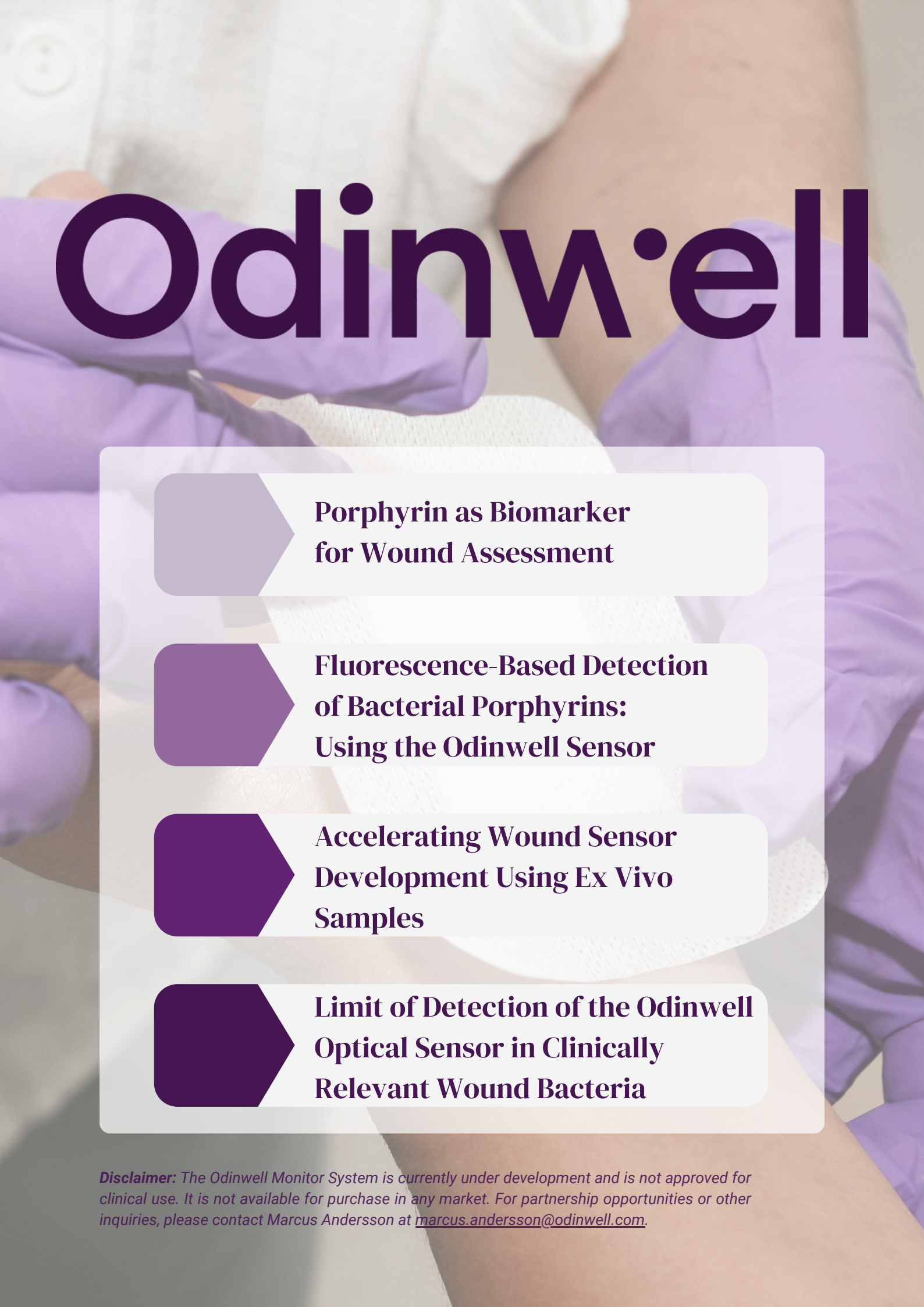

Odinwell

Porphyrin as Biomarker for Wound Assessment

Fluorescence-Based Detection of Bacterial Porphyrins: Using the Odinwell Sensor

Accelerating Wound Sensor Development Using Ex Vivo Samples

Limit of Detection of the Odinwell Optical Sensor in Clinically Relevant Wound Bacteria

Disclaimer: *The Odinwell Monitor System is currently under development and is not approved for clinical use. It is not available for purchase in any market. For partnership opportunities or other inquiries, please contact Marcus Andersson at marcus.andersson@odinwell.com.*

Porphyrin as Biomarker for Wound Assessment

Bacterial Fluorescence: Molecular Basis and Emerging Clinical Applications

Fluorescence is a photophysical phenomenon in which a molecule absorbs light at a specific wavelength and subsequently emits light at a longer wavelength. Many bacterial species produce fluorescent compounds endogenously, with porphyrins being the most prevalent, followed by molecules such as pyoverdine. Porphyrins are cyclic tetrapyrrole structures that play essential roles in bacterial biological functions, including oxygen transport [1]. These compounds exhibit fluorescence when excited by violet light, typically emitting a characteristic red fluorescence.

Although the photophysical basis of bacterial fluorescence has long been observed in laboratory settings [2], its translation into clinical practice is a more recent development. Fluorescence is increasingly being harnessed in the growing field of diagnostic innovation, as such in wound care [3].

Clinical Application of Bacterial Fluorescence

In wound care, fluorescence imaging has emerged as a powerful tool for real-time assessment of bacterial burden. Devices such as the MolecuLight i:X™ and Reveal® FC allow clinicians to visualize bacterial presence directly on the wound surface, guiding decisions around cleaning, debridement, and antimicrobial use, improving treatment outcomes [3, 4].

Most bacteria commonly found in wounds produce porphyrins. Given that infected wounds typically harbour polymicrobial communities, porphyrins serve as a robust and clinically relevant biomarker for bacterial colonization [5]. Empirical studies have demonstrated that fluorescence imaging can detect bacterial loads exceeding clinically significant thresholds (i.e., ≥10^4 CFU/g) [3, 5, 6], even in wounds that appear visually clean. This technology has shown particular utility in the management of chronic wounds [6].

Odinwell Sensor System: A Novel Approach to Wound Assessment

While devices such as MolecuLight i:X™ and Reveal FC are classified as point-of-care tools the Odinwell Sensor system operates on a fundamentally different principle. Rather than relying on fluorescence imaging, it utilizes spectrometric data to provide continuous, quantitative insights into the wound environment.

This approach offers several *key advantages:*

- **Miniaturized, Continuous Monitoring:** Odinwell's compact sensor technology can be integrated directly into or onto wound dressings, enabling continuous monitoring over time. As a connected device, it transmits data remotely to healthcare professionals, allowing for off-site assessment and timely intervention between clinical visits. This capability introduces new opportunities for proactive and personalized wound care.
- **Quantitative Bacterial Analysis**: Unlike imaging-based systems, Odinwell provides numerical data that can be used to quantify bacterial load, estimate growth rates, and, to some extent, infer bacterial species present. These metrics are critical for understanding wound progression and tailoring treatment strategies.
- **Data-Driven Insights:** The generation of structured, longitudinal data opens the door to advanced analytics. In the future, this could support predictive modeling, early detection of complications, and optimized treatment pathways through modeling and AI-driven decision support.

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Fluorescence-Based Detection of Bacterial Porphyrins: Using the Odinwell Sensor

Background and Objective

The early identification of bacterial colonization in wounds is critical for preventing infection-related complications and optimizing treatment strategies. Certain bacterial species are capable of metabolizing 5-aminolevulinic acid (ALA) into porphyrins—intermediates in the heme biosynthesis pathway—which exhibit characteristic red fluorescence when excited by blue-violet light (approximately 405 nm). This biochemical property provides a potential diagnostic marker for bacterial presence, enabling non-invasive detection through bacterial autofluorescence [1,2].

The objective of the study was to evaluate the ability of the Odinwell sensor to detect porphyrin fluorescence produced by clinically relevant bacterial species following exposure to ALA, under conditions simulating wound environments.

Methodology

Three bacterial species commonly associated with wound infections—*Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli*—were cultured on ALA-supplemented substrates. Incubation was carried out at 34–37 °C for 2, 3.5, and 5 hours. A mixed-species sample was also included [3].

The experimental protocol was adapted from established porphyrin detection methods, including the porphyrin production test described by Lund and Blazevic [4] and the differentiation method developed by Kilian [5].

Fluorescence measurements were performed through wound dressing film using the prototype sensor. The system employed a 405 ± 5 nm excitation source, and emission spectra were recorded across the 300–900 nm range, with emphasis on the 600–700 nm interval where porphyrin-associated fluorescence is expected. Each sample was measured at the point of maximum signal intensity.

Results

- All three bacterial species produced detectable fluorescence within 2 hours of incubation, see Figure 1.
- Fluorescence intensity varied spatially, reflecting heterogeneity in bacterial distribution.
- *S. aureus* exhibited a distinct spectral profile compared to the Gram-negative species, suggesting potential for taxonomic discrimination, see Figure 2.
- The mixed-species sample also yielded measurable fluorescence, indicating the sensor's applicability in polymicrobial conditions.

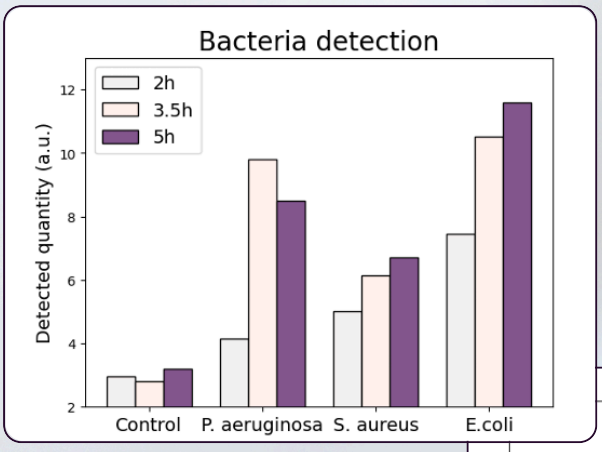
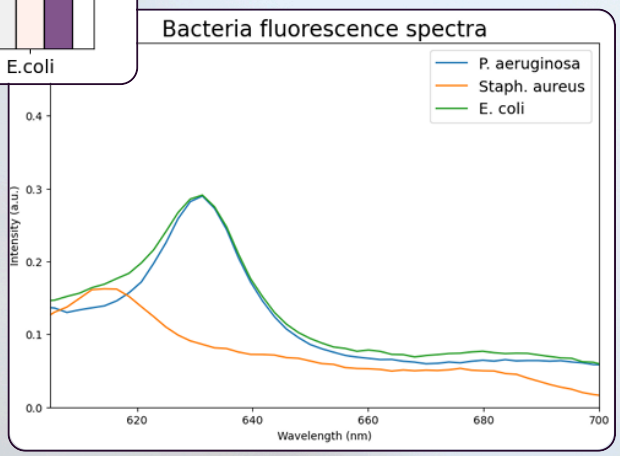


Figure 1

Displays the fluorescence levels of three wound-associated bacterial species, *P. aeruginosa, S. aureus, and E. coli,* alongside a control sample after 2, 3.5, and 5 hours of incubation.

Figure 2

Illustrates the spectral profiles of three distinct bacterial species, highlighting clear differences in peak wavelengths between Gram-positive (*S.aureus*) and Gram-negative (*E.coli & P. aerginosa*) bacteria.



Conclusion

These findings provide preliminary validation of the sensor's capability to detect porphyrin fluorescence from wound-associated bacteria. The results support the potential of this technology for early, non-invasive detection of bacterial colonization in wounds. Furthermore, the observed spectral differences between bacterial species may offer opportunities for species-level discrimination in future clinical applications.

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Accelerating Wound Sensor Development Using Ex Vivo Samples

Introduction

Smart wound dressings offer a promising step toward personalized care by enabling real-time monitoring and early infection detection [1]. However, introducing new technologies into clinical practice poses technical and regulatory challenges, including biocompatibility, sterility, and data transmission, which hinder clinical adoption. Clinical trials are also time-consuming and resource-intensive, especially in early development.

To overcome these barriers, this study evaluates used wound dressings as a non-invasive platform for sensor testing. By analyzing retained exudate, we assess the sensor's ability to detect clinically relevant markers without direct patient involvement, an approach that accelerates validation and design optimization before clinical use.

Here, we tested Odinwell's fluorescence-based sensor on both the wound-facing and external sides of dressings.

Methodology

Used wound dressings were obtained with the patient's informed consent via a biological sample broker (SmartSample, UK) after ethical approvals were received from both the collection country (ISTANBUL MEDIPOL UNIVERSITY: E-10741096-291.3.01-6781) and in the country where the tests were conducted (Swedish Ethical Review Authority Dnr 2025-01863-01). The used wound dressings were from patients with ulcers that had clear signs of infection. The samples were frozen to -80˚C within 20 minutes from harvesting and shipped to a laboratory for storage and testing (Industridoktorn®, Sweden). After identification of fluorescent areas, using a UV flashlight in combination with laser safety spectacles that block the UV light while transmitting red light, the Odinwell sensor system was used to spectrophotometrically analyze the emitted light using a 405 nm light source as excitation for the porphyrin and pyoverdine biomarkers. Data was acquired for different types of sensors to compare their performance, and repeated measures were taken for each sample and each sensor. The spectral data was then analyzed using Odinwell's developed algorithm to receive numerical data.

Results

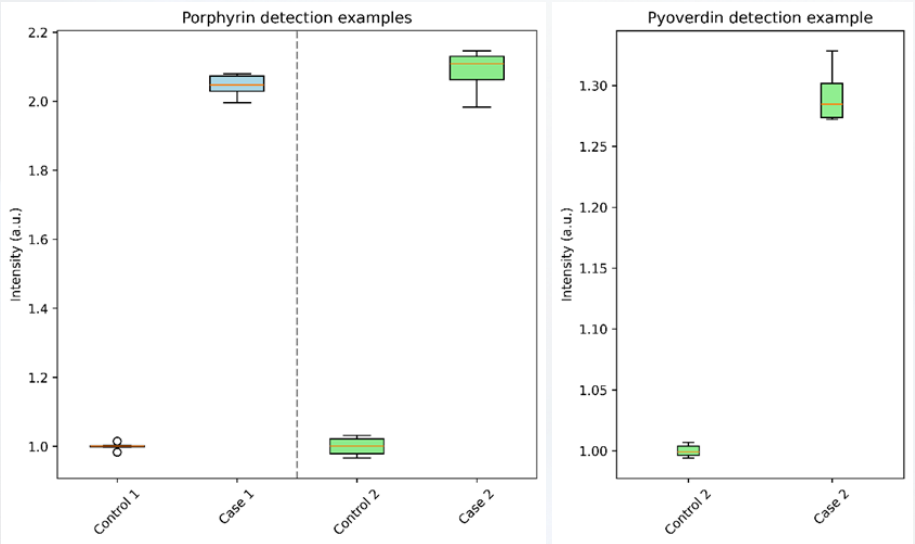

Figure 1 & 2 *present example data from two wound dressings that exhibited bacterial autofluorescence. The intensity corresponding to porphyrin and pyoverdine fluorescence was compared to the intensity of unused sterile dressings (Control) acquired during the same measurement session. Each box in the plot represents repeated measurements from one dressing using one sensor type, illustrating variability and signal strength across samples.*

Discussion and Future Outlook

In this work, we demonstrated that Odinwell's system is capable of detecting multiple biomarkers associated with bacterial presence directly from used wound dressings. Spectral data can provide a detailed insight into the type of bacteria, such as differentiating between types of porphyrin and pyoverdine, opening new possibilities for assisted wound assessment. For example, coproporphyrin III is associated with biofilm formation [2,3], and pyoverdine can help assess the virulence of *P. aeruginosa* in infected wounds, especially where biofilm formation is a concern [4].

The testing method is still being refined, and these results are preliminary. Not all samples showed fluorescence under the torch inspection; however, when fluorescence was visible during the inspection, it was also detectable by the sensor. A key area for improvement is the handling and processing of used dressings to preserve wound exudate integrity and ensure sensor readings reflect the in vivo environment.

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Limit of Detection of the Odinwell Optical Sensor in Clinically Relevant Wound Bacteria

Background

Early detection of bacterial activity in wounds is essential for preventing infection-related complications, yet conventional diagnostic methods typically identify infection only after bacterial loads become clinically significant [1]. Odinwell's optical sensor is designed to capture bacterial autofluorescence signals directly within wound-like environments, potentially enabling earlier identification of bacterial proliferation than current clinical benchmarks.

The objective of this study was to determine the sensor's limit of detection for porphyrin-producing bacteria typically found in chronic wounds, thereby assessing its sensitivity and evaluating its potential role in advancing early infection monitoring.

Materials & Methods

Bacterial colonies of *E. coli* and *S. aureus* were inoculated into Lysogeny broth and Tryptose Phosphate broth respectively, and both were supplemented with 1 mM 5-aminolevulinic acid and incubated at 37 °C for 24 hours under shaking conditions. Serial dilutions of each culture were prepared in Phosphate Buffered Saline, and aliquots were applied onto 4 cm² filter papers in triplicates. Fluorescence measurements were obtained using the Odinwell sensor. For each dilution series, colony-forming unit (CFU) counts were determined by plating on LB agar. CFU/cm² values were calculated based on the quantified colonies and the corresponding filter paper area. Each dilution was compared with the bacteria-free negative control using a one–tailed Mann–Whitney U test, starting from the highest concentration and proceeding stepwise to lower concentrations. The smallest dilution showing a statistically significant increase in fluorescence was identified, and p-values were Bonferroni-corrected for multiple comparisons.

Results

For both *S. aureus* and *E. coli*, fluorescence was detectable down to approximately 10^3 CFU/cm², which therefore represents the lower limit of detection for the sensor across the tested species. As shown in Figure 1, the sensor response increased in intensity with increasing bacterial surface concentration. The lowest detectable concentration significantly higher than the bacteria-free negative control was 1400 CFU/cm² ($P_{Bonf} < 0.0001$).

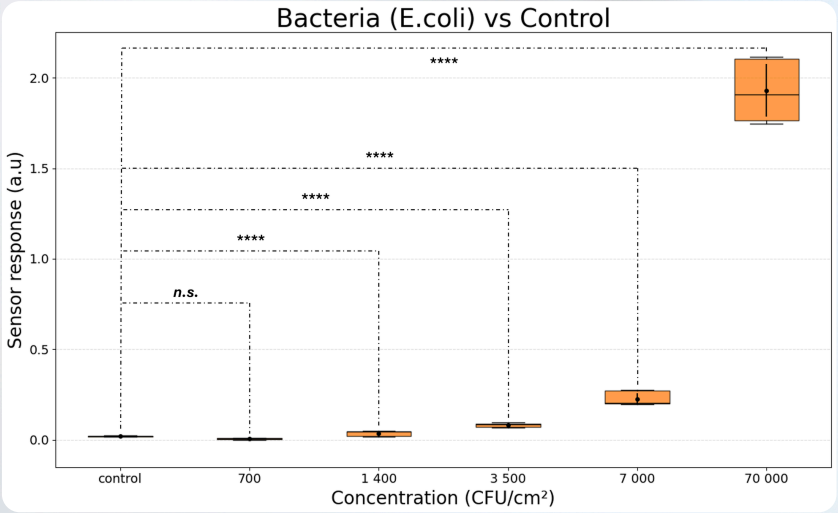

Figure 1. A representative graph of sensor response versus bacterial surface concentration in CFU/cm² for E. Coli.

Discussion

The Odinwell sensor successfully detected porphyrin in tested bacterial species, achieving a detection limit of approximately 10^3 CFU/cm². As the measurements in this experiment were obtained through surface-level detection, it is appropriate to compare them to the Levine technique, which has a diagnostic infection cutoff of 10^5 CFU/cm² [2-3]. Swab-based method remains the most clinically used technique for quantifying bacterial burden in wounds, due to its cost-effectiveness compared to tissue biopsy [4]; however, they still require skilled personnel, financial and logistical resources, and results are obtained only after 24-72 hours. In contrast, the Odinwell sensor provides instantaneous results without swabbing or sample handling required.

Conclusion

These results indicate that the Odinwell sensor can detect bacterial loads even earlier than the established clinical infection threshold, offering a sensitive, instantaneous, and non-invasive approach for early surface-level detection. This capability suggests strong potential for the sensor to complement existing diagnostic methods and support earlier clinical decision-making in wound management.

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