Incorporation of a pH-Sensitive Indicator Into Wound Dressings to Improve Monitoring of Wound Healing Progression

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INTRODUCTION

The pH level of a wound, and its exudate, is a significant indicator of a wound's progression through the healing process. The pH of healthy intact skin is acidic (pH≈4-6), whereas it becomes slightly alkaline (pH≈7.4) from exposure to physiological fluids from the body after skin damage.[1,2,3] As the wound heals, the wound pH shifts toward neutral and eventually becomes acidic again. However, proliferating bacteria within the wound could cause the wound pH to rise to >9 due to their alkaline byproducts.[2,4] Chronic wounds also tend to exhibit high pH levels (pH≈7.15-8.9), potentially from necrotic tissue leading to tissue hypoxia.[3,5]

Previously, clinicians measured the pH of wounds with glass pH microelectrodes. These devices are fragile, time consuming, only provide a single-point measurement, and cannot be integrated into a dressing. New solid-state pH sensors have been developed that could be integrated into a dressing, but they are relatively expensive and require additional hardware and/or software to operate. Recently researchers have begun to explore the use of anthocyanins, which is a class of naturally occurring, pH-sensitive pigments extracted from various fruit and vegetable sources. When exposed to a solution, the pH of the medium can alter the intensity and color of this type of halochromic pigment. Additionally, anthocyanins are inexpensive and the safety of this class of compounds has been well demonstrated through extensive research conducted over the years.

This study explores the use of an anthocyanin based halochromic compound that can be easily and economically incorporated into a wound dressing material to provide clinicians a means to monitor the patient's wound state, all through a simple visual examination of the dressing's color.

MATERIALS & METHODS

Test Articles

In this study, an anthocyanin-based halochromic compound (ABHC) extracted from a natural, plant-based source and processed into a stable powder was incorporated into a medical-grade polyurethane foam used in wound dressings. The PU foam samples were treated by submersing the entire sample in an aqueous dispersion of the ABHC for a brief period of time and subsequently drying them. The ABHC remained bound to the surface of the PU foam through electrostatic interaction and/or hydrogen bonding that occurs between the polymer functional groups and hydroxyl groups present on the anthocyanin molecules. A non-treated PU foam control along with two separate test articles containing different concentrations of the ABHC were evaluated in this study.

Table 1. Test samples examined in this study and a brief description of each sample

Sample	Description
Test article 1	Non-treated PU foam control, no ABHC treatment
Test article 2	PU foam material treated with a low loading level of ABHC additive
Test article 3	PU foam material treated with a high loading level of ABHC additive

pH-Responsive Color Change

The pH-responsiveness of the ABHC treated foam test articles was evaluated by inoculating samples of the test articles with 25 µL of clear pH buffer solutions at 7 discrete physiologically relevant pH values, ranging from 4 to 10. The colorimetric transition that occurred following exposure to each buffer solution was monitored for up to 15 minutes and photographic images were taken of both the inoculated surface of the sample (representing the wound contacting surface) and the non-inoculated, outward facing surface.

Fluid Absorbency

To assess the capacity of the test articles to absorb fluid from moderate to heavily exuding wounds, a protocol based on BS EN 13726-1:2002 was used. In short, a 5 cm x 5 cm sized sample was cut from each test article, measured to determine the sample area (A) and weighed in a dry state (M_0). The test article samples were placed into separate petri dishes and immersed completely in a volume of simulated wound exudate (Test Solution A), prewarmed to 37 ± 1°C, that corresponded to 40 times the mass of the sample being examined. The test article samples in the petri dishes were transferred to an incubator and allowed to soak for 30 ± 1 min at 37 ± 1°C. Each sample was then removed from the test solution and excess fluid was allowed to drip off for 30 ± 5 seconds. The dressing samples were then reweighed (M_t). Absorptive capacity was expressed as the mass of solution absorbed per 100 cm² and was calculated as follows:

$$Absorptive\ Capacity\ \left(\frac{g}{100\ cm^2}\right) = \frac{(M_t - M_0) \times 100}{A}$$

Fluid Retention

In order to evaluate the ability of the different test articles to retain fluid when under compression, a custom test was performed.[6,7] After carrying out the free swell test, each test article sample was placed onto a polypropylene perforated surface, with the patient contacting surface facing downward. A 7 cm x 7 cm Plexiglass compression plate was placed on top of the test article sample to help ensure homogenous pressure distribution over the entire sample. A pressure equivalent to the recommended compressive force used for venous leg ulcer compression bandaging (40 mm Hg) was applied to the test article sample using weights amounting to 1,350 g. After 1 min of compression, the test article sample was reweighed (M_r). Fluid retention capacity was expressed as the mass of solution retained per 100 cm² and was calculated as follows:

$$Fluid\ Retention\ Capacity\ \left(\frac{g}{100\ cm^2}\right) = \frac{(M_r - M_0) \times 100}{A}$$

In addition, the percentage of fluid retention was calculated by comparing the ratio of the fluid held after compression (M_r) to the absorptive capacity (M_t).

$$\%\ Fluid\ Retention\ = \frac{(M_r)}{(M_t)} \times 100\%$$

Conformability

The impact that the ABHC treatment had on the bending conformability (i.e., stiffness) of the treated foam material was determined by evaluating the bending length of each test article. Bending length was determined using a protocol based on the cantilever procedure described in EN 1644-2, Annex E. In short, 25mm x 200mm samples of each test article were cut. A Shirley stiffness tester was used to manually determine the overhang length of these samples, which were measured on the face and back of both ends of the samples for a total of four readings per sample. The overhang length was divided by 2 to calculate the bending length.

RESULTS

Figure 1. Photographic images of samples from each test article examined in this study.

Test Article 1 (Untreated Control)	Test Article 2 (Low ABHC Loading Level)	Test Article 3 (High ABHC Loading Level)
		

Figure 2. Photographic images capturing the colorimetric transition that occurs on test article 2 (PU foam treated with the low ABHC loading level) when exposed to pH values ranging from 4 to 10.

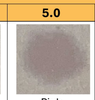
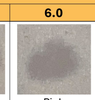
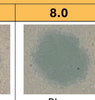
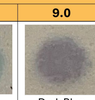
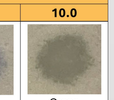
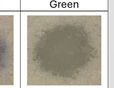
Sample	pH Values						
	4.0	5.0	6.0	7.0	8.0	9.0	10.0
Inoculated Surface "Wound Contacting Surface"	 Pink	 Pink	 Pink	 Light Purple	 Blue	 Dark Blue	 Green
Non-Inoculated Surface "Outward Facing Surface"	 Pink	 Pink	 Pink	 Light Purple	 Blue	 Dark Blue	 Green

Figure 3. Photographic images capturing the colorimetric transition that occurs on test article 3 (PU foam treated with the high ABHC loading level) when exposed to pH values ranging from 4 to 10.

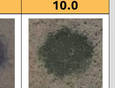
Sample	pH Values						
	4.0	5.0	6.0	7.0	8.0	9.0	10.0
Inoculated Surface "Wound Contacting Surface"	 Pink	 Pink	 Pink	 Purple	 Blue	 Dark Blue	 Dark Green
Non-Inoculated Surface "Outward Facing Surface"	 Pink	 Pink	 Pink	 Purple	 Blue	 Dark Blue	 Dark Green

Table 2. Summary of the average fluid absorbency, percent fluid retention, and conformability values for each test article and the percent change in those variables relative to the untreated control.

Sample	Fluid Absorbency		Fluid Retention		Conformability	
	Average Fluid Absorbency ($g/100cm^2$)	% Change in Fluid Absorbency (Relative to Untreated Control)	Average % Fluid Retention	% Change in % Fluid Retention (Relative to Untreated Control)	Average Bending Length (mm)	% Change in Bending Length (Relative to Untreated Control)
Test Article 1 (Non-treated Control)	25.72	N/A	98.39%	N/A	22.20	N/A
Test Article 2 (Low ABHC Loading Level)	25.49	-0.90%	97.82%	-0.59%	22.67	+2.12%
Test Article 3 (High ABHC Loading Level)	25.41	-1.23%	97.72%	-0.68%	22.40	+0.916%

DISCUSSION

The ABHC treated test articles demonstrated a pH-dependent color shift from bright pink at pH 4, pink at pH 5-6, purple at pH 7, blue at pH 8, dark blue at pH 9 and green at pH 10. These colorimetric changes align well with the physiological pH values of wounds during the healing process. The purple to blue to green color shift that occurs as the pH transitions above 7.0, could be used to alert clinicians not only of the presence of a bacterial infection and/or necrotic tissue within the wound but also provide an approximate location within the wound of such issues. Moreover, at low exudate levels (replicated by inoculating samples with only 25 µL of pH buffer) the ABHC not only exhibited a notable colorimetric change at the inoculated surface (representing the wound contacting surface), but the color change was also observable on the outward facing, non-inoculated surface. This may allow clinicians to more easily monitor the pH of wound sites without requiring them to remove the wound dressing, which could negatively impact the progress of an otherwise healing wound.

The ABHC treatment did cause a minor pink beige tint to the otherwise white color of the non-treated PU foam material. Aside from this aesthetic effect, an assessment of several key performance metrics for wound dressings indicated that ABHC treatment did not negatively impact the performance of the PU foam. The ABHC treatment level appeared to correlate with a very small decrease in absorptive capacity and the fluid retention values, but these changes were not statistically significant at the low (p=0.56 and 0.26, respectively) and high (p=0.60 and 0.18, respectively) ABHC loading levels examined in this study. Additionally, the ABHC treatment did not appear to result in a statistically significant impact on the conformability/stiffness of the PU material at the low or high loading levels (p=0.34 and 0.54, respectively).

CONCLUSIONS

- At the high loading level, the ABHC treated PU foams exhibited a visually distinguishable color change at different physiologically relevant pH values associated with wound healing and impaired wounds.
- Even at low inoculum volumes, the pH-responsive color change was observable on both the wound contacting surface and the outward facing surface of ABHC treated PU material. This could allow clinicians to quickly and easily monitor wound site pH without having to remove the dressing.
- Absorbency, fluid retention, and conformability/stiffness were not impacted by the ABHC additive or the ABHC treatment process used in this study.
- The study demonstrates that ABHC treated dressings provide a means by which clinicians can monitor pH changes across the entire wound and use this additional information to better assess the wound state, evaluate the efficacy of the therapeutic treatment, and potentially reveal the location/severity of bacterial infections and/or necrotic tissue.

REFERENCES

(1) Arafa, A. A., Nada, A. A., Ibrahim, A. Y., Sajkiewicz, P., Zahran, M. K., & Hakeim, O. A. (2021). Preparation and characterization of smart therapeutic pH-sensitive wound dressing from red cabbage extract and chitosan hydrogel. International journal of biological macromolecules, 182, 1820–1831.

(2) (Wang, Y., Guo, M., He, B., & Gao, B. (2021). Intelligent Patches for Wound Management: In Situ Sensing and Treatment. Analytical chemistry, 93(11), 4687–4696.

(3) Alsahag, M., Alisaac, A., Al-Hazmi, G. A. A., Pashameah, R. A., Attar, R. M. S., Saad, F. A., & El-Metwaly, N. M. (2023). Preparation of carboxymethyl cellulose/polyvinyl alcohol wound dressing composite immobilized with anthocyanin extract for colorimetric monitoring of wound healing and prevention of wound infection. International journal of biological macromolecules, 224, 233–242.

(4) Rahimi, R., Ochoa, M., Parupudi, T., Zhao, X., Yazdi, I., Dokmeci, M., Tamayol, A., Khademhosseini, A., Ziaie, B. (2016). A low-cost flexible pH sensor array for wound assessment. Sensors and Actuators B: Chemical, 229, 609-617.

(5) Gethin, G. (2007). The significance of surface pH in chronic wounds. Wounds UK, 3 (3), 52-56

(6) Mennini, N., Greco, A., Bellingeri, A., De Vita, F., & Petrella, F. (2016). Quality of wound dressings: a first step in establishing shared criteria and objective procedures to evaluate their performance. Journal of wound care, 25(8), 428–437.

(7) Debashish, C., Roman, M. (2015). In Vitro Comparison of the Absorption and Retention Capabilities of Two Superabsorbent Wicking Dressings. Poster presented at the meeting of Wound, Ostomy, and Continence Nurses Society, San Antonio, TX.