AFRL

WEARABLE PLATFORM FOR WOUND INFECTION MANAGEMENT: A SYNTHETIC BIOLOGY APPROACH

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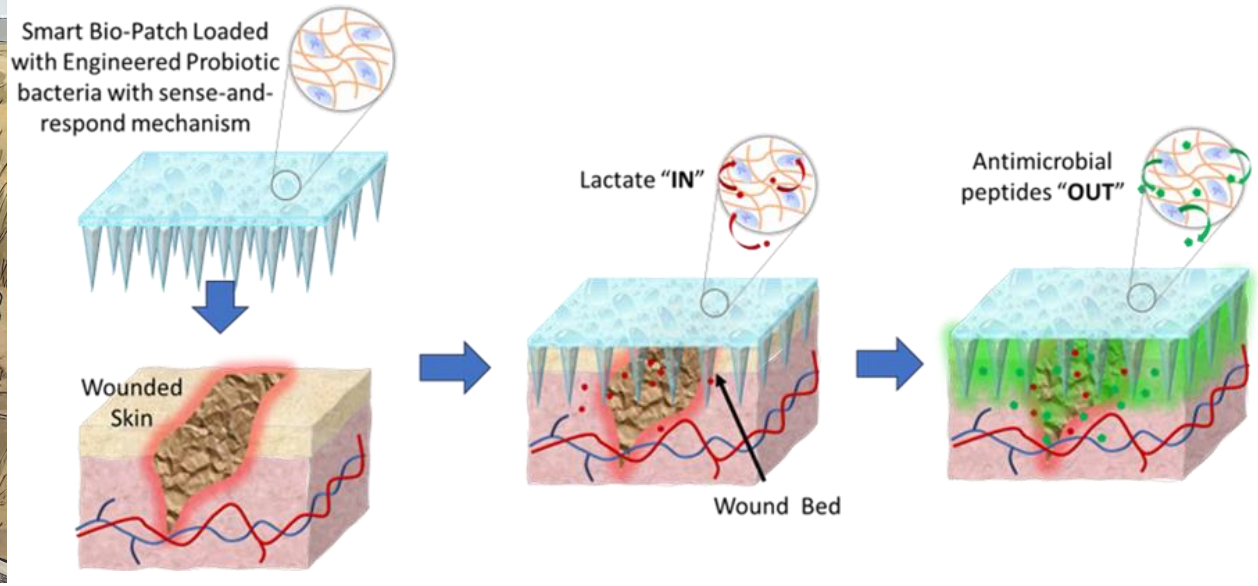
Wound Infection is a Significant Issue in the Field

PROBLEM: Over 20% of combat wounds become infected, and this percentage increases with prolonged transit time (Tribble et al., 2019; Petfield et al., 2022). This percentage is expected to rise significantly during future conflicts, especially with Agile Combat Employment (ACE) tactics. The field currently lacks the knowledge and capabilities required for next-generation wound management and effective prevention of wound infections.

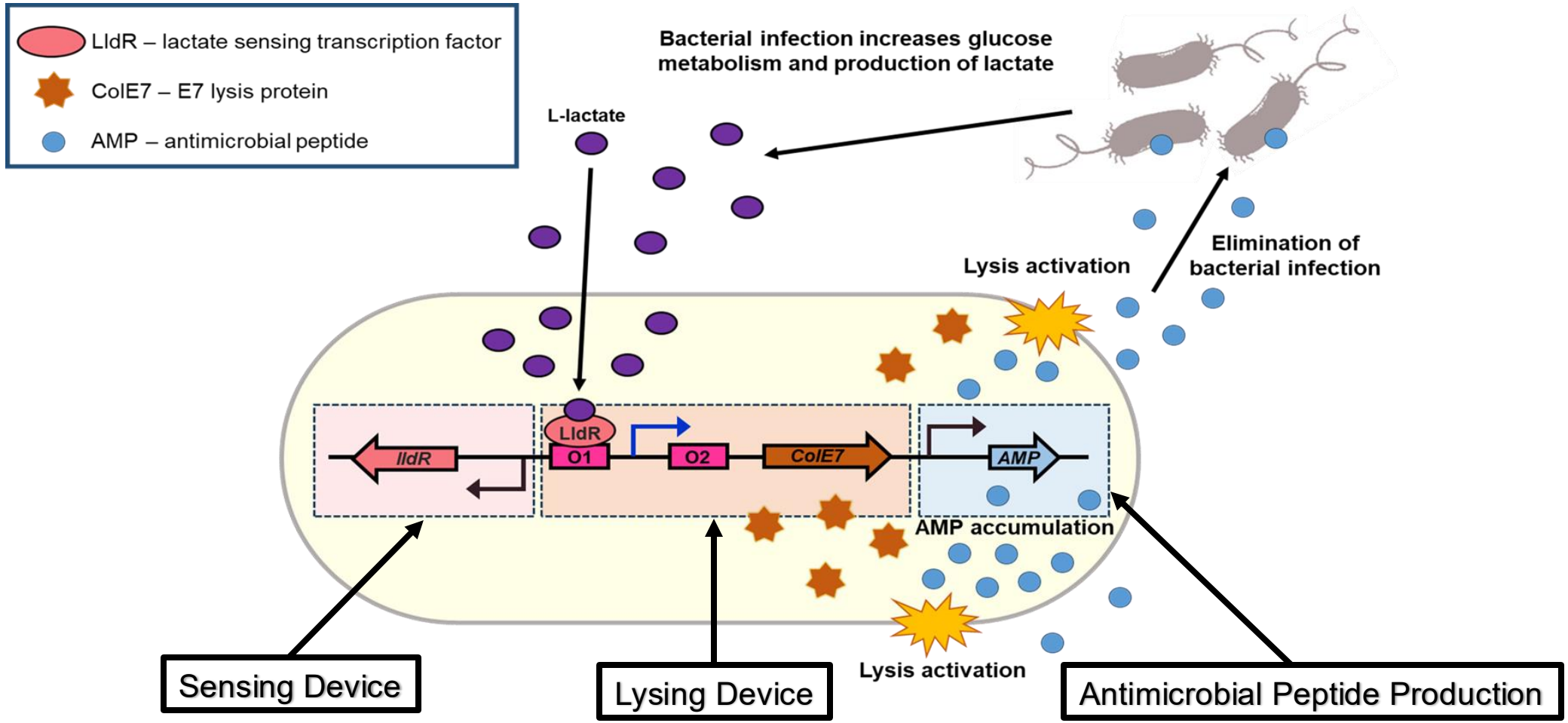
SOLUTION: ***A microneedle bio-patch to control and mitigate local infection before critical care can be administered*.**

Infection monitoring sensor with a closed-loop mechanism for treatment of wound-related complications.



Microneedle bio-patch loaded with probiotic bacteria (*E. coli* Nissle 1917) that sense infection biomarker (lactate) in wound space and produce antimicrobial compounds (peptides) to stop infection at the point of injury.

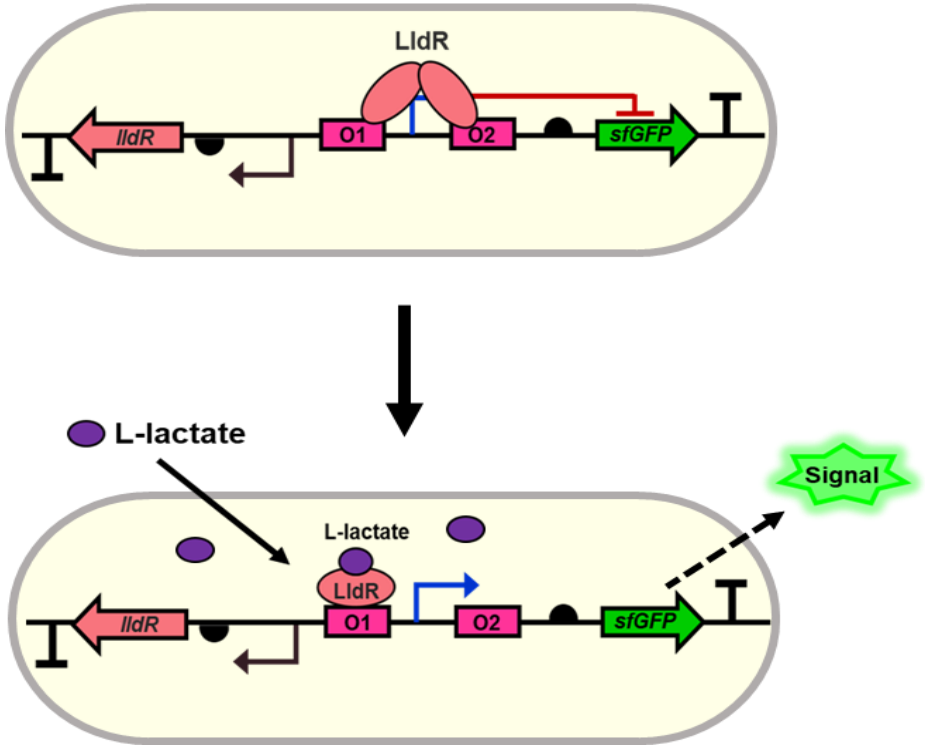
A synthetic biology-inspired closed-loop system can sense a specific biomarker related to a condition and autonomously produce a mitigation response through a genetically encoded feedback loop.



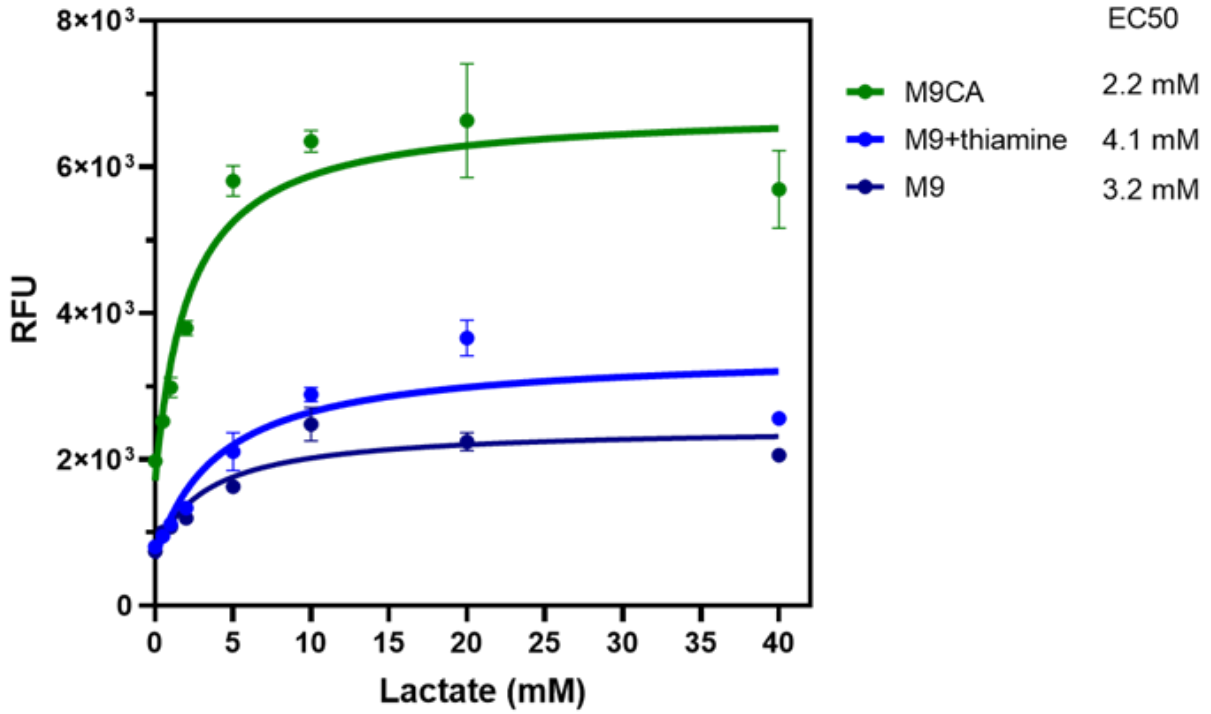
Cell-Based Biosensor with Sense-and-Respond Mechanism: *Sensing Device*

The LldR transcriptional regulator from *E. coli* activates gene expression in the presence of L-lactate, a biomarker for infection.

Engineered *E. coli* Nissle 1917



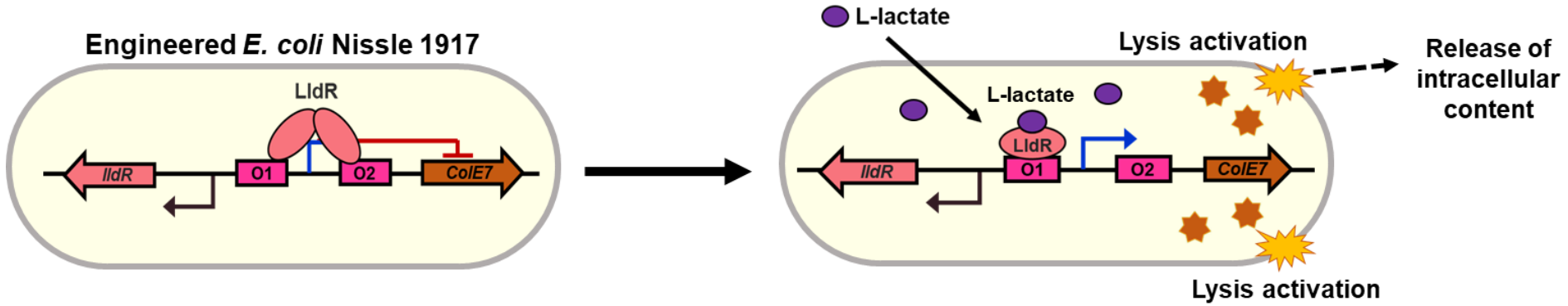
Biosensor performance in different media



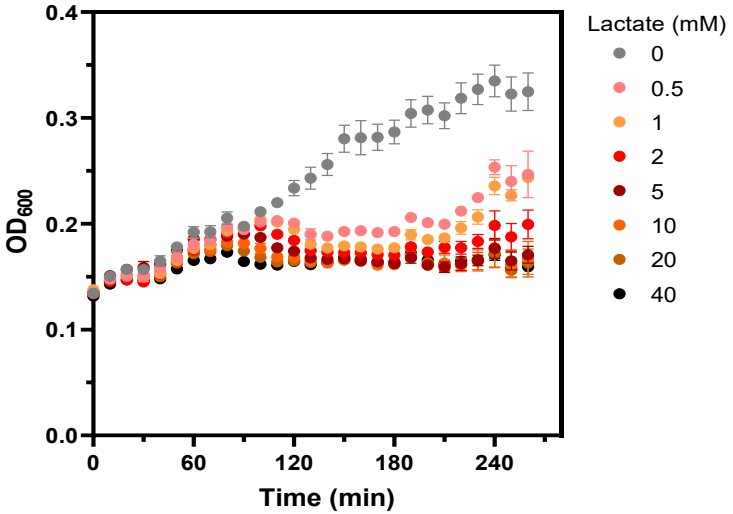
The sensor operates in the range of physiological and elevated concentrations of lactate (1-20 mM).

Cell-Based Biosensor with Sense-and-Respond Mechanism: *Lysing Device*

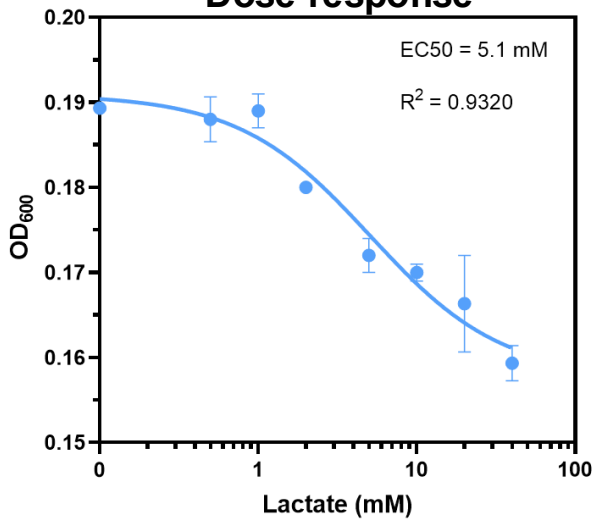
The E7 lysis protein lysis engineered *E. coli* cells in response to L-lactate, releasing their intracellular content.



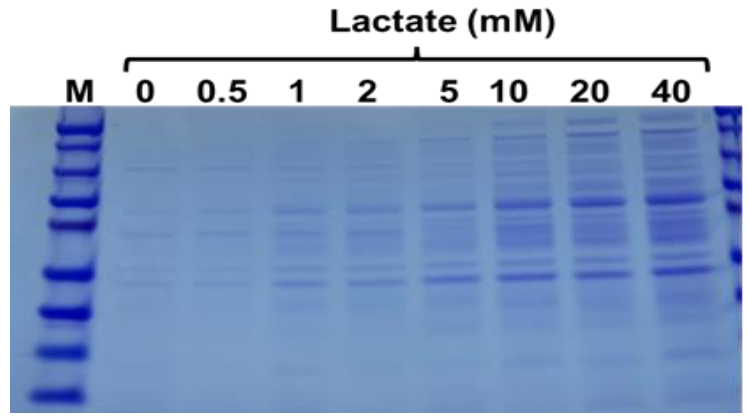
Growth curves



Dose-response



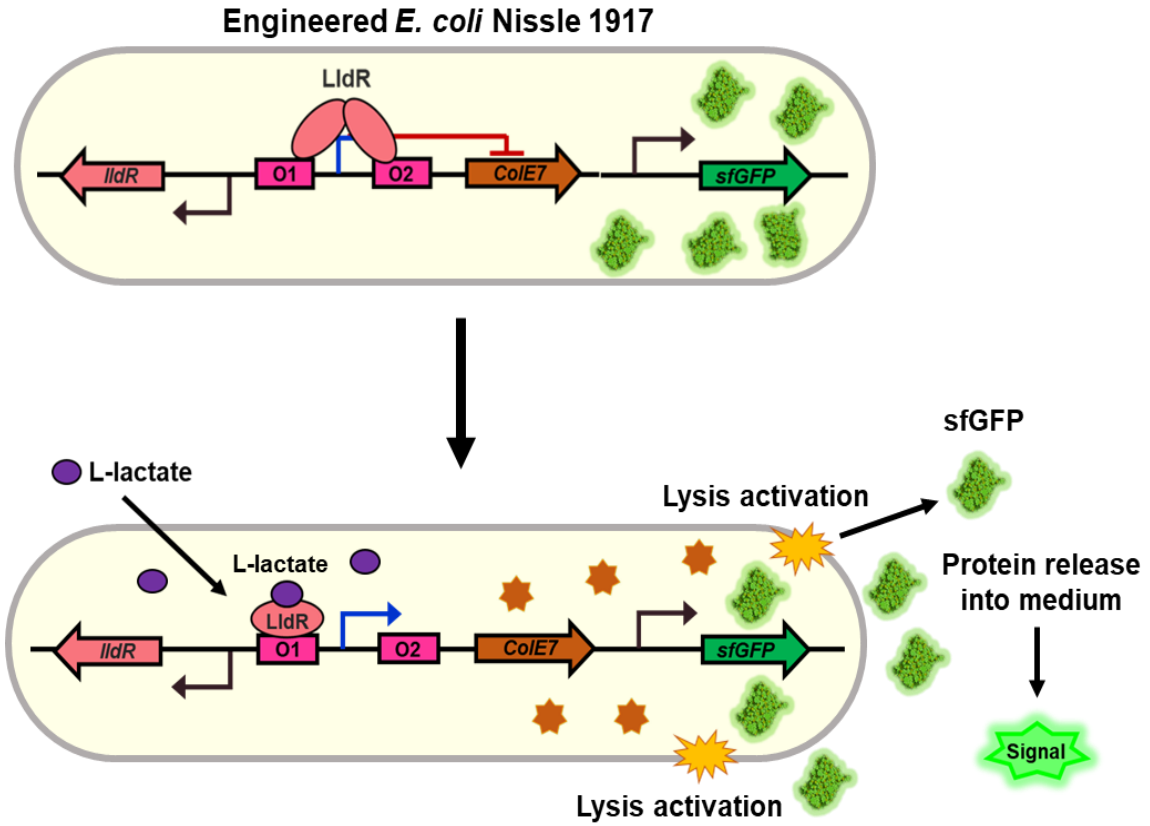
Detection of proteins released into medium



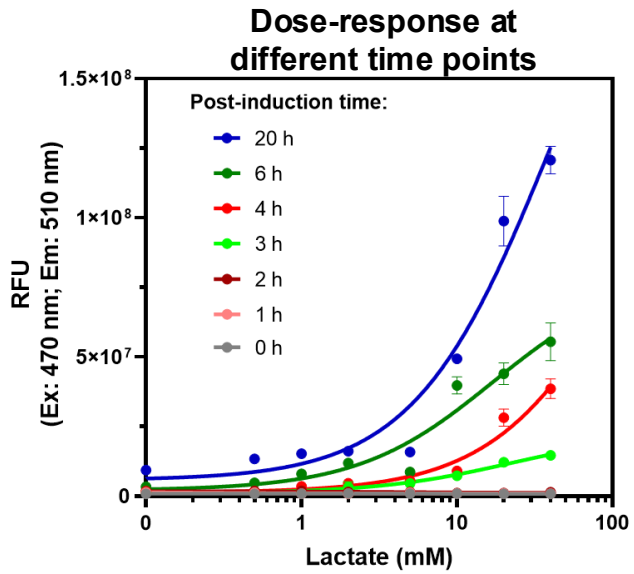
Lactate induces the lysis of *E. coli* cells in a dose-dependent manner.

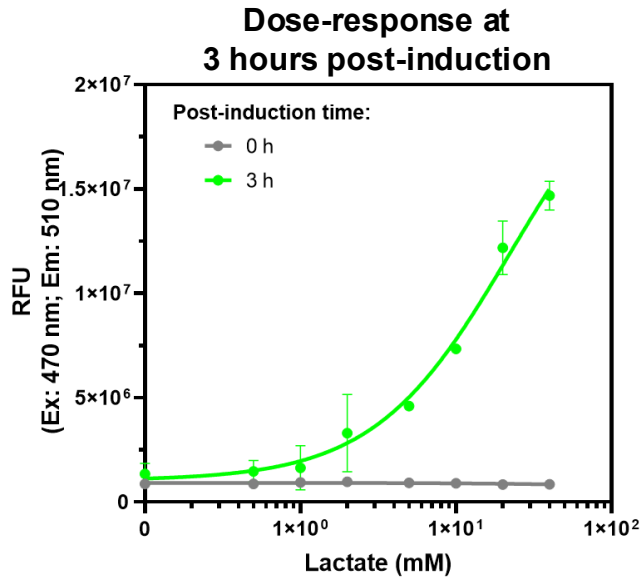
Cell-Based Biosensor with Sense-and-Respond Mechanism: *Protein Release*

Lysing device is coupled with a constitutively expressed green fluorescent protein (sfGFP) gene.



Supernatant fluorescence

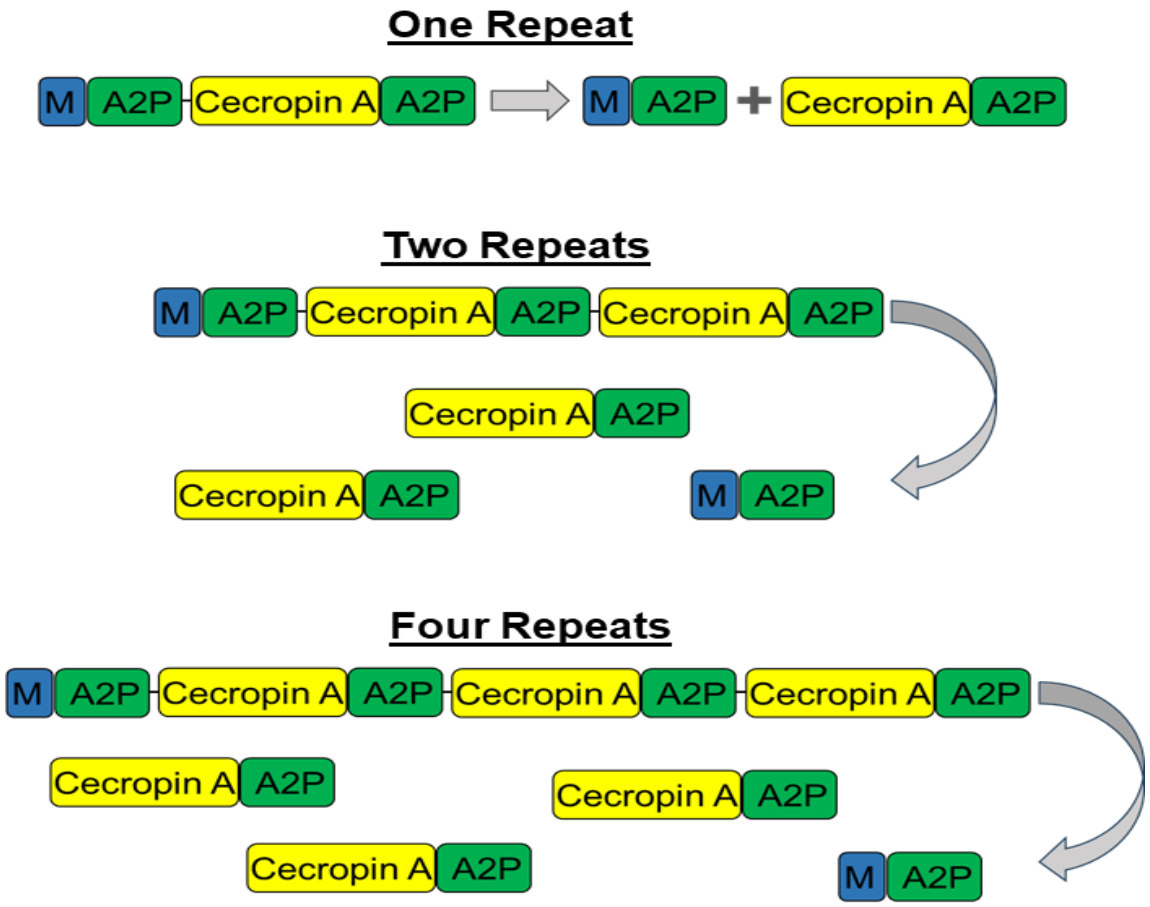




sfGFP release increased with lactate concentration, becoming detectable as early as 3 hours post-induction at lactate concentrations above 2 mM.

Cell-Based Biosensor with Sense-and-Respond Mechanism: *Cecropin A Production*

To increase Cecropin A production in engineered *E. coli* cells, a gene encoding several repetitive sequences of the antimicrobial peptide separated by A2P self-cleaving peptide sequences was created. During translation, A2P separates one Cecropin A sequence from another, while A2P itself remains at the C-terminus of the previous Cecropin A peptide.



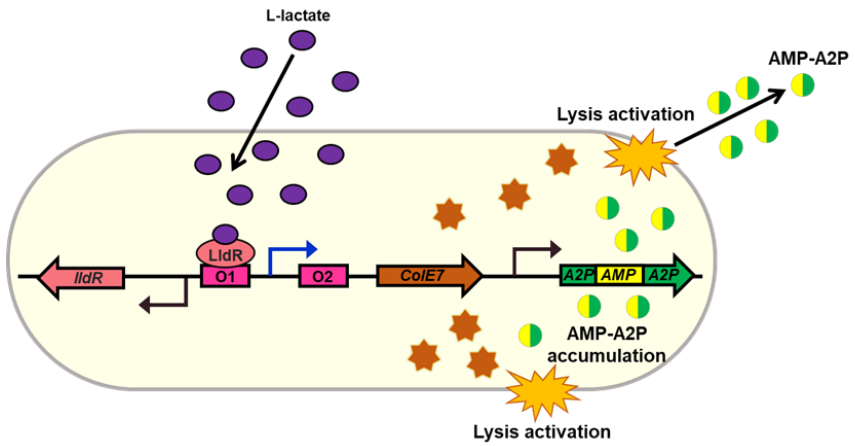
Peptide structures predicted with AlphaFold

Cecropin A

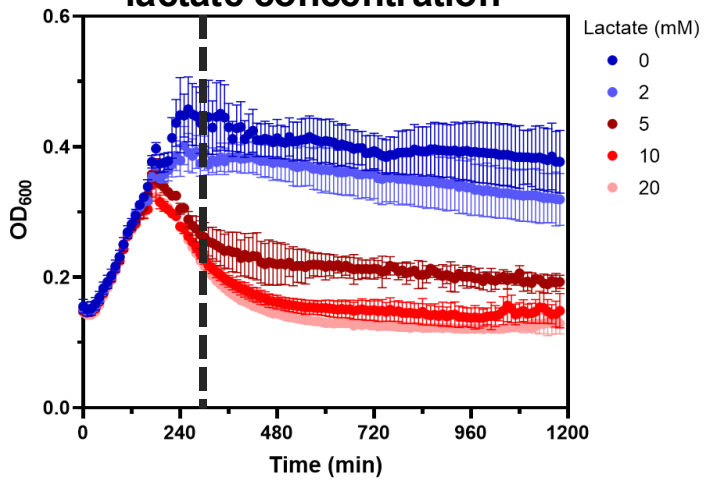
Cecropin A-A2P

Addition of the A2P peptide does not disrupt the alpha helix structure of Cecropin A.

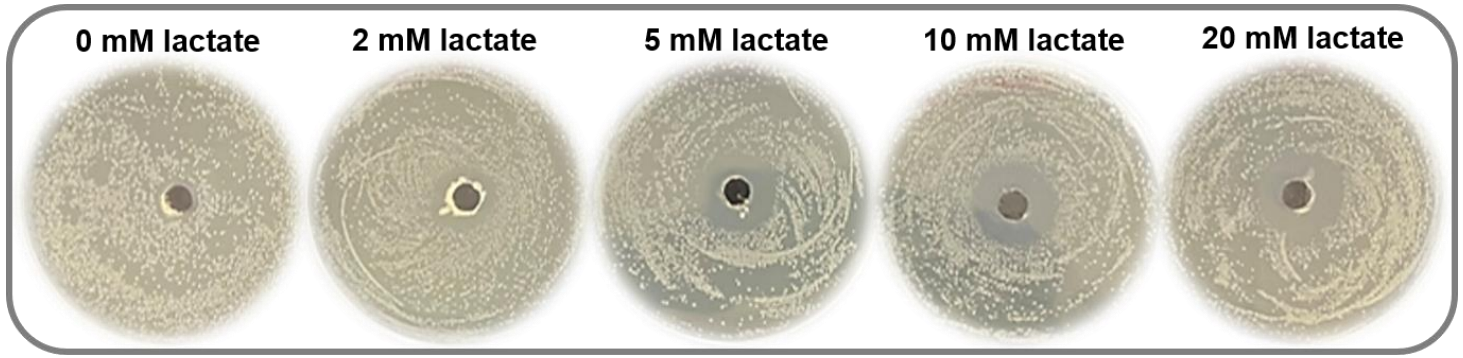
Cell-Based Biosensor with Sense-and-Respond Mechanism: *Cecropin A Release*



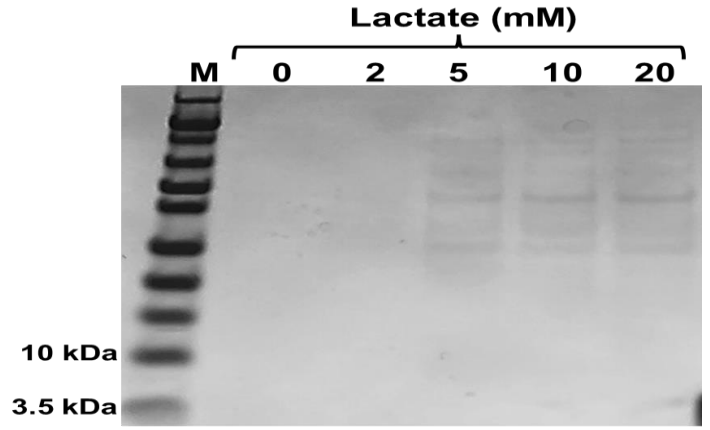
Growth curves of *E. coli* cells in the presence of different lactate concentration



Antimicrobial activity of produced Cecropin A-A2P: *dose-response*



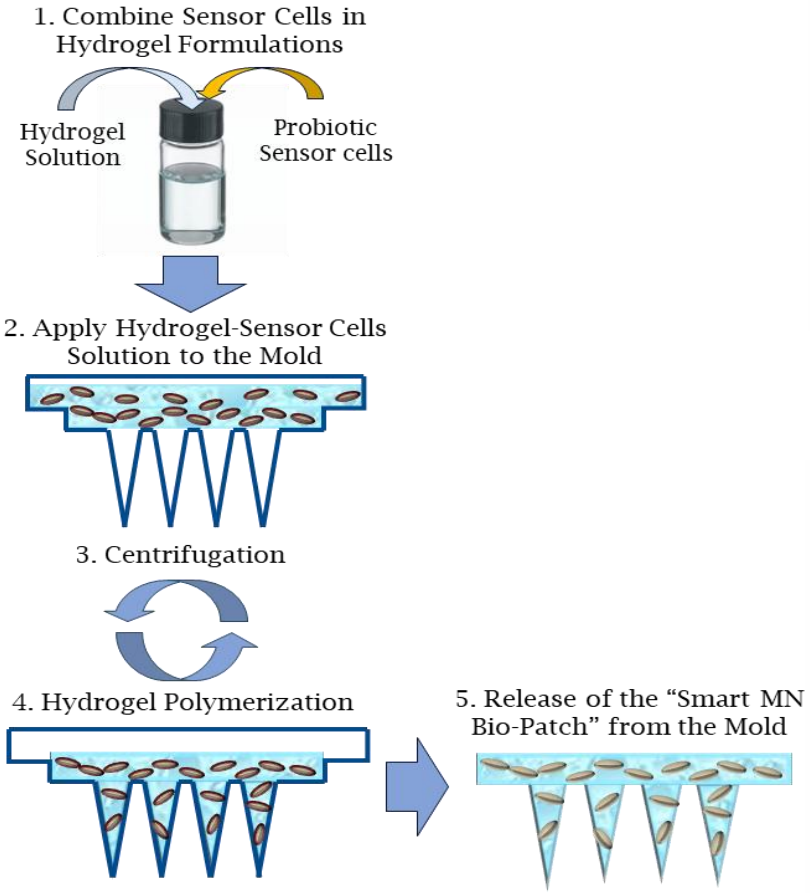
Detection of proteins released into medium

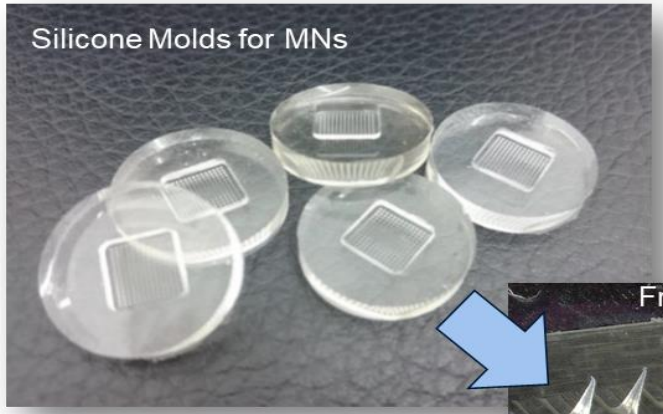


Supernatants from lactate-induced engineered *E. coli* inhibited *E. coli* DH5α growth in a dose-dependent manner.

Wearable Platform: *Hydrogel-Based Microneedle Bio-Patch*

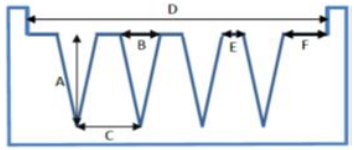
Protocol for creating microneedle (MN) bio-patch:
hyaluronic acid (HA)-polyethylene glycol (PEG) crosslinked hydrogel

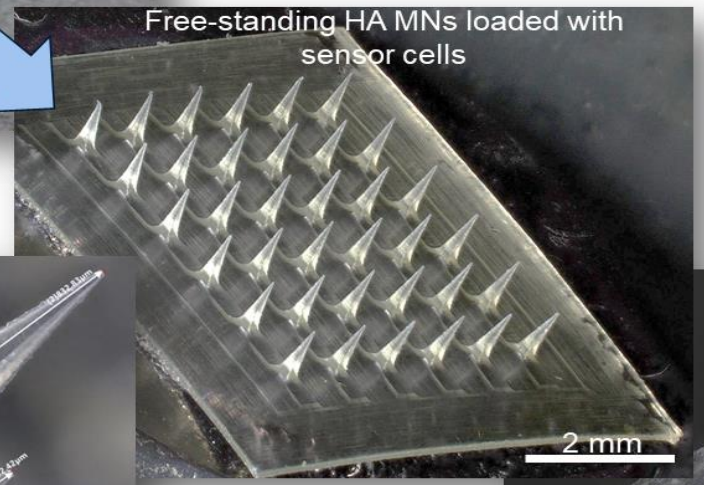


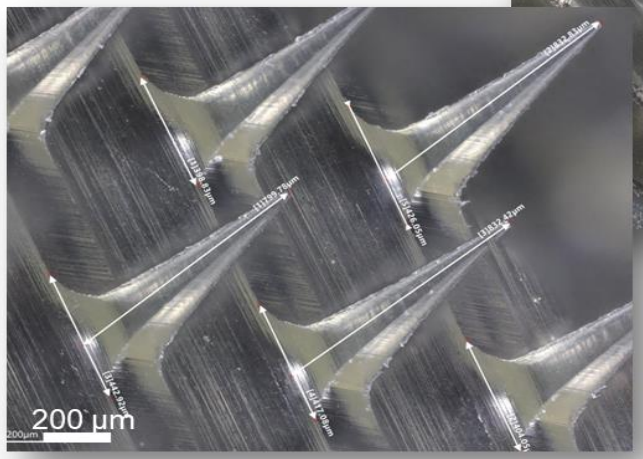


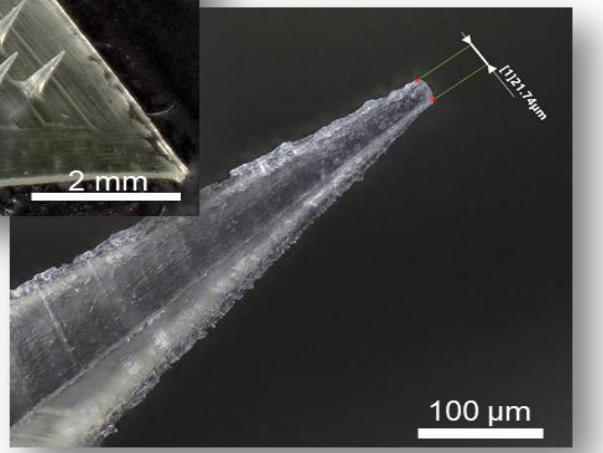
Silicone Template with 6 x 6 microneedle array:

- Patch size: 10 mm x 10 mm
- Needle height (A): 1 mm,
- Needle base (B) 250 um,
- Needle pitch (C): 1 mm,





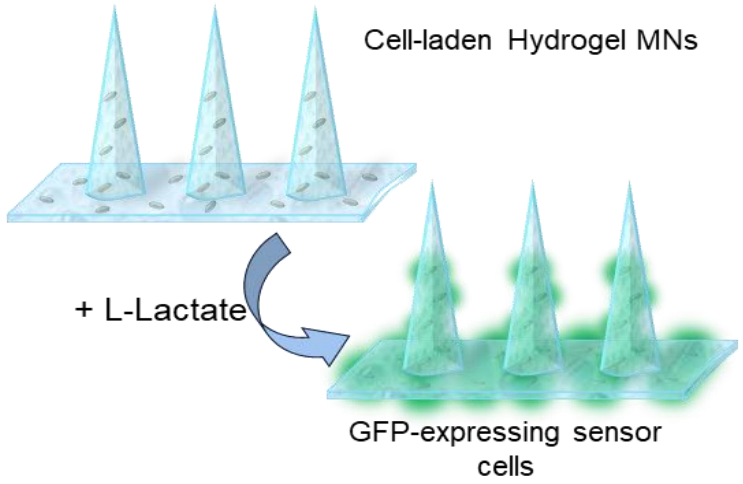




Fabricated uniform hydrogel-based microneedles with optimized pore size and swelling properties.

Hydrogel-Based Microneedle Bio-Patch: *Activation of Lactate Biosensor*

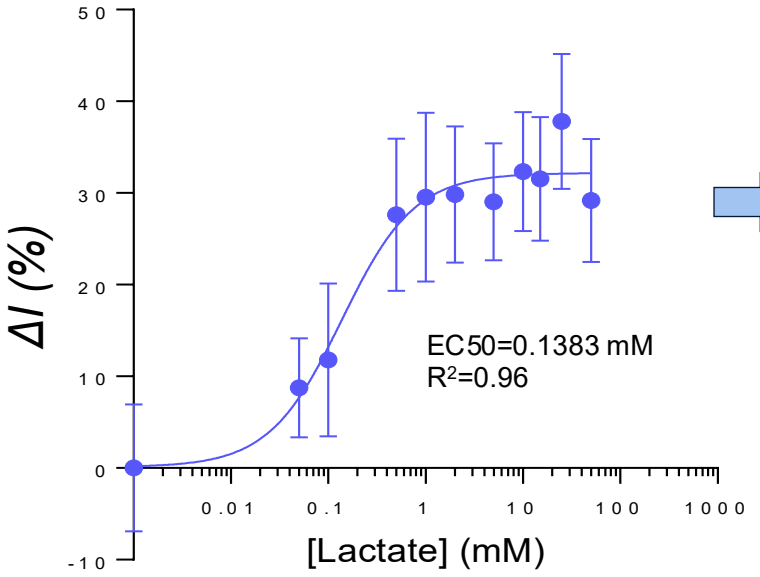
Response of engineered *E. coli* Nissle 1917 cells encapsulated in HA-PEG hydrogel microneedles.



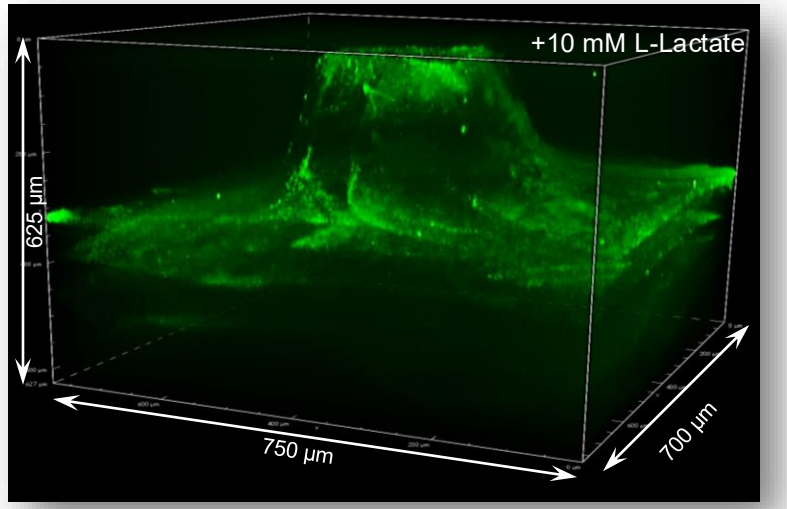
Conditions for encapsulation and stimulation:

- Cells loadings: 15-20% of the total hydrogel weight
- Incubation: 37°C, for 24 h
- Ex=440nm/Em=510nm
- Dose response was calculated based on the end point data after 24 h

Dose-response of *E.coli* Nissle encapsulated in HA-PEG hydrogel



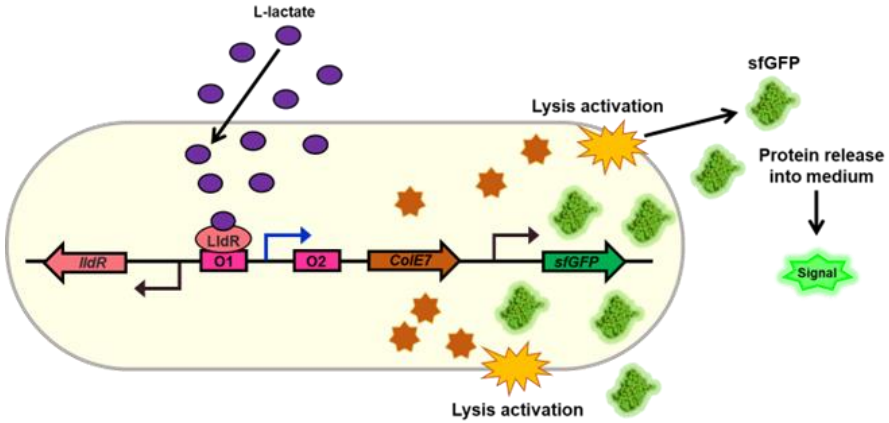
Confocal image of a GFP expressing cell-laden hydrogel microneedle after stimulation with L-lactate



Biosensor is active in hydrogel-based microneedles upon stimulation with lactate.

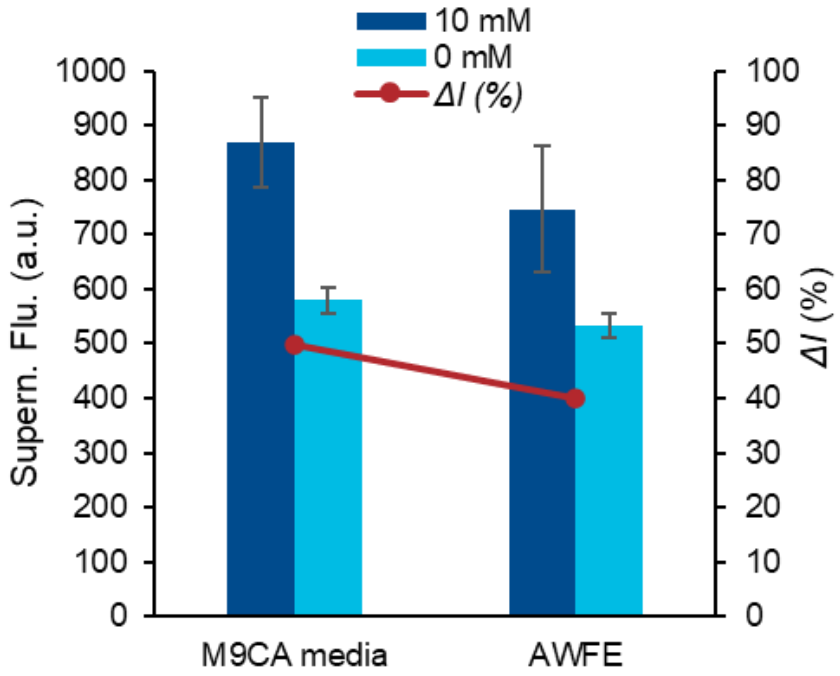
Hydrogel-Based Microneedle Bio-Patch: *Protein Release*

Bio-patches were stimulated with L-lactate in media or artificial wound fluid exudate (AWFE).

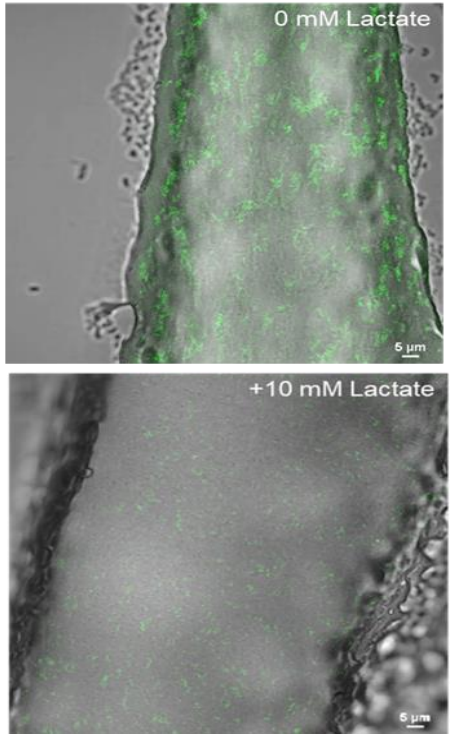


Hydrogel microneedles

sfGFP fluorescence recovered from supernatant



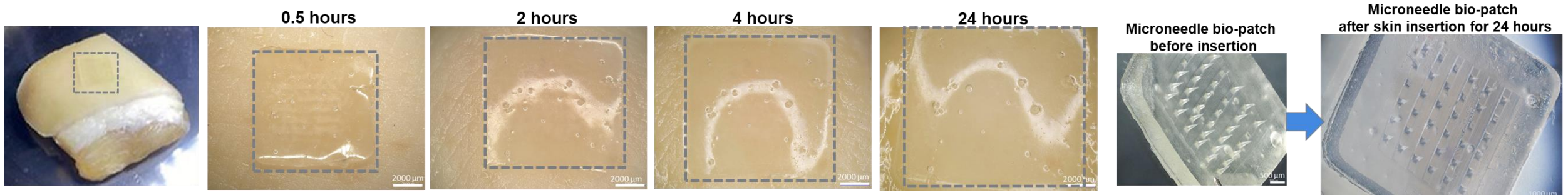
Bio-patch stimulated with 10 mM lactate in media



An increase in fluorescence of supernatants collected from bio-patches was observed in the presence of lactate, due to the release of sfGFP.

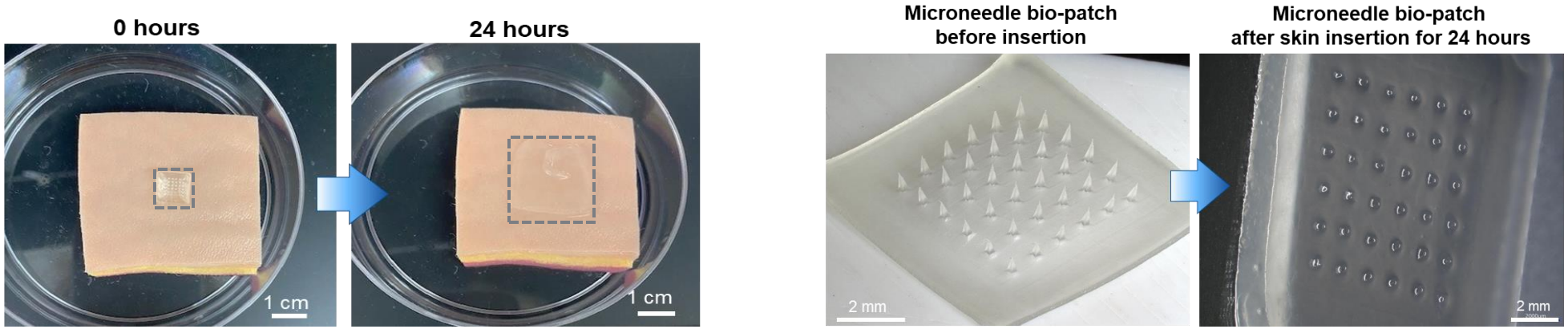
In Vitro Evaluation of the Bio-Patch Design: *Insertion Studies*

Microneedle bio-patch was inserted into porcine skin infused with lactate in artificial interstitial fluid (ISF).



Swelling of the bio-patch after 0.5 hours contact with the skin allows for a ***passive extraction*** of the biomarkers in ISF.

Microneedle bio-patch was inserted into synthetic skin tissue (SynDaver) infused with lactate in artificial ISF.



Bio-patch absorbed ISF and increased in mass by 4-5 folds.

The microneedle bio-patch can penetrate the skin; it is stable for more than 24 hours – does not pill; it is robust after removal from the skin tissue.

Summary

- ❑ Engineered closed-loop cell-based biosensor with sense-and-response mechanism: lactate sensing and production of antimicrobial peptide, Cecropin A.

- ❑ Developed and characterized the hyaluronic acid-based hydrogel microneedle bio-patch:
  - *Bio-patch composition:*
    - hyaluronic acid (HA) - base biopolymer (15-17% w/v), crosslinking density and length of the cross-linking agent - 5-10% w/v
    - cells loading - 5-15% v/v, cells metabolic stage - early exponential phase
  - *Swelling:*
    - between 4-15 folds depending on hydrogel composition
  - *Shelf life:*
    - up to 3.5 mon. without loosing their function
  - *Cells leakage from the hydrogel patch:*
    - not observed (fresh or 3.5 mon. old)
  - *Skin penetration:*
    - can penetrate porcine and artificial skin
    - stable for more than 24 hours
    - robust after removal

- ❑ Demonstrated development of a hydrogel-based microneedle bio-patch with engineered probiotic bacteria to sense bacterial infection biomarker, lactate.

- ❑ Demonstrated lactate-dependent release of a recombinant protein, produced in *E. coli* Nissle cells, from the bio-patch.

Acknowledgments

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Jorge Chávez

Irina Drachuk

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QUESTIONS?