FLEXISENSE: PH-GUIDED PRECISION DRUG DELIVERY AND MONITORING USING FLEXIBLE ELECTRONIC TECHNOLOGY

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ABSTRACT:

The growing prevalence of bacterial chronic wounds impacts approximately 0.5% of the total population in the United States, exerting a considerable economic strain on healthcare systems due to the management requirements. However, one main complication associated with such wounds are the heterogeneous nature of the bacterial population within the surface. Improper and rampant use of antibiotics in such wound bed could potentially lead to the development of antibiotic resistance and further complicate the treatment process. To address this need, we have investigated the use of pH responsive polymers to selectively deliver the antibiotics to the specific locations of the wound. Increase in the bacterial population accompanied by the pH change triggers the delivery of the antibiotics into the selective area of the wound bed. Additionally, integration of a closed loop feedback system with the patch allows continuous monitoring of the drug release kinetics within the wound area. Overall, the current technology provides a seamless way of personalized medicine to deliver antibiotics in accordance with the individuals requirements and monitor the drug release kinetics within the wound bed.

KEYWORDS:

Wound care, pH sensitive polymer, Feedback system, printed flexible electronics.

INTRODUCTION:

Chronic non-healing wounds, affecting approximately 8.2 million people in the United States alone, constitute a significant healthcare challenge in resent time. These wounds typically arise from extensive skin lacerations, often post-surgical, yet persist due to underlying factors such as diabetes, immunocompromised states, or advanced age. The annual cost of such wound treatment is about $31.7 billion from Medicare beneficiaries alone, with surgical infections being the most prevalent.[1] With bacterially infected chronic non healing wounds being the most prevalent, a primary complication associated with such wounds is their considerable heterogeneity over the infected wound surface.

Traditional chronic wound treatments include debridement, wound dressings, and compression bandages to protect the wound from the development of an infection. However, upon infection, antibiotics are considered as the first line of defence in treating such wounds, offering localized and targeted therapy to eradicate bacterial pathogens. Despite their cost effectiveness, application of antibiotics into the unnecessary regions of the wound bed can trigger the development antibiotic resistant bacterial strains which further complicates the treatment process. Additionally, it is also improbable to account for the bacterial heterogeneity and selectively administer drugs during the direct topical administration into the wound bed. Various real time monitoring sensors and metrics have been developed to provide objective evaluation of their own status and controlled forms of antibiotic delivery with microneedles or slow releasing pumps. However, such pumping systems are expensive, and need critical attention from a technician to ensure proper working of the sensor.

In this scenario, monitoring changes in wound pH as a marker to detect the onset of infection has gained significant popularity. [2,3] As a wound becomes infected, continuous release of proteases during the inflammatory stage and proliferation of bacteria cause an increase in pH above 7 [4]. Thus, many designs have been geared towards the detection of increases in pH in wound healing applications. In a work by Rahimi *et. al.* a wearable potentiometric pH sensor was manufactured using a stretchable substrate and conductive ink[5]. Though these techniques are effective to monitor the wound progression, it does not provide a targeted way of delivering antimicrobial as a feedback mechanism.

To address this need, the pH responsive systems are experimented to deliver antibiotics into specific locations of the wound bed. A “smart” pH-responsive hydrogel will swell or compress as pH-changes, which results in the delivery of therapeutics such as antibiotics into the wound bed[6]. Although these designs are simple and capable of delivering therapeutics based on changes in pH, synthesis of drug carriers is typically a complex process[7]. An effective alternative route to circumvent this complex process is to use pH-sensitive acrylic copolymers that dissolves at particular pH thereby releasing antimicrobials to the wound bed. These polymers offer several advantages over a hydrogel-based delivery approach, including easier processability using common solvents, high stability, and improved bonding to substrates, all of which are important when considering sensor design.

Hence, this work reports a multifunctional impedimetric multisensor array capable of monitoring local changes in the wound and triggering the release of therapeutics. The multisensor array was manufactured through scalable processes, including screen-printing and automated dispensing onto a flexible PET substrate. As a proof-of-concept, four interdigitated electrodes (IDEs) arranged in a 2x2 array were developed using facile screen-printing technology to monitor four separate regions within the same wound environment. These IDEs were coated with an antibiotic-infused pH-sensitive polymer to facilitate stimulus-responsive release of antibiotics. The sensing system comprises the flexible, disposable multisensor array and a solid electronic impedance analysing system integrated with a wireless communication chip for ease of communication as shown in **Figure 1**. The array can be incorporated as a patch into traditional

978-1-940470-05-4/HH2024/$25©2024TRF
DOI 10.31438/TRF.HH2024.35

wound dressings for continuous monitoring of wound health. As local sites of infection occur, the dissolution of the pH-sensitive coating is captured through the impedance analysing unit, while the therapeutic is released at the specific site of infection. This system thus detects the onset of infection while also delivering an antibiotic agent capable of preventing further proliferation of bacteria. Through this wireless passive system, clinicians can monitor the status of multiple patients simultaneously, identifying infections before they spread, while avoiding the overuse of antibiotics, which can lead to antibiotic resistance.

EXPERIMENTAL SECTION:

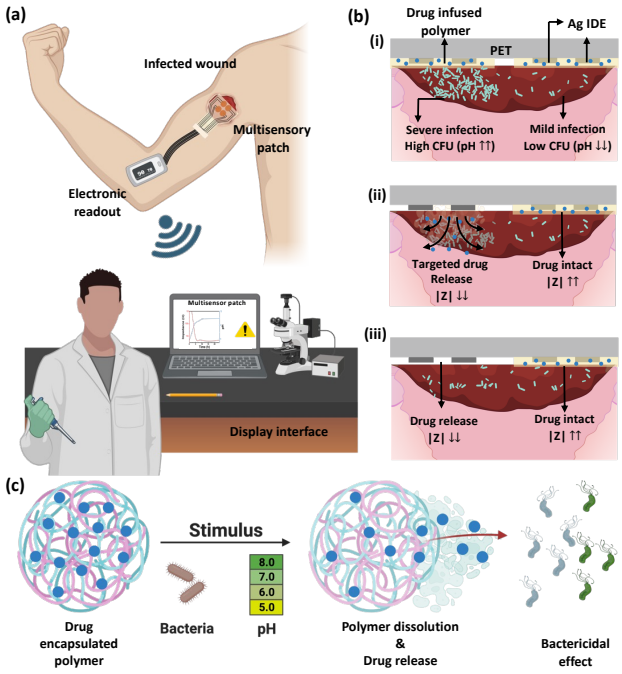

Figure 1. (a) Overall thematic idea of the FlexiSense patch, (b) targeted release of the antibiotics to selected region of the wound, and (d) drug release kinetics analysis.

The polyethylene terephthalate (PET) substrate used for the development of the printed electronic traces were purchased from McMaster-Carr (IL, USA). A sample of Eudragit® FS30D copolymer was kindly donated by Evonik Industries Ag® (Essen, Germany). Clear pH buffers (5, 6, 7, 8, and 9), Clindamycin HCl, Tryptic soy broth (TSB), and agar powders were purchased from Sigma Aldrich® (MO, USA). Crystal violet, Dulbecco Modified Eagle Medium (DMEM), and Fetal Bovine Serum (FBS) were purchased from ThermoFisher Scientific™ (MA, USA). PET sheets used for the development of the printed electronic traces were purchased from McMaster-Carr (IL, USA).

Electronics. Arduino WiFi 1010, 8:2 Multiplexer, AD5933 impedance analyzer, DS3231 Real Time Clock board, and all electrical components were purchased from Digikey.

Electrode fabrication process. Electrode patters for the flexible patch was initially designed in CAD software and developed into a Ni screen. **Figure 2a**. shows the manufacturing procedure for the multimodal smart sensing patch. Each array is printed onto a PET substrate through standard screen-printing procedures using a commercial silver ink. The PET surface was initially plasma treated for 2 mins before starting the screen-printing process to aid proper adhesion. After drying the surface at room temperature for 4 h, each IDE is coated with a 120 µm film through a facile drop-casting technique. In this work, a polymeric composite of Eudragit FS30D (dissolves at a pH > 6.8) and clindamycin HCl, a broad-spectrum antibiotic which is known to be effective in treating most bacterial strains was coated onto the IDE electrodes. Finally, the rest of the sensor area is passivated using a biocompatible silicone-based flexible layer. Additionally, the reader module used for the impedance measurement is shown in **Figure 2b**. The final printed electrode and the entire assembled model of the FlexiSense patch is shown in **Figure 2c-d**.

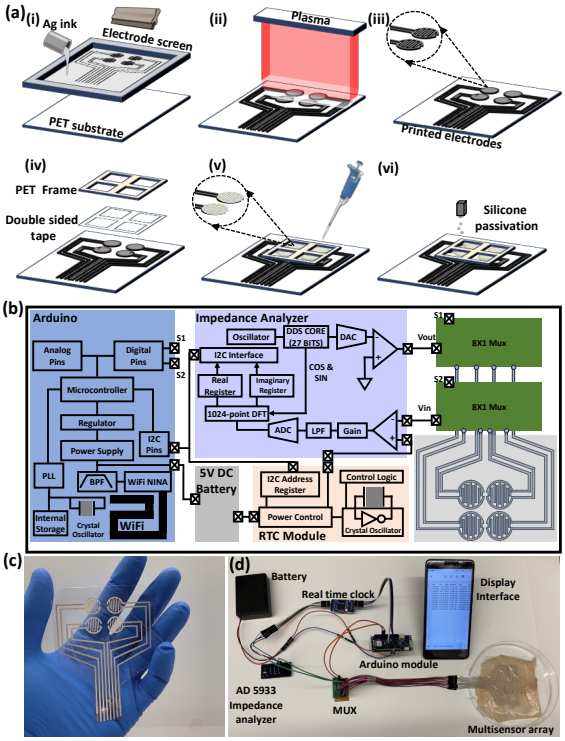

Figure 1. (a) Fabrication process of the Ag/AgCl electronic patch development, (b) the electronic readout module, (c) fabricated electrode patch, and (d) assembled real time detection module.

Dissolution characterization was performed with the developed patch by exposing polymer crystal violet dye composite to various pH buffer solutions from pH 5- 9. Drug release kinetics and the polymer dissolution kinetics were monitored by measuring the absorbance and the change in electrical impedance of the patch respectively. The analysis was performed for a time period of 24 h with measurements performed at the time points of 0, 1, 3, 6, 12 and 24 h.

Antibacterial activity of the developed patches was analysed by exposing the polymeric composite against two common pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Overnight culture of the bacterial populations was washed and resuspended at different pH buffer solutions (pH 5-9). Next, the polymeric composite was exposed to the buffer solutions followed by sampling of the bacterial solution for a time period of 24 h. The obtained bacterial solutions were serially diluted and plated in TSB pates to obtain the absolute bacterial counts. Biocompatibility assessment of the developed composite patch and the manufacturing process was confirmed by exposing the components to NIH/3T3 fibroblast cells. The cell viability and the fluorescent cell imaging tests were performed to validate the biocompatibility of the *FlexiSense* patch. Finally, the developed patch system was validated by artificially creating a bacterially infected wound in an *ex-vivo* porcine model and applying the patch system. For this test, a superficial would was carefully developed using a sterile surgical scalpel and adding 8-log CFU of *P. aeruginosa* infection. The change in the bacterial population was analysed by sampling the section of the wound using a sterile biopsy punch while continuously monitoring the impedance.

RESULTS:

The dissolution of the FS30D coating in different pH buffers was validated through two methods, as depicted in **Figure 3a**. Top row shows five vials containing buffers of increasing pH, from left to right pH 5, 6, 7, 8, and 9, at time t=0, 3 and 24 h. No dissolution of the polymeric composite coated on the IDE electrode was observed at time t=0 h. On the other hand, no dissolution of the polymer was observed inside the vial with pH buffer of 5 and 6 at t=3 and 24 h. In contrary, buffers of pH 7 or greater, observed a clear dissolution of the polymeric composite and thereby release of the dye was observed. **Figure 3b-c.** shows quantitative results for impedance of the electrodes and polymer dissolution exposed to different pH buffer solutions. As expected, the pH 5 and 6 (below the threshold) showed minimum reduction in the impedance profile with a maximum release of 8% and 12% respectively, followed by a plateau. On the other hand, in solutions above the threshold, 100% dissolution of the polymeric surface with a 3-fold reduction in the impedance characteristics. Here, the stability below the solubility threshold is clear as there is no significant difference in dissolution percentage between early and prolonged exposure to the buffer. Above the solubility threshold, FS30D presents rapid dissolution and load release accompanied with a clear decrease in the impedance characterises.

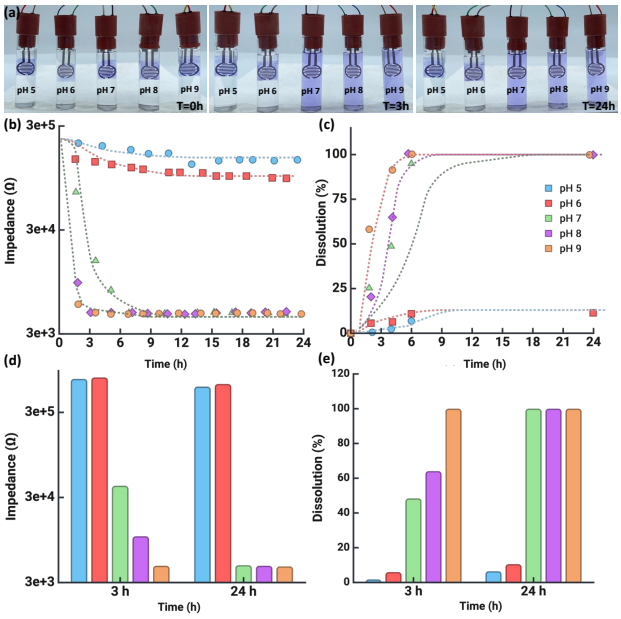

Figure 3. (a) Time lapse images of the polymeric dissolution of the polymeric composite exposed to different pH buffers, (b) shows the continuous impedance monitoring data with (d) summary and (c) the % dissolution overtime and (e) dissolution summary.

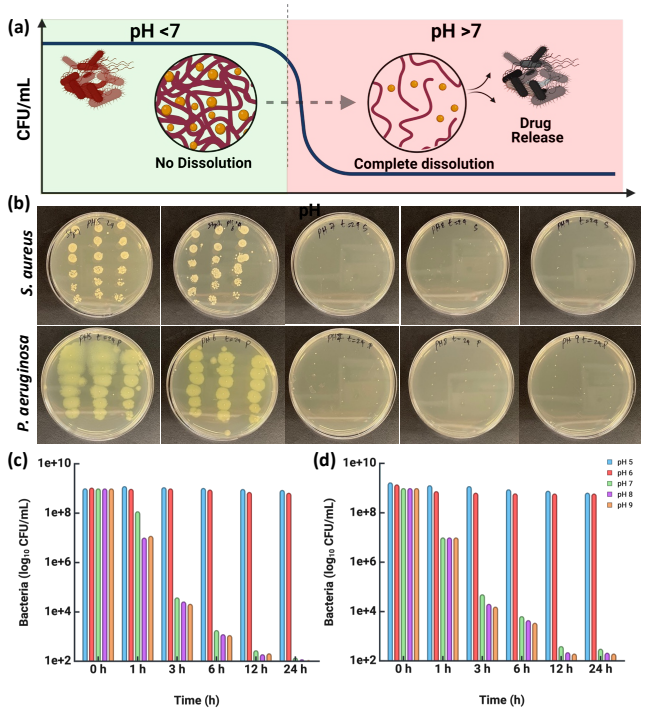

Figure 4. (a) Time lapse images of the polymeric dissolution of the polymeric composite exposed to different pH buffers, (b) shows the continuous impedance monitoring data with (d) summary and (c) the % dissolution overtime and (e) dissolution summary.

Antibacterial activity of the developed patches tested against 9-logs of two common skin bacterial pathogens namely *P. aeruginosa* and *S. aureus* (**Figure 4**). The overall bacterial concentrations were maintained at 9-log CFU/mL in the buffer solution at the initial time before starting the experiment. After 3h, a clear 4-log reduction in the bacterial colonies were observed with the pH buffers of 7, 8, and 9. On the other hand, the lower buffer solutions did not show any significant change in the bacterial populations. The reduction trend was observed to be continued until time 24 h while the pH 5 and 6 remained undeterred. These results clearly validate the antibacterial properties of the polymer composite patch to eradicate the bacterial populations with increase in pH.

Next, the biocompatibility of the different components of the

wound patch and the manufacturing process was validated against NIH/3T3 cells over 7 days, through cell viability and live/dead staining analysis. **Figure 5a.** shows viability percentages for each sample on day 1, day 3, and day 7, calculated through an MTT cell proliferation assay. Overall, all components possess a high degree of biocompatibility, with cell viability percentages above 80% except to that of the Ag trace. This was probably due to the leaching of the screen-printed silver ions into the cells thereby observing slight increase in toxicity. However, upon plasma treatment, the printed traces were sintered thereby reducing the effect of silver leaching. Additionally, live-dead staining images were performed to visually observe the cell viability in a microscopic fluorescence image of samples on day 1 and day 7 (**Figure 5b**). Based on these images, each component presented a high level of biocompatibility, with live cells proliferating extensively over the surface. It is necessary to note that in this design, there is no expectation for the silver trace to contribute towards the patch's antibacterial functionality. Similarly, there is no intention for the plasma treatment to either enhance any material/antibacterial property other than improving the adhesion strength between the flexible substrate and the FS30D coating. Yet, the plasma treatment indirectly improved the biocompatibility of the silver trace as well as being effective in increasing the adhesion strength. These results confirm a reduced potential for cytotoxic damage to skin epithelial cells as they are exposed to the FS30D coating which dissolves upon bacterial proliferation.

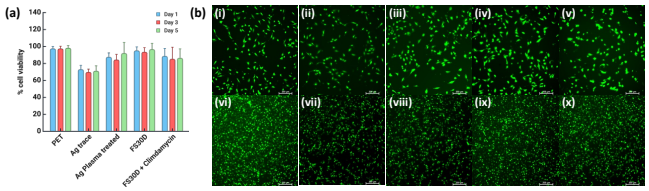

Figure 5. Biocompatibility assessment. (a) Shows the % cell viability of the components and observed using MTT cell proliferation assay and (b) live-dead staining images of (i,vi) PET, (ii,vii) Ag, (iii,viii) Plasma treated Ag, (iv,ix) FS30D, and ((v,x) FS30D+clindamycin at Day1 and Day 7 respectively.

Finally, the developed sensor was tested on an *ex-vivo* porcine model as shown **Figure 6a**. The change in pH was observed using a simple strip test and the impedance was monitored overtime. As observed from Figure 6b-c, a clear decrease in the impedance value and reduction in the bacterial populations were observed with increase in pH triggered by the development of the infection. In summary, the developed *FlexiSense* system exhibits promising potential in revolutionizing wound monitoring and antibiotic delivery, offering a comprehensive solution for effective and personalized wound care.

CONCLUSION:

This work introduces a novel method for real-time wound pH monitoring and delivery of antibiotic using a multifunctional flexible multisensor array patch. The developed patch demonstrated pronounced bactericidal efficacy at elevated pH levels against various bacterial population while maintaining the biocompatibility for safe application. As a proof-of-concept, the patch was tested ex-vivo utilizing pig skin as a surrogate for the wound environment which pronounced effciaiy of the patch with sharp decrease in the bacterial populations within 24h of exposure. On the whole the current technology provides an effective way to selectively deliver

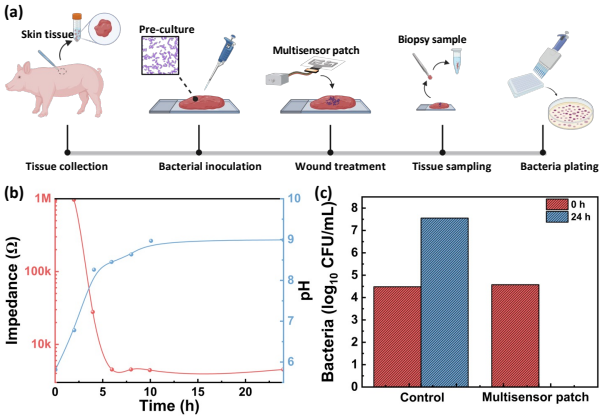

Figure 6. Ex-vivo analysis. (a) Experimental process of ex-vivo analyses, (b) continuous monitoring infected wound patch and pH change and (c) total bacterial count of the wound bed with and without the FlexiSense patch.

antibiotics into the specific regions of the infected wound bed while monitoring the infection status in from the wound bed.

REFERNCES:

[1] S. R. Nussbaum, M. J. Carter, C. E. Fife, J. DaVanzo, R. Haught, M. Nusgart, D. Cartwright, *Value in Health* **2018**, *21*, 27.

[2] P. Mostafalu, A. Tamayol, R. Rahimi, M. Ochoa, A. Khalilpour, G. Kiaee, I. K. Yazdi, S. Bagherifard, M. R. Dokmeci, B. Ziaie, S. R. Sonkusale, A. Khademhosseini, *Small* **2018**, *14*, 1.

[3] R. Rahimi, U. Brener, S. Chittiboyina, T. Soleimani, D. A. Detwiler, S. A. Lelièvre, B. Ziaie, *Sens Actuators B Chem* **2018**, *267*, 198.

[4] L. R. Bennison, C. N. Miller, R. J. Summers, A. M. B. Minnis, G. Sussman, W. McGuiness, *Wound Practice & Research: Journal of the Australian Wound Management Association* **2017**, *25*, 63.

[5] R. Rahimi, M. Ochoa, T. Parupudi, X. Zhao, I. K. Yazdi, M. R. Dokmeci, A. Tamayol, A. Khademhosseini, B. Ziaie, *Sens Actuators B Chem* **2016**, *229*, 609.

[6] H. Jiang, M. Ochoa, J. F. Waimin, R. Rahimi, B. Ziaie, *Lab Chip* **2019**, *19*, 2265.

[7] J. Shan, X. Zhang, B. Kong, Y. Zhu, Z. Gu, L. Ren, Y. Zhao, *Chemical Engineering Journal* **2022**, *444*, 136640.

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