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Article

Development of a pH-sensing hydrogel wound dressing for early infection monitoring for all skin color types

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SUMMARY

Wound infections cause significant morbidity and mortality. Increase in wound pH has been correlated with infection and may indicate infection before symptoms occur. However, little work on hydrogel wound dressings that can detect wound pH has been done. We therefore aimed to optimize a cost-effective pH sensing wound dressing that changes color to indicate infection. We hypothesized that using a collagen solution containing 0.13mM bromothymol blue (BTB) will permit a visible color change that is more visible than other hydrogel materials, as collagen is often used in wound healing applications and can be loaded with other materials. BTB was selected as the pH-indicating dye because it provides a color change detectable to the unaided eye. We tested several variables - hydrogel materials and concentration, amount of BTB, addition of BTB before/after solidification, and wound bed conditions - to find the optimal hydrogel that provided a distinct color change across a range of pH 5.0-8.0 on all six Fitzpatrick skin colors (1 = fairest to 6 = most pigmented). The optimal hydrogel consisted of 3mL of a 66.7 mg/mL gelatin solution with 0.13mM BTB added after solidification and showcased the greatest range in color from pH 5.0 to pH 8.0 and had a color change that was visually detectable and statistically significant (in pH 5.0 compared to pH 7.25 and 8.0) on all skin color types. Our work may provide a method for early detection of wound infection for patients with difficult access to care, such as in the developing world.

INTRODUCTION

Surgical site infections (SSIs) are the most prevalent and costly hospital-acquired infections (1). SSIs happen around the globe but occur in significantly higher rates in low- and middle-income countries, such as those in Sub-Saharan Africa. In Tanzania, for example, the prevalence of SSI is 41.9%, compared to just 2.5% in Europe (2). Approximately 20% of healthcare associated infections are SSIs, affecting around 2-5% of patients undergoing surgery nationally (3). Furthermore, SSIs take away from one's daily routine, increasing a patient's time in the hospital by about 9.7 days (1). SSIs can lead to lasting impairments and can significantly increase risk of death by 11-fold compared to patients without SSIs (3). Additionally, of patients with SSIs, 75% of deaths were caused by SSIs (3). Severe non-healing infected wounds can lead to bacterial spread into the bloodstream and subsequently other body parts, which can lead to end-organ damage, amputation, and even death (4).

SSIs are typically identified clinically by symptoms (worsening pain) or signs such as redness, swelling, and pus formation, but these signs and symptoms usually do not present until later stages of infection (5). The recurring limitation to early identification of wound infection is that the patient or doctor must first detect symptoms or signs of infection (5). Surgical infection usually requires a patient to travel to a healthcare clinic for a medical professional to make the diagnosis (6). A method of detecting possible wound infection earlier prior to the manifestation of signs or symptoms may allow for earlier treatment and quicker resolution of infection to improve quality of life and mortality. Several elegant devices have been developed for identifying early infections in wounds, such as smartphone programs, wearable devices, and flexible electronics (7). These devices may be limited in their real-world utility due to the high cost, complexity of readout, and availability. If the method of wound infection can be easily visually discerned with a clear, simple readout and without needing special equipment, it would allow widespread accessibility in poorer socioeconomic settings or in developing countries. Allowing patients to self-monitor their wounds would help overcome transportation barriers to wound infection diagnosis.

The pH of a skin wound has been identified as a major indicator of wound infection (8). Although many variables such as age, ethnicity, sebum, sweat, soaps, and cosmetic products can affect skin pH, the pH value of healthy skin is relatively consistent for all humans (9). The acid mantle makes up the outermost layer of the epidermis and keeps skin at a pH value of 4.2-5.6 in healthy adults and children (9). Skin heals best in an acidic environment, which promotes the formation of regenerative tissue and manages microbial levels (9). In contrast, alkalinity in the skin promotes both bacterial growth/infection and skin non-healing. Infected skin wounds typically have a pH of 7.0-9.0 (5). There is a significant body of literature that suggests an alkaline pH of a wound is associated with bacterial infection, and measurement of wound pH can be a potential diagnostic and therapeutic indicator of infection in wound healing (8, 10-12). In skin infections, pH increases before any clinical symptoms of infection occur (9). Pathogenic bacteria, which are bacteria that can override most bodily defense systems and cause disease, often release ammonia, which causes the pH of a wound to increase (4). When the skin pH rises above 6, pathogenic bacteria can proliferate more readily, leading to further worsening infection and further impairing wound healing in a relentless cycle (4). Antimicrobial peptides, which are part of the innate immune system, also often function best in acidic skin environments (13).

Hydrogels are crosslinked polymer chains with a 3-D structure that can absorb and retain fluids well (14). Hydrogels have been demonstrated to aid in wound healing because of their biocompatibility, absorption of excess fluid, and flexibility (14). We chose to use hydrogels for this study because of their

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transparency and excellent contact with the wound surface area, in addition to said advantages. Natural hydrogels, gelatin, collagen, and agar-agar were chosen due to their high availability (14). Using hydrogels for a pH-sensing wound dressing would allow the dressing to be read out without having to remove the bandage, possibly peeling off healing tissue or introducing new bacteria.

To ensure that this pH-sensing hydrogel wound dressing was compatible with all skin color types, swatches of colors across the Fitzpatrick scale were used (15). The hydrogel was placed on a background of different Fitzpatrick skin colors. Then, the color difference of the hydrogel was analyzed through visual inspection and measuring color using a colorimeter. This demonstrated if the hydrogel was able to display a color change on different skin colors. The Fitzpatrick scale consists of 6 skin color types; skin color 1 is the lightest, and skin color 6 is the darkest. Widely used by dermatologists, the Fitzpatrick scale is traditionally used to specify how different skin color types respond to sun exposure (15).

The ideal pH indicator for early detection of infected wounds would be one that has a rapid, high-contrast, visually detectable colorimetric change in the range of pH 4-9 (from normal and non-infected skin pH of 4-6 to infected skin pH of 7-9). We chose bromothymol blue (BTB) because of its drastic color change of non-physiological colors (bright yellow to bright blue) that occurs from pH 5 to pH 8. Other biological indicators, such as turmeric (which changes from yellow to dark red as alkalinity increases), and phenol red (which also changes from yellow to hot pink), have color changes that can be confused with increasing redness from bleeding, inflammation, or hyperpigmentation (16). Phenol red has also been found to have irreversible mutagenic effects if ingested (17). To be clinically useful, a pH-sensing bandage would need to be accurate, stable, and produce a distinct color change that can be interpreted with the unaided eye without elaborate equipment or training. Additionally, BTB was used in this study because it is yellow in an acidic environment, green in a neutral pH, and blue in a basic environment. Specifically, BTB has a significant color change from yellow-green to blue at pHs above 6, which corresponds to the pH of infected wounds. BTB has also been used in other wound dressings due to its low toxicity and strong biocompatibility (18).

The purpose of this project was to determine the optimal makeup of a pH-sensing hydrogel wound dressing using different hydrogel materials and BTB concentrations that would have the greatest range in color from yellow in acidic environments to blue in alkaline environments. Based on prior research showing that a pH-sensing hydrogel that cross-linked BTB to bacterial nanocellulose had strong conformability, mechanical rigor, water vapor transmission, cellular nontoxicity, we hypothesized that the optimal hydrogel would be composed of a 6.67% collagen solution with 0.25mL of 1.6mM bromothymol blue added after solidification (5). Collagen is commonly used in wound healing applications because of its matrix, which can absorb large amounts of water without compromising its structure (19). Additionally, collagen hydrogels can be loaded with other elements, such as antibiotic and anti-inflammatory bioactive materials, so a collagen hydrogel has the potential to retain bromothymol blue well (19). Furthermore, we hypothesized that the greatest concentration of bromothymol blue tested, 0.25mL of 1.6mM BTB solution, would allow for the greatest vibrancy and range in color. We chose the largest volume of BTB to be 0.25mL, as it would allow for a vivid and uniform color without compromising transparency or oversaturating the hydrogel, potentially affecting hydrogel stability. We also hypothesized that adding bromothymol blue into the hydrogel after solidification and allowing the dye to be adsorbed into the hydrogel would create a more visible readout. Heat can

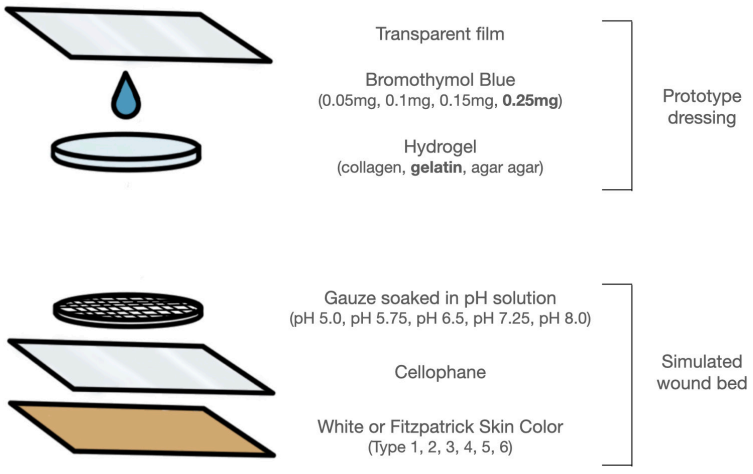

Figure 1: Layers of prototype dressing and simulated wound bed model. The prototype wound dressing consisted of a transparent film dressing covering a hydrogel with bromothymol blue (BTB) incorporated. Different hydrogel materials (collagen, gelatin, and agar-agar) were tested, as well as different amounts of BTB. The simulated wound bed consisted of gauze soaked in pH solutions (varying from pH 5.0 to pH 8.0) placed on cellophane, which was then placed on a white control or one of the six Fitzpatrick skin colors background.

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disrupt the structure and functional properties of a molecule. While not tested with BTB, it is known that the temperatures involved in collagen hydrogel formation can interfere with integrated components (19). Thus, we formed a preliminary claim: a wound dressing composed of collagen and 1.6mM BTB would enable color-based pH detection across different skin tones.

RESULTS

The goal of this project was to determine the optimal hydrogel composition that had the greatest range in color from yellow to blue for the easiest patient readout. We hypothesized that the optimal hydrogel would be composed of collagen and 1.6mM BTB. Several variables—hydrogel material, concentration of hydrogel material, amount of BTB per hydrogel, addition of BTB before and after solidification, and amount of gauze (used to simulate the wound bed)—were tested to find the optimal hydrogel that could show a distinct color change from yellow to blue across a range of pH 5.0-8.0 on all six Fitzpatrick skin color backgrounds **(Figure 1)**. The color of the hydrogel was then measured using a colorimeter. Given that a solid hydrogel was tested, and spectrophotometers require a sample to be in solution, a colorimeter was used for this study because of its ability to quantify color differences on solid surfaces (20). Colorimeters are also more cost-efficient than spectrophotometers.

We discovered that all collagen hydrogels had a minimal change in color from pH 5.0 to pH 8.0. The pH values were modeled by creating solutions with pH 5.0, 5.75, 6.5, 7.25, and 8.0 using baking soda, vinegar, and distilled water. The hydrogels were then placed on top of gauze, which was used to simulate the wound bed, soaked in a pH solution **(Figure 1)**. The b-value (negative value is blue, positive value is yellow) was persistently negative, meaning the hydrogel was unable to become yellow, as it was expected to in an acidic pH (**Figure 2A, 2B)**. When hydrogels were made by adding the BTB before solidification of the collagen hydrogel, the hydrogels became more vibrant blue as a greater concentration of BTB was used, but the hydrogels remained around the same color in all pHs (**Figure 2A**). Similarly, when hydrogels were made by adding the BTB after solidification of the collagen hydrogel, the hydrogels also remained blue throughout all pHs (**Figure 2B**). Thus, regardless of the method of incorporation of BTB and concentration of BTB in the hydrogel, the collagen hydrogels were unable to become yellow in an acidic pH, so they were not suitable for this technology.

Instead, we determined that gelatin was the optimal hydrogel material that successfully exhibited a visible color change from pH 5.0 to pH 8.0 **(Figure 2C, 2D)**. Hydrogels that were formulated by adding BTB before the solidification of the hydrogel successfully changed from yellow in acidic environments to blue in alkaline environments but did not have even distribution of the BTB throughout the hydrogel (**Figure 2C**). The BTB became light in color in the gelatin, so it was difficult to evenly mix in the BTB into the liquid hydrogel solution. Conversely, the gelatin hydrogels created by adding BTB after solidification demonstrated more even distribution of dye and consistent color throughout the hydrogel (**Figure 2D**). The negative control hydrogels (which were not infused with BTB) showed almost no change in b-value (slope -0.75, median, n=3) as the pH increased **(Figure 2D).** Hydrogels composed of 6.67% agar-agar were not functional, as they could not be removed from the mold without breaking apart (data not shown).

With regard to hydrogel concentration, the hydrogels made using a higher concentration of gelatin (8.89% gelatin solution) did not exhibit as great a color differential compared to using a lower concentration of gelatin (6.67% gelatin solution). The hydrogels made using an 8.89% gelatin solution did not produce a blue color throughout pH 5.0 to pH 8.0 **(Figure 2D)**. The 6.67% gelatin hydrogel allowed excellent contact with the simulated wound because it was malleable and conformed to irregular surfaces. This allowed for a thorough readout for the entire wound and possible identification of the nidus of infection.

We found that the optimal pH-sensing wound dressing consisted of 3mL of a 6.67% gelatin solution with 0.25 mL of 1.6mM BTB added after solidification of the hydrogel. The hydrogels were created as discs 3.75 cm in diameter (large enough to cover a medium-sized wound). After the hydrogel was solidified at room temperature for 15 minutes, 0.25 mL of 1.6mM BTB was evenly distributed on the surface of the hydrogel. The difference between b-values of the BTB-infused hydrogels from the lowest pH (5.0) to the highest pH (8.0) increased as the amount of BTB per disc was increased (**Figure 2D**). This hydrogel was remade in nine separate repeat trials to ensure reproducibility. This optimal hydrogel was able to exhibit a color change that was relevant to the pH of non-infected and infected wounds from a very positive b-value in pH 5.0 (median: 33.89, n=9), which correlates to a very yellow color, to a very negative b-value in pH 8.0 (median: -21.26, n=9), which correlates to a very blue color **(Figure 2D)**. This hydrogel exhibited a visually detectable color change across all six Fitzpatrick skin color types. Thus, this hydrogel could be used for the detection of infection prior to the onset of clinical symptoms in patients living in areas with poor access to care and with different skin pigmentation.

In regards to usability on all skin color types, the color of the hydrogel remained relatively consistent throughout all six Fitzpatrick skin color types **(Figure 3)**. The slope of the trend line of median b-values of the optimal hydrogel on a white background from pH 5.0 to pH 8.0 of these nine trials was -15.77. The slope of the trend line of median b-values for Fitzpatrick skin types 1 though 6 ranged from -14.96 to -12.30, which is a slight deviation (5-22% difference) from the white background control of -15.77 (data not shown).

As the Fitzpatrick skin color scale background became darker, the slope decreased only minimally, indicating that there was less contrast between the b-value of pH 5.0 and pH 8.0. The greatest median b-value of pH 8.0 was -17.58 on type 4 Fitzpatrick skin color background, and the least median b-value of pH 8.0 was -21.43 on white control background. The b-value of pH 5.0 decreased as the background became darker, meaning the hydrogels appeared less yellow. The greatest median b-value at pH 5.0 was 33.60 on white background, and the least median b-value of pH 5.0 was 27.08 on type 6 Fitzpatrick skin color background. Thus, the BTB-infused hydrogels allow detection of a visually significant color change from pH 5.0 to pH 8.0 in the white and all 6 Fitzpatrick skin color backgrounds. Throughout all backgrounds, the

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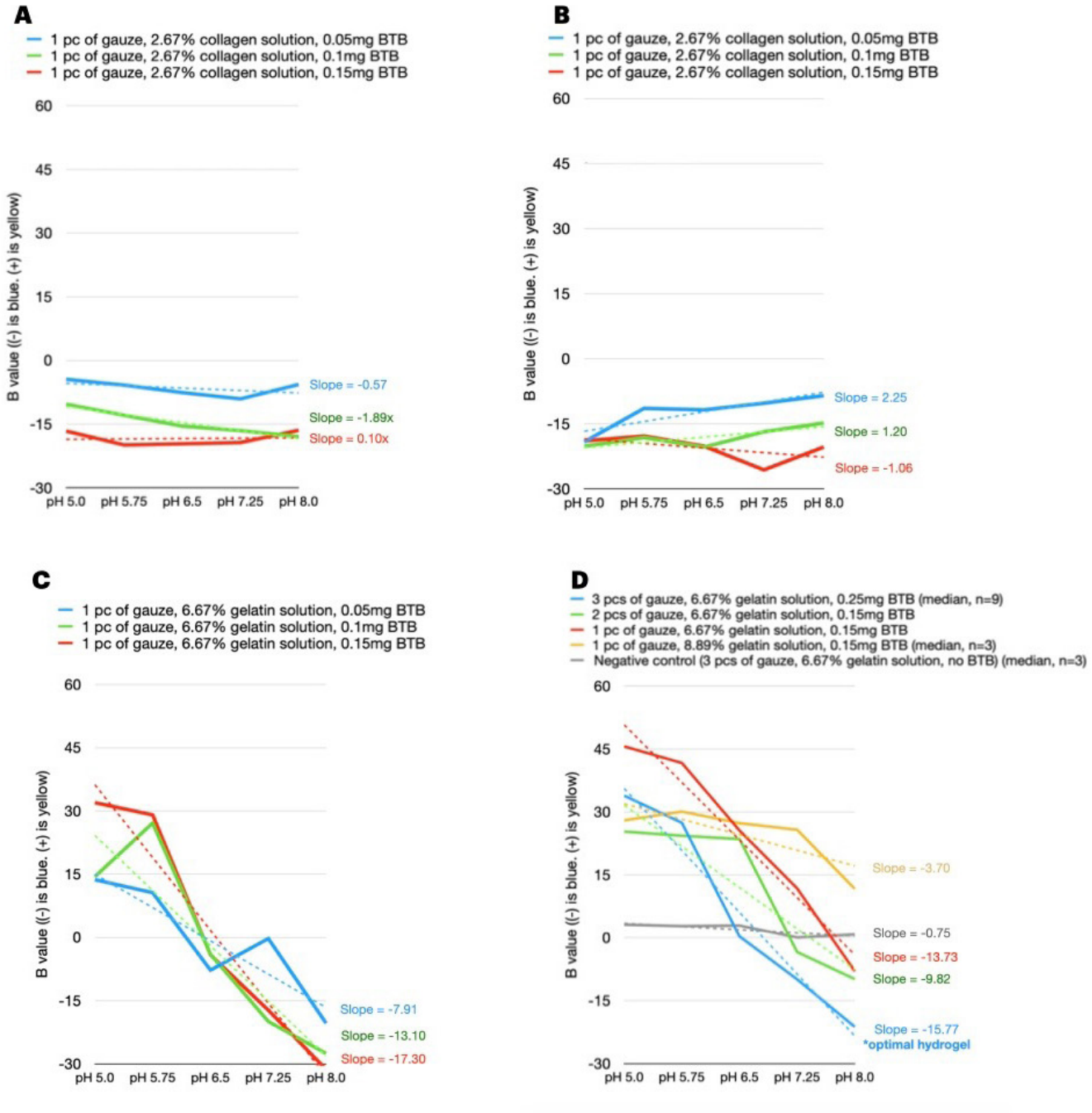

Figure 2: B-value of hydrogels made with collagen or gelatin with 0.1% BTB added before and after solidification. The line graphs show b-values of after 10 hours in pH 5.0-8.0. **(A)** The blue, green, and red lines demonstrate the b-values of 2.67% collagen hydrogels with 0.05mg, 0.10mg, and 0.15mg of BTB, respectively, on one piece of gauze (simulated wound bed) with BTB added prior to solidification. **(B)** The blue, green, and red lines demonstrate the b-values of 2.67% collagen hydrogels with 0.05mg, 0.10mg, and 0.15mg of BTB, respectively, on 1 piece of gauze with BTB added after solidification. **(C)** The blue, green, and red lines demonstrate the b-values of 6.67% gelatin hydrogels with 0.05mg, 0.10mg, and 0.15mg of BTB, respectively, on 1 piece of gauze with BTB added prior to solidification. **(D)** The negative control hydrogel consisted of a hydrogel with no BTB and had minimal change in color (slope of the trendline was -0.75). The blue line demonstrates the b-values of a 6.67% gelatin hydrogel with 0.25mg of BTB on 3 pieces of gauze with BTB added after solidification. The green line demonstrates the b-values of a 6.67% gelatin hydrogel with 0.15mg of BTB on 2 pieces of gauze. The red line demonstrates the b-values of a 6.67% gelatin hydrogel with 0.15mg of BTB on 1 piece of gauze. The yellow line demonstrates the b-values of an 8.89% gelatin hydrogel with 0.15mg of BTB on 1 piece of gauze. The gray line demonstrates the b-values of a 6.67% gelatin hydrogel with no BTB on 1 piece of gauze. All experiments not otherwise specified were performed as single trials.

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b-value of the hydrogels in pH 5.0 and 5.75 had relatively similar positive b-values, and the hydrogels were very yellow. The b-value in pH 6.5 was around 0, and the hydrogel was either slightly yellow or slightly blue. The b-values of pH 7.25 and 8.0 were both blue **(Figure 3, Figure 4)**.

The Kruskal-Wallis test with Dunn's multiple comparison test was used to find the lowest pH at which there was a significant statistical change in b-value compared to the b-value in pH 5.0. It was found that there was a significant statistical change of the optimal hydrogel in pH 5.0 and hydrogel in pH 7.25 and pH 8.0 on white and all six skin colors of the Fitzpatrick scale ($p<0.05$) (**Figure 5**).

The optimal hydrogel maintained a color differential between the different pHs that could be visually discriminated up to the longest incubation time (100 hours) (**Figure 6**). At 10 hours, the median b-values (n=9) at pH 5.0, 5.75, 6.5, 7.25, and 8.0 were 33.89 (very yellow), 37.44 (very yellow), 0.31 (greenish), -9.93 (blue), and -21.26 (very blue), respectively. For the mid-range pHs of 5.75, 6.5, and 7.25, the b-values tended to decline with incubation time (hydrogels became bluer). After 5 minutes incubation and after 1 hour incubation, the b-values at pHs 6.5 and 7.25 were positive (yellow), but after 10 hours incubation, the b-values at pHs 6.5 and 7.25 dropped closer to zero (became less yellow and bluer), such that it became difficult to visually distinguish the color differential between pH 5.75, 6.5, and 7.25 at 100 hours. Importantly, though, throughout all incubation times (5 minutes, 1 hour, 10 hours, 24 hours, 100 hours), the b-value for pH 5.0 remained quite positive (hydrogels maintained a yellow color). Additionally, throughout all incubation times (5 minutes, 1 hour, 10 hours, 24 hours, 100 hours), the b-value for pH 8.0 remained quite negative (hydrogels maintained a dark blue color) **(Figure 6)**. Given hydrogels typically last about 3 days before needing to be changed, the ability of the hydrogel to detect color differentials between pH 5.0 and pH 8.0 should last the duration of most hydrogel dressings (21). Thus, a hydrogel consisting of 3mL of a 6.67% gelatin solution with 0.25mL of 1.6mM BTB was determined to be the optimal hydrogel capable of a robust color change from yellow in acidic simulated wound beds to bright blue in alkaline simulated wound beds across all 6 Fitzpatrick skin colors for up to 100 hours after placement.

DISCUSSION

The goal of this study was to determine the optimal composition of a BTB-infused hydrogel that would allow for the greatest range in color from yellow in acidic pHs to blue in alkaline pHs. This hydrogel could potentially be used for the early detection of wound infection. The optimal hydrogel, which was composed of 3mL of a 6.67% gelatin solution with 0.25mL of 1.6mM BTB, exhibited a color change relevant to differences in pH of infected and non-infected wounds that was not only visually detectable, but also statistically significant.

Gelatin, collagen, and agar-agar are all natural hydrogels, which are biocompatible and are non-toxic. The gelatin forms a gentle adhesion to the wound site and may be less likely to damage tissue when removed compared to gauze dressings that dry and adhere to the wound, causing possible damage to the healing wound when removed (22). Additionally, these hydrogel materials were all chosen for their wide availability and relatively low cost (14). Of the different hydrogel materials, gelatin successfully demonstrated a significant range of color when infused with BTB and that could retain its shape while still conforming to the wound bed. None of the hydrogels made using collagen, even using both BTB incorporation

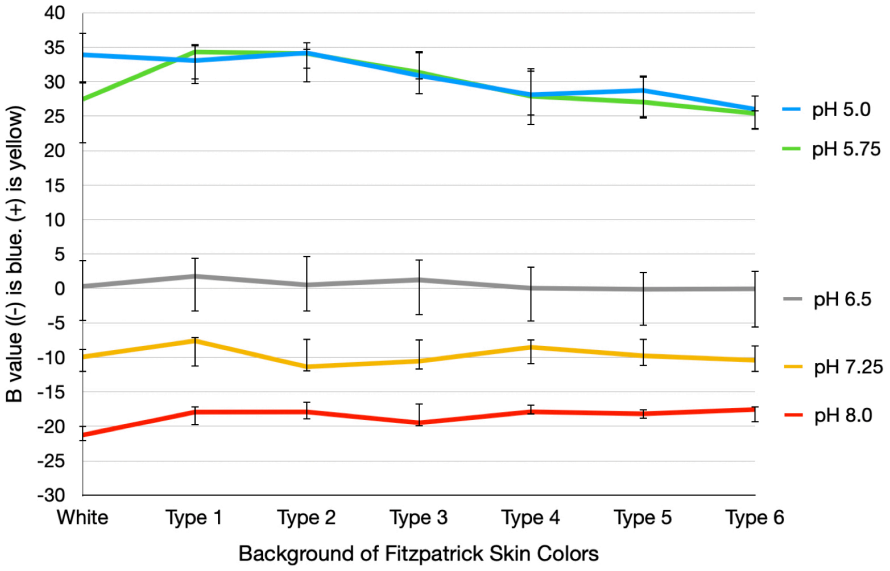

Figure 3: Median b-values after 10 hours of optimal hydrogels made with gelatin and 0.1% BTB added after solidification across different skin type backgrounds. The b-values of the hydrogel remained relatively consistent across all backgrounds, median shown (n=9). In pH 5.0 and 5.75, the b-values were positive (very yellow), in pH 6.5, the b-values were around 0 (slightly yellow or slightly blue), and in pH 7.25 and 8.0, the b-values were negative (blue).

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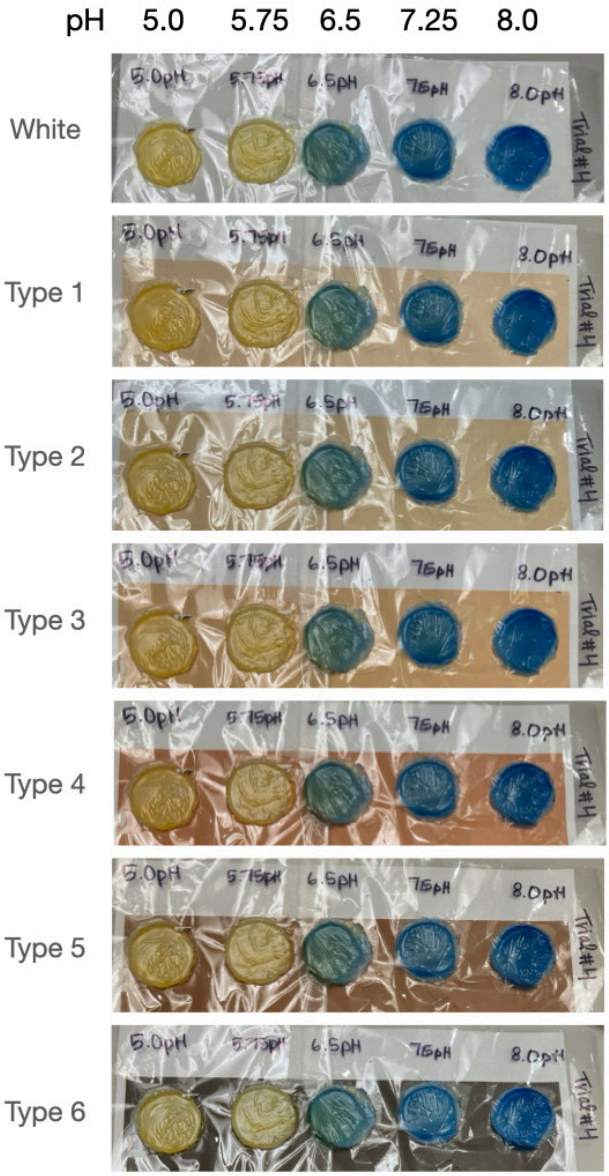

Figure 4: Representative images of 6.67% gelatin hydrogels with BTB on different Fitzpatrick skin color backgrounds. Images taken after 10 hours of incubation. The optimal hydrogel exhibited a color difference from very yellow in pH 5.0 to very blue in pH 8.0 that was easily visible across all backgrounds. These backgrounds included a white control background and six Fitzpatrick skin colors (1=lightest, 6=darkest).

methods, different concentrations of collagen, and different concentrations of BTB, produced a yellow color unless placed in a very acidic environment (pH 2.0) (**Figure 2C, 2D**). This could have been caused by the existing pH of the collagen that interfered with the ability of the BTB to adapt to the pH of the gauze or the collagen affecting the molecular structure of the BTB. The isoelectric point, or the pH at which the molecule carries no net electrical charge, of collagen is between 7 and 8 (23). This intrinsic pH, which would result in a blue color, may explain why the collagen hydrogels were unable to become yellow. The hydrogels made using agar-agar were not mechanically robust and lacked the flexibility that the gelatin and collagen hydrogels exhibited, which prevented them from being removed from the mold. Additionally, using a greater concentration of hydrogel material impaired the color-changing abilities of BTB, as the b-value decreased only minimally **(Figure 2B)**.

The color change of the hydrogel was not only visually detectable, but also statistically significant in all skin types tested. The b-value of the hydrogel in pH 5.0 was statistically significantly greater compared to the b-value of the hydrogel in pH 7.25 and 8.0, which correlates to the pH of infected wounds (pH 7-9) across all six Fitzpatrick skin types (5). The significance is that this hydrogel wound dressing can potentially detect wound infection in a wide spectrum of people of different skin pigmentation. Given that color changes may

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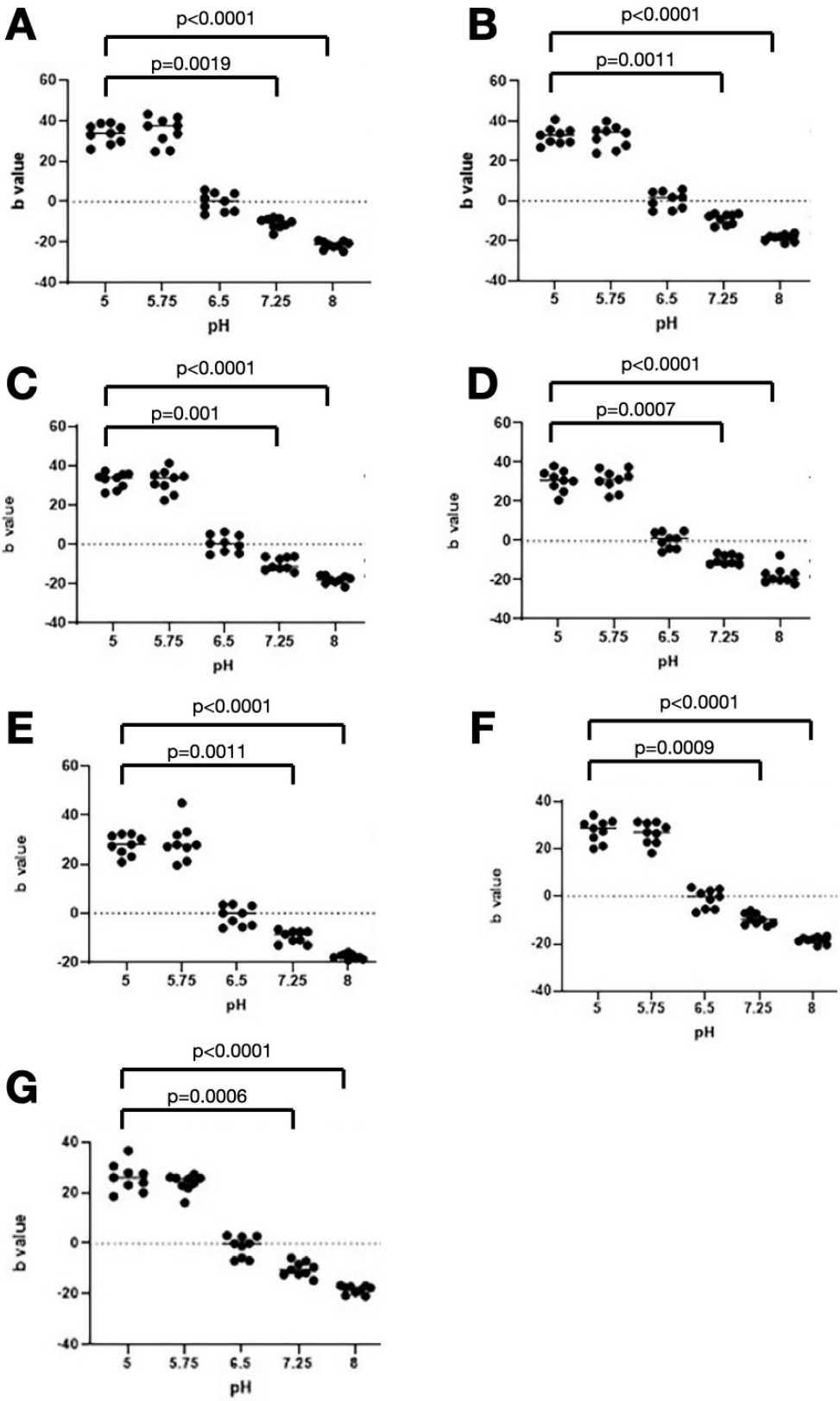

Figure 5: B-value of optimal hydrogel after 10 hours in pH 5.0 compared to other pHs on all Fitzpatrick skin types. B-values at varying pHs were compared to pH 5.0 from hydrogels on **(A)** a white background, **(B)** a background of skin type 1, **(C)** a background of skin type 2, **(D)** a background of skin type 3, **(E)** a background of skin type 4, **(F)** a background of skin type 5, **(G)** a background of skin type 6 of the Fitzpatrick scale.

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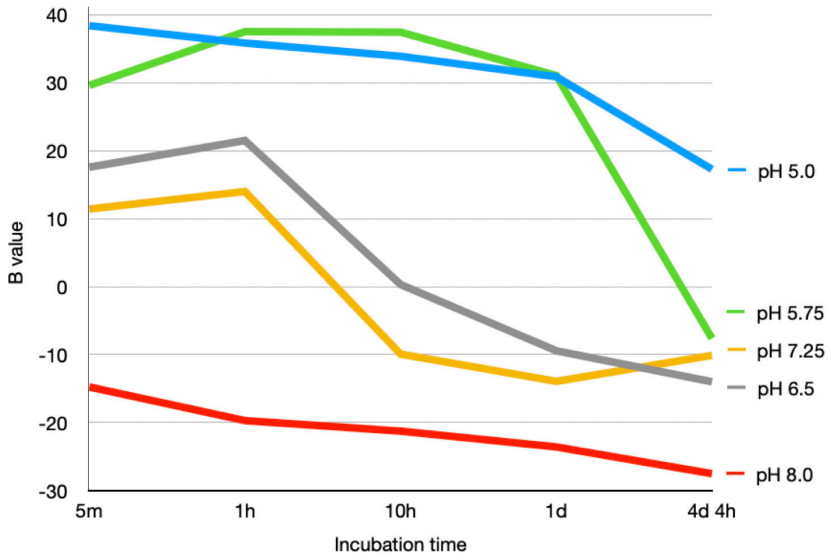

Figure 6: B-value of optimal hydrogel on a white background over time at different pHs. This line graph displays the b-value of a hydrogel consisting of a 6.67% gelatin solution with 0.25mL of 1.6mM BTB at different incubation times. Throughout all incubation times, the b-value of the hydrogel in pH 5.0 remained quite positive (yellow), and the b-value of the hydrogel in pH 8.0 remained quite negative (very blue) (n=1).

be easier to visually detect on lighter skin pigmentation, our data showing that the hydrogel successfully exhibits a visually detectable and statistically significant color change on light and dark Fitzpatrick skin colors increases its scope. Thus, many patients with poor access to care in developing countries, often having people with darker skin pigmentation, could benefit from having this hydrogel dressing.

Regarding time sensitivity of the hydrogel wound dressing, we found that the color change could last up to 100 hours. At 10 hours, the color readout was most distinguishable between the pHs. Importantly, though, throughout all incubation times, the b-value for pH 5.0 remained quite positive (hydrogels maintained a yellow color), and the b-value for pH 8.0 remained quite negative (hydrogels maintained a dark blue color). The significance of this finding is that the color of this hydrogel can be maintained for up to 100 hours. BTB has been demonstrated to be well tolerated by primary skin keratinocytes and fibroblasts via cell proliferation, motility, and viability studies and in porcine wound models (5).

Limitations include color instability over extended periods of time, the absence of human studies, and the lack of model infections with microbes. Over time, all b-values of all hydrogels at all pHs slowly declined, becoming bluer. Future directions could include culturing the hydrogels to assess whether the color change over time is due to microbial growth or natural breakdown of BTB or other mechanisms. Future experiments will also explore whether the BTB-adsorbed hydrogel can detect dynamic changes not only from acidic to alkaline conditions as a wound becomes infected but also changes from alkaline back to acidic conditions as an infection resolves. In the future, it would also be important to test the BTB-infused hydrogels on human skin to see if there are any adverse reactions such as contact dermatitis or breakdown of the gel at body temperature. Another limitation of this hydrogel is that it cannot identify the microbe or characterize the extent of bacterial colonization in the case of an active infection. An exciting extension of this study would be to explore other indicators that could permit a color change in the presence of a specific pathogen. However, this current hydrogel is still clinically useful and broadly applicable, as it allows the detection of multiple different pathogens.

In conclusion, the optimal pH-sensing wound dressing consisted of a BTB-infused gelatin hydrogel which demonstrated an easily visually detectable and statistically significant color change from yellow in pH 5.0 to blue in pH 7.25 and 8.0 in all 6 Fitzpatrick skin color types.

This hydrogel was also cost-efficient ($0.40/dressing). This pH sensing hydrogel dressing prototype would be used as a method of wound monitoring and potentially early detection of infection prior to the onset of clinical symptoms.

MATERIALS AND METHODS

Hydrogel formulation

Collagen (ZJchao, B09DDBGCYD), agar-agar (Kate Naturals, 810002444135), and gelatin (Knox, 043000048672) were all tested in order to find the optimal hydrogel. 3 mL of a hydrogel was poured into a circular silicone mold 4.5 cm in diameter. To prepare each hydrogel solution, 75mL of distilled water was boiled. For the collagen hydrogel solution, 1 tablet of collagen (2g) was dissolved in 75mL of distilled water (2.67%). For the gelatin hydrogel solution, different amounts of gelatin were dissolved (5g and 6.66g) in 75mL of distilled water (6.67% and 8.89%). For the agar-agar solution, 4g of agar was dissolved in 75mL of distilled water (5.33%).

We used a 0.1% solution of BTB (Aldon Corp, IS11076), which had a molarity of 1.6 mM. The BTB was dropped on top

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of the hydrogel once the hydrogel hardened, and the hydrogel sat for 30 minutes at room temperature for the dye to be absorbed. In other batches, the 1.6mM BTB was added to the hydrogel solution prior to solidification and mixed using a stirrer. We also varied volume of 1.6mM BTB (0.05mL, 0.1mL, 0.15mL, 0.25mL).

After the dyed hydrogel has set, it was placed on a piece of transparent medical dressing (All Health, 017276234480). To test the visibility of the color change, pieces of circular gauze (CVS Health, #893119) (4.5 cm in diameter) were soaked in different concentrations of vinegar (for acidic pHs) or pure sodium bicarbonate (for basic pHs) and water to create solutions with pHs of 5.0, 5.75, 6.5, 7.25, and 8.0. The pH 5.0, 5.75, 6.5, 7.25, and 8.0 solutions were tested using a pH meter to confirm solution pH immediately before submerging the gauze in the solutions.

pH detection

We further diluted distilled white vinegar at 5% acidity with distilled water to the needed acidic pHs. Pure sodium bicarbonate was further diluted with distilled water to the needed alkaline pHs. The positive control in this study was originally a pH paper test strip (EASYTEST, 860011903536) on a piece of substance-soaked gauze to verify the pH of each substance for every substance tested; however, it was found that the test strips had a difficult readout and the colorfastness was ephemeral. Another option was to directly measure the pH of the gelatin using a pH meter (PmoYoKo, 00721678690132), but doing so could have led to permanent damage of the pH meter. Instead, the positive control was that the pH solutions of vinegar, baking soda, and water were tested using a pH meter immediately before submerging the gauze in the solutions. The pH meter was calibrated using standard buffer solutions (BOJACK, B09DCP4HNH).

The gauze was placed on clear cellophane covering paper shaded with six different Fitzpatrick skin colors to simulate skin wounds **(Figure 1)**. A bandage of dyed hydrogel and transparent medical dressing was placed on top of the piece of gauze. The color change was recorded after 5 minutes, 10 hours, 24 hours, and 100 hours using a colorimeter (Beley, MOT255-382-1133584414). Colorimeters measure color in terms of L, a, and b-values (12). The L value represents the light or darkness of a color, and the value of L increases as a color becomes lighter. The a-value represents how green or red a color is. A positive a-value represents the redness of a color, while a negative a-value represents the greenness of a color. The b-value represents how blue or yellow a color is. A positive b-value represents the yellowness of a color, while a negative b-value represents the blueness of a color. These values are the measurement of human-perceived colors, meaning the intensity of redness/greenness, blueness/yellowness, and lightness/darkness (12).

Statistics

We compared the slope of the color changes (difference in b-values) to find the hydrogel with the greatest change in color. After we determined the optimal color change, the non-parametric Kruskal-Wallis test with Dunn's multiple comparisons test was used to find the pH that would result in a significant statistical change in b-value compared to the b-value of the hydrogel in pH 5.0. We used the Kruskal-Wallis test with Dunn's multiple comparisons test due to the fact that there was not enough data to assume a normal distribution, and that multiple b-value groups in different pHs were compared. These tests were carried out using GraphPad Prism. We also graphed the colorfastness to determine the longevity of accurate readout. The negative control of this study was a hydrogel without any BTB on a piece of gauze dipped in pH solutions. After the optimal hydrogel consistency and BTB concentration for the greatest color change were determined, this optimal BTB-infused hydrogel was remade and retested in nine separate repeat experiments to demonstrate reproducibility.

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