep decline in lactate concentration; this confirms robust sensor cell function and indicates that a therapeutic response can be initiated within the first hours of application. Conversely, empty hydrogels showed only passive diffusion, with lactate absorption steadily increasing until saturation at the 6-hour mark, which is consistent for hydrogel system (Figure 9).

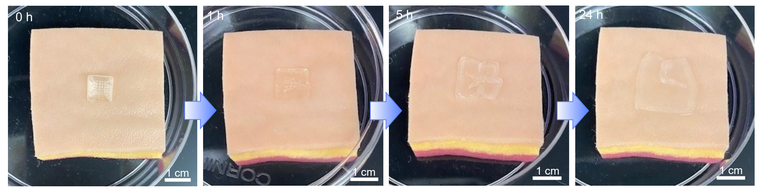

***Figure 8**. Ex vivo application of bio-patches on SynDaver tissue for 24 hours.*

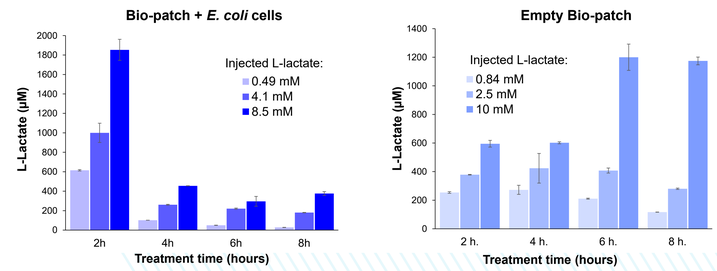

***Figure 9**. Kinetics of L-lactate absorption by bio-patches during application on SynDaver muscle tissue model.*