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The influence of different dressings on the pH of the wound environment

Stephen D Milne, EngD, Biomedical Engineering, University of Strathclyde, Glasgow

Patricia Connolly, PhD, Biomedical Engineering, University of Strathclyde, Glasgow

Patricia Connolly, Biomedical Engineering, University of Strathclyde, Wolfson Centre, Glasgow, G4 0NW, Scotland, UK. Telephone: 0141 548 3034

Abstract

The pH level of a wound has been shown to have a significant influence over various aspects of the healing cascade such as cell proliferation and Matrix metalloproteinases (MMPs) levels. It is known that some of the modern wound dressings in current use can markedly affect the pH of wounds to which they are applied. We have therefore conducted a simple study to examine the effect of some of these new materials upon a simulated wound environment. This study investigates the pH influence of a number of wound dressings (Manuka honey dressing; sodium carboxymethylcellulose hydrofiber dressing; polyhydrated, ionogen-coated, polymer mesh dressing; and a protease modulating collagen cellulose dressing) in real time to assess the potential changes that may occur to the wound environment. From this the effect of local buffering can be observed and pH changes in real time are reported. It was found that the dressings all had low pH of below pH 4 with the lowest being the protease modulating collagen cellulose which had a pH of 2.3. The dressing with the strongest acid concentration was the polyhydrated, ionogen-coated, polymer mesh dressing. The low pH and strong acidic nature of the dressings investigated indicate that they may play a role in influencing the healing process in a wound.

keywords

Keywords: pH, wound dressing, honey, MMP modifying dressing

Introduction

Modern wound dressings are known to influence the healing conditions of a wound to provide an improved healing environment at the wound bed. For the general patient population the healing conditions of the wound bed can be somewhat modified by the wound dressings applied to it by adjusting moisture¹, pH² and bacterial count.³ There are a number of wound dressings that are known to include constituents that alter the pH of a wound. For example, the active components of some advanced wound dressings contain acids such as citric acid⁴ and acetic acid.⁵ Manuka honey dressings contain gluconic acid which has a naturally low pH this has been proposed as one of its main methods improving the healing condition of the wound bed.² The acidic strength of the dressings are unknown and what effect they will have on the pH of the wound when applied has not been routinely investigated. Thus there is a need to study in more depth the pH altering qualities of advanced wound dressings and consider how this may affect the wound on the immediate application of the dressing and as healing progresses.

The pH of a wound can influence many different parts of the healing process such as cells, bacteria and MMP's.⁶ Some of the key cells in the wound healing process fibroblasts, neutrophils and platelets have been shown to be effected by the pH level of the wound with cell migration and production of proteases being affected by change in pH levels.⁷ When the skin is broken the internal pH of the body is exposed moving the pH from the acidic skin (on average just below pH5) towards the slightly alkali pH of the internal body. The pH of chronic wounds has been reported to be in the range from 5.45-8.9.⁸⁻¹⁰ The pH level in the wound is influenced by many different factors such as the body's physiological pH level, oxygen levels, bacterial load and healing stage of the wound.¹¹¹²

For this study a printed, disposable pH sensor has been developed which will allow pH measurements to be taken directly from the wound bed or from freshly sampled wound exudate. This new sensor enables more real time data to be gathered to further the understanding of the effect of pH on the wound healing process. This investigation focuses on using the printed pH sensor to monitor the change in local pH of the wound environment that is induced by the application of wound dressings.

The local pH effect of modern wound dressings on the wound bed has not been studied in any depth, due to the difficulties of placing the traditional pH sensors in this environment, and this research aims to observe how modern wound dressings influence pH and how this might modify the wound healing conditions. Importantly, the experimental design allows for real time changes in pH to be monitored.

Materials and Methods

This study investigates the pH of four different wound dressing types and their influence on a wound bed environment created using horse serum as a substitute for human wound fluid. Similar studies have used bovine serum as a substitute for human wound fluid.¹³ The use of horse serum diluted with a physiological salt solution (Solution A) enables the pH response of the wound dressings to be observed in a similarly buffered environment to that of human wound exudate and allows for the expected time dependent pH response to be measured upon application of the dressing to the wound and exudate environments.¹⁴¹⁵¹⁶

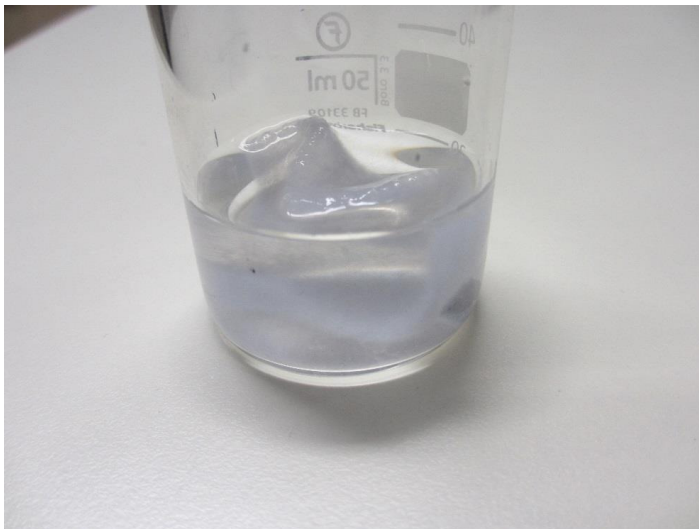


Figure 1 Example of a wound dressing hydrated in 20ml of Solution A. The sensor is placed in the liquid to monitor pH change.

The testing of wound dressings consisted of two testing stages:

1. The testing the pH of wound dressings while immersed in a beaker of Solution A (**Figure 1**), an unbuffered ionic solution used to mimic wound exudate ionic content in wound dressing testing.¹⁷
2. Real time pH monitoring in a wound bed simulation after application of wound dressing (**figure 2**).

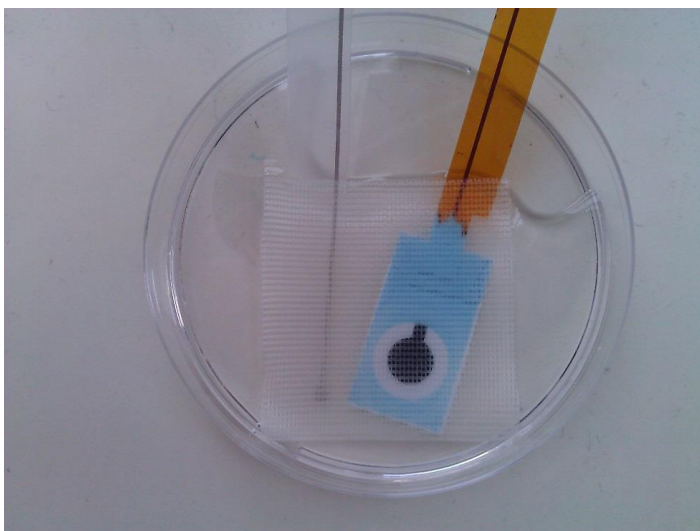


Figure 2 Wound bed environment simulation showing screen printed pH sensor and screen printed Ag/AgCl reference electrode immersed in Solution A in petri dish with gauze dressing applied.

Four different dressing types that were suspected to have an influence on local wound pH were tested. Two of the dressings tested are marketed as MMP modifying dressings: the polyhydrated, ionogen-coated, polymer mesh dressing, and the protease modulating collagen cellulose dressing. The third dressing is a manuka honey based dressing which stimulates the healing process, has antibacterial and antimicrobial properties that reduce infection and that it helps maintain a moist wound environment.¹⁸ The final dressing tested is a hydrofiber based dressing made from sodium carboxymethylcellulose this turns to a gel like substance when it comes into contact with liquid. The gel helps to maintain a moist wound environment which results in an improved healing rate.¹⁹

Dressings were unpacked from the sterile packs and cut into 5cmx5cm squares for testing. Dressing samples were used immediately or discarded.

Solution A and Horse serum solutions

Solution A was prepared from 142mM NaCl, 2.5mM CaCl₂·2H₂O in distilled water.¹⁷ Diluted horse serum mixture was prepared from 50% Solution A and 50% horse serum (donor horse serum, Harlan Seralab S-0004A). Diluted horse serum was used to reduce the viscosity of the horse serum mixture. This dilution will reduce concentration of buffering elements in the protein which reduces the buffering capacity of the serum.

Dressing pH testing

The pH of each of the wound dressings when hydrated in a beaker of Solution A was measured. The wound dressing were immersed in 20ml of Solution A and mixed then left for 20 minutes to ensure mixing of dressing constituents with Solution A.

The pH measurements of the dressings in the beaker, shown in **figure 1**, were made on a pH meter (Fisher brand Hydrus 300) with a glass pH electrode (Fisher scientific).

Real time pH testing in a simulated wound bed

The wound dressings were then tested in a simulated wound bed to mimic the application to a real world environment and measure the temporal pH response.

The pH sensors used for determining the pH in real time are manufactured using a screen printing method and an ion selective membrane. The reference electrode employed was a screen printed Ag/AgCl electrode supplied by Ohmedics (Ohmedics, UK). The voltage response of the pH sensor was recorded on a Solartron 1286 electrochemical interface (Solartron). The pH electrodes were calibrated in pH 4 and pH 7 solution prior to each wound dressing test.

The sensor and reference couple were then placed beside each other in a standard petri dish and 10ml of horse serum mixture added to the sensors (as shown **figure 2**). The base voltage (pH) was recorded for 120 seconds before application of the dressing. The 5cmx5cm dressing square was placed onto the sensors and liquid

solution and was left with the sensor voltage being measured until the voltage had stabilised. All dressings were tested 3 times with new dressings and sensors for each experiment.

Results

Hydrated wound dressing pH testing

Dressing type	pH meter pH measurement (pH) \pm SD
Manuka honey	3.49 \pm 0.10
Sodium carboxymethylcellulose hydrofiber	4.51 \pm 0.02
Protease modulating collagen cellulose	2.30 \pm 0.03
polyhydrated, ionogen-coated, polymer mesh dressing	3.25 \pm 0.01

Table 1 Measured pH after hydration of wound dressing in 20ml Solution A using glass electrode pH meter (n=3).

The results shown in **table 1** illustrates the pH of the dressings when immersed in 20ml of Solution A as shown **figure 1**. The pH of the tested dressings varies from a pH of 4.51 to a pH of 2.3.

Wound environment pH testing

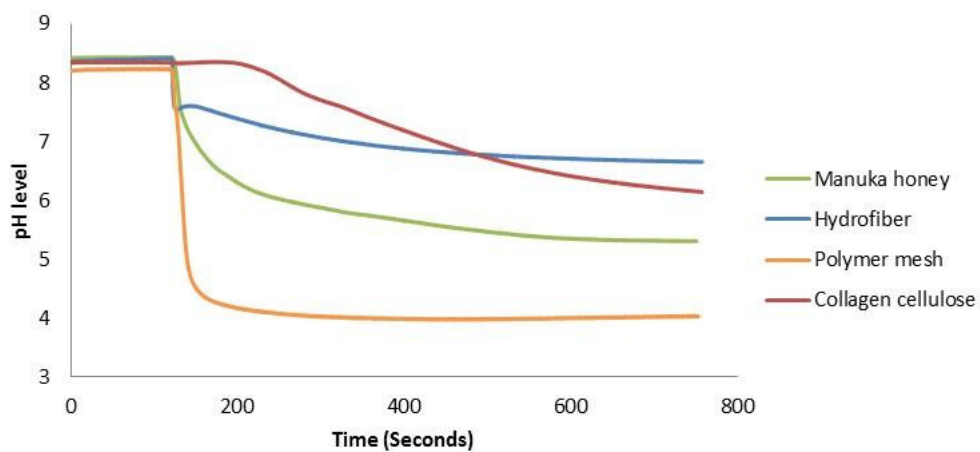


Figure 3 Average pH response in horse serum wound bed simulation environment after application of dressing after 120 seconds.

Figure 3 shows the change in pH level when the wound dressings are applied after 120 seconds. The pH response over the time period details the real time pH response of the horse serum/Solution A mixture after application of the dressings. The responses shown are the average pH response from three experiments on each dressing type. The protease modulating collagen cellulose dressing takes 1800 seconds to reach its final value of pH 5.41 due to the extended time for settling to a steady pH only the first 800 seconds of response is shown in **figure 3**.

Dressing type	pH in horse serum of wound dressings \pm SD	pH change of Horse serum after immersion of wound dressing \pm SD
Manuka honey	5.30 \pm 0.08	3.09 \pm 0.09
Sodium carboxymethylcellulose hydrofiber	6.65 \pm 0.08	1.71 \pm 0.1
Protease modulating collagen cellulose	5.41 \pm 0.18	2.93 \pm 0.19
polyhydrated, ionogen-coated, polymer mesh dressing	4.02 \pm 0.06	4.18 \pm 0.19

Table 2 Summary of pH change after application of wound dressing

Table 2 details the pH of the pH change induced in a horse serum simulated wound bed. The results shown are the mean values of pH response after n=3 measurements in the wound bed simulation environment. The second column details the average pH of the horse serum solution measured after 20 minutes. The third column details the average change in pH from before application of dressing to the final measured pH after dressing application.

Discussion

pH of wound dressings

The four wound dressings when immersion in Solution A show that all the dressings show an acidic pH. The polyhydrated, ionogen-coated, polymer mesh dressing, and protease modulating collagen cellulose dressing are especially acidic at pH 3.25 and pH 2.3 respectively. The pH of these dressings is similar to lemon juice as this has a pH level between 2-3.²⁰ This acidic pH may produce discomfort when applied to the wound.

The pH values recorded in Solution A only indicates the local pH of the wound dressings in an ionic solution but this does not provide information on the total concentration of the acid in the dressings. The final pH at the wound site will depend on the buffering capacity of the exudate in combination with the concentration of acid in each dressing. Higher concentrations of acid within the wound dressings will cause a greater change in pH of the buffered wound fluid. The changes in pH will be closer to the buffered horse serum/Solution A simulated wound bed solution discussed below.

Wound bed simulation

The results show that the buffering capacity of horse serum/Solution A mixture plays a significant role in the pH response influenced by the wound dressings. Wound exudate is composed of fluid leaking from the capillaries containing the proteins found in the blood serum. To use horse serum as a substitute for wound exudate it was diluted with Solution A to give a more realistic protein content and viscosity.²¹ The buffering capability of the horse serum/Solution A mixture prevents the pH change depending on the concentration of the acid used in the wound dressings. The horse serum components are made up of salts (Na^+ , Ca^{2+} , HCO_3^- , Cl^-), proteins (Albumin, globulins), lipids (cholesterol) and water. Of these components the proteins, bicarbonate ions and phosphate ions play a role in buffering the pH of the serum solution.¹⁴

The sodium carboxymethylcellulose hydrofiber dressing is the weakest acidic concentration out of all dressings tested it also has the highest pH out of all tested in Solution A. From this data it is predicted that the sodium carboxymethylcellulose hydrofiber dressing will not have much influence on the pH of a wound the dressing. It is chiefly meant to hydrate the wound and does not make any claim of modifying properties other than maintaining moisture in the wound.

The protease modulating collagen cellulose dressing induced a pH change of 2.93 in the horse serum/Solution A. The pH response of protease modulating collagen cellulose dressing shows that it is the slowest of all dressings to alter pH after initial immersion in the simulated wound bed solution. These results suggest that the protease modulating collagen cellulose dressing has a slow release/mixing of pH modifying ingredients when in contact with the pH buffering horse serum solution.

The manuka honey dressing produced the second highest change in pH when used in the horse serum/solution A wound bed simulation. The overall change in horse serum solution pH was 3.09 after the application of the dressing. The honey naturally includes a number of acidic components, with gluconic acid having the highest concentration, each honey has different amounts and types of acid depending on honey type and concentration.²² It has been reported that the acidity of honey is the main factor in its antibacterial properties.²³ The dressings are covered in a thick coating of honey that will result in higher molar concentration of the acids in the honey which will account for the large change in pH in the horse serum solution. This coating also is responsible for the fast initial drop in pH with the honey being able to mix easily with the horse serum/Solution A.

The polyhydrated, ionogen-coated, polymer mesh dressing showed the greatest change in pH of 4.18. The dressing surface is a mesh that the active components are coated around this results in faster dissolving of the active components on contact with the horse serum/Solution A, this can be observed with the fast drop in pH after dressing application. The data sheets for the polyhydrated, ionogen-coated, polymer mesh dressing state that it uses citric acid to lower pH, however, it does not state the concentration. The large change in pH indicates that the polyhydrated, ionogen-coated, polymer mesh dressing has the strongest acid concentration out of all the

dressings tested. When applied to a wound the polyhydrated, ionogen-coated, polymer mesh dressing will produce the largest change in pH.

The low pH found across a broad range of commonly used wound dressings raises further questions about how the pH of these dressings will influence the healing process. The reduction of pH has been previously shown in an in vitro model to reduce the proliferation of the MMP's.⁸ Elevated MMP activity has been found to cause delayed wound healing through degradation of collagen matrixes vital to the healing process.²⁴¹⁹ Protease activity peaks at between pH7 to pH8 and decreases rapidly in the presence of acidity. If wound pH is reduced from pH 8 to a pH of 4 then the MMP enzyme activity would drop by 80%.⁸ The low pH of the MMP modifying dressings (polyhydrated, ionogen-coated, polymer mesh dressing, and protease modulating collagen cellulose dressing) suggests that this plays a major role in reducing the MMP activity.¹⁹

However, studies conducted on cell proliferation and migration of fibroblasts and keratinocytes have shown that pH has a large influence of over these important wound healing processes. It was found that optimum proliferation of these cells occurs between a pH of 7.2 and 8.3 with a sharp reduction of cell proliferation out with this pH range.⁷ Fibroblast cell migration rate was also found to be reduced in an acidic wound environment.²⁵ These cellular studies suggest that the cells need a neutral pH close to the body's internal pH level of 7.4 to perform at their optimum level.

The studies on the effect of pH on protease level, cellular migration and cell proliferation give contrasting data about the optimum pH for different stages of the healing process. An acidic pH will help reduce protease activity but will act to reduce cell migration and proliferation in the wound. Further research needs to be conducted to understand how the pH of these dressings potentially effects the vital components of the healing process.

Conclusion

The results of this investigation highlight the potential of wound dressings to change the pH of the wound healing environment. The dressings investigated displayed

different pH levels, on initial application and over time, in a simulated wound bed solution and had differences in total acidic concentration which indicates that the choice of dressing can be an important factor in influencing the pH conditions within a wound. The effect of altering the pH in the wound is not fully understood and needs to be studied further to determine the optimal pH level for wound healing. One important aspect of that will be to have the ability to monitor pH temporally in real wound environments and the printed pH sensor described in this work could be adapted for such studies. With further research into its effects the monitoring and controlling of pH could play an important future role in objective wound healing treatments.

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