The Effects of a Hydroconductive Dressing on Wound Biofilm

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Bacteria possess the ability to cause infection in two very distinct ways.[1] The first way is when an individual bacterium with its unique genome uses one portion of its genes to stay a free-floating, motile cell (planktonic phenotype) that has a strategy in a host environment to breach and kill cells with its virulence factors to create a source of nutrition. The second way is that the very same bacterium can up-regulate a separate group of genes, which lets it attach to a host structure. Once attached to the host, the bacterium secretes a polysaccharide matrix around itself and its progeny. When this small group reaches a sufficient number (quorum), signaling molecules (quorum-sensing molecules) direct the gene expression of each bacterium throughout the colony. This lets a community of bacteria develop within the protection of the matrix, which gives colony defenses against host immunity, including antibodies and white blood cells.[2] Given that a biofilm requires attachment, it cannot use the host tissue to which it is adhered for a nutritional source and, therefore, successful biofilm uses local inflammation to produce plasma exudate on which it can nourish itself.[3]

Excess exudate causes poor wound healing outcomes. Many strategies have been employed to decrease wound exudate including antibiotics, topical antiseptics, edema management and control of inflammation. However, most wound care strategies include removal of the exudate once it is formed. For decades, moist, interactive wound care has been utilized to manage exudate to improve wound healing. [4,5] It would be of great importance if a dressing had the ability not only to manage the exudate, but also to suppress the formation of the exudate at its source.

Chronic wounds have a large amount of biofilm on their surfaces and acute wounds have very little biofilm.[6] The presence of biofilm is sufficient to explain the hyperinflammatory milieu that is the hallmark for chronic wounds. Chronic wounds have elevated proinflammatory cytokines such as tumor necrosing factor, gamma interferon, interleukins 1, 6 and 8, and a host of other inflammatory cytokines.[7] The chronic wound environment is also highly proteolytic, with elevated levels of matrix metalloproteinases (MMPs) 2, 8 and 9, along with elastase.[8] Additionally, at a cellular level, chronic wounds are associated with excessive neutrophils.[9] This biochemical and cellular phenomenon of the chronic wound is also seen in other chronic infections.

Another strong argument for biofilm's role in the nonhealing of wounds is host cellular senescence. Cellular senescence is evident by host cells that are unable to undergo cell division (shed),[10] unable to migrate[11] and, most importantly, unable to apoptose.[12] Apoptosis is the strategy the host uses to clear damaged or infected cells. By producing wound bed senescence, the biofilm prevents the host from removing the secure attachment for the biofilm while also preventing the wound's healing.

The activity of biofilm is controlled by quorum sensing molecules that diffuse throughout the biofilm community. The nutritional source is host plasma. Therefore, decreasing the dwell time of the plasma and other fluids within the wound biofilm may diminish the ability of biofilm to produce host inflammation, host cell senescence and subvert host immunity.

Other technologies targeting rapid removal of wound exudate include negative pressure wound therapy. In previous studies, it was shown that the bacterial numbers increased with negative pressure wound therapy. Yet, there was significant improvement in wound healing outcomes. There was no evidence that this was due to decreased dwell time for quorum-sensing molecules or nutrient molecules from the plasma.

Our study focused on the ability of a dressing with the properties of being able to generate high capillary pressures capable of the rapid removal of wound exudate. It was hoped that, with the rapid removal of wound exudate, the biofilm's ability to produce persistent inflammation and host cellular senescence would be diminished. It was also important to determine if rapid removal of exudate decreased bacterial numbers on the surface of the wound.

Methods

Ten patients with nonhealing, moderate to highly exudative venous leg ulcers (lasting more than 30 days) were identified and consented to participate in a small cohort study (Western IRB

TABLE 1. A significant reduction is seen in wound volume for nine of the 10 patients in the study. Two patients actually went on to full wound healing within the 4 weeks of the study.

Patient ID	Initial Volume (cm^2)	Final Volume (cm^2)	% Healed
22517	0.07	0.00	100.0%
22632	1.10	0.09	91.7%
9510	2.18	2.14	1.7%
23008	0.48	0.28	41.6%
23262	9.43	4.75	49.6%
16358	5.58	3.01	46.1%
13711	0.08	0.00	100.0%
3035	1.82	1.11	39.0%
15623	1.18	0.23	80.5%
22822	2.94	0.89	69.7%
		Avg	62.0%

TABLE 2. The beginning cycle threshold (CT) numbers compared with the final CT numbers for the 10 evaluable patients are shown. The CT number indicates how many times the sample had to be doubled before a signal could be obtained. The number of doublings required to obtain a signal is directly related to how much of the target DNA is in the original sample. The more bacteria present, the smaller the CT number. Four patients showed an increase in bacteria over the 4 weeks of the study.

Patient ID	Initial Cycle Threshold Number	Final Cycle Threshold Number	Bacteria Change
23262	25.73	26.10	Less
15623	28.50	28.31	More
2308	16.81	27.05	Less
3035	18.75	26.99	Less
16358	19.95	18.28	More
9510	28.85	19.78	More
22822	22.85	24.51	Less
22632	27.63	22.24	More
13711	27.11	0	Less
22517	27.41	0	Less

#20101569). The average age of the study participants was 56.3 years (42 years old to 68 years old). There were six males and four females, and four of the patients were under management for diabetes. There were no other significant comorbidities.

Each patient was subjected to evaluation at each visit (weeks 0, 1, 2, 3, and 4) for a total of five visits over a 4-week period. At weeks 0 and 4, all wound metrics recorded and 5 mm punch biopsies were performed for comprehensive molecular evaluation (polymerase chain reaction [PCR] and sequencing), plus scanning electron microscopy. The molecular diagnostics were conducted by PathoGenius Laboratories. The biopsies were sent for scanning electron microscopy evaluation at the Center for Biofilm Engineering.

All wounds were managed under a general treatment regimen that included standard-of-care techniques. Measurements were obtained using Aranz Silhouette (Aranz Medical) equipment adhering to the manufacturer's recommendation. The venous leg ulcers were assessed clinically, and then cleaned with a nontoxic, non-antimicrobial product. The wounds were then sharply debrided to manage the surface accumulation of slough, devitalized tissue, and any other debris. DrawTex dressings were then applied. A multilayer compression wrap was then applied to provide management of lower-limb edema. The dressings were changed on a Monday/Wednesday/Friday basis until the next clinic visit.

Results

Table 1 shows that nine of 10 patients showed 40% or more healing within the 4-week duration of the study. Only one wound failed to heal, but it did not show any deterioration. Two wounds healed completely, and one wound healed 92%.

To quantify the amount of bacteria on the wound pre- and post-treatment, real-time PCR methods were used. As seen in **Table 2**, two of the patients healed and, of the remaining eight, four had slight increases in bacterial numbers, and four had some decreases in the number of bacteria. Given that the real-time PCRs on the pre- and post-treatment samples were run on the same plate, the cycle threshold numbers are comparable.

Discussion

The use of the Drawtex hydroconductive dressing did improve clinical outcomes. There was less maceration and less erythema of the wounds. More importantly, their wound healing trajectories improved: three wounds were healed or almost healed within the 4-week duration of the study. This is better than expected for these types of chronic wounds.

There did not seem to be a significant correlation between the reduction of wound biofilm and wound healing. This does not preclude the possibility that decreasing dwell time of the exudate diminished the effect of the biofilm on the host wound. In fact, drying the wound biofilm may artificially increase the density of bacterial cells within the sample taken. This would be reported as an increase in bacterial numbers per gram of tissue. Regardless, the positive effects on healing from the rapid removal of wound exudate do not appear to be dependent on the reduction of bacterial numbers.

The ability of the hydroconductive dressing to rapidly remove wound exudate improves wound healing, but not by the mechanism of reducing the number of bacteria present. Therefore, further investigation will need to be conducted, possibly focusing on microbial and host transcriptomes, to determine if the rapid removal of exudate is related to nutrient depletion, disruption of quorum sensing, or unknown mechanisms. ■

References

1. Kim M, Ashida H, Ogawa M, et al. Bacterial interactions with the host epithelium. *Cell Host Microbe*. 2010;8(1):20–35.
2. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science*. 1999;284(5418):1318–1322.
3. Wolcott RD, Rhoads DD, Dowd SE. Biofilms and chronic wound inflammation. *J Wound Care*. 2008;17(8):333–341.
4. Ratliff CR. Wound exudate: an influential factor in healing. *Adv Nurse Pract*. 2008;16(7):32–35.
5. Hourigan LA, Linfoot JA, Chung KK, et al. Loss of protein, immunoglobulins, and electrolytes in exudates from negative pressure wound therapy. *Nutr Clin Pract*. 2010;25(5):510–516.
6. James GA, Swogger E, Wolcott R, et al. Biofilms in chronic wounds. *Wound Repair Regen*. 2008;16(1):37–44.
7. Gohel MS, Windhaber RA, Tarlton JF, et al. The relationship between cytokine concentrations and wound healing in chronic venous ulceration. *J Vasc Surg*. 2008;48(5):1272–1277.
8. Trengove NJ, Stacey MC, MacAuley S, et al. Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. *Wound Repair Regen*. 1999;7(6):442–452.
9. Diegelmann RF. Excessive neutrophils characterize chronic pressure ulcers. *Wound Repair Regen*. 2003;11(6):490–495.
10. Preston GM. Metropolitan microbes: type III secretion in multihost symbionts. *Cell Host Microbe*. 2007;2(5):291–294.
11. Mills E, Baruch K, Charpentier X, et al. Real-time analysis of effector translocation by the type III secretion system of enteropathogenic Escherichia coli. *Cell Host Microbe*. 2008;3(2):104–113.
12. Mimuro H, Suzuki T, Nagai S, et al. Helicobacter pylori dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach. *Cell Host Microbe*. 2007;2(4):250–263.