Abstract: Although excessive exudate has been associated with poor wound healing outcomes, exudate is still not well understood in the pathophysiology of chronic wounds. Wound exudate is believed to be the result of wounds that are trapped in a persistent, hyper inflammatory state. Biofilm, bacteria of multiple species living in community, has multiple well-defined molecular pathways that produce hyper inflammation. The exudate that is produced in wounds is a potentially important nutrient source for biofilm; therefore, rapidly removing exudate may rob the biofilm of important nutrients and suppress its negative effects. Methods. A hydroconductive fiber dressing that possesses excellent capillary action properties was utilized to rapidly remove wound exudate in 10 patients. Results. The data demonstrate an average 62% reduction in wound volume for these 10 wounds over a 4-week period. Two wounds completely closed during the 2 weeks, and all but 1 wound significantly improved. Only 6 of the 10 wounds showed fewer bacteria at the end of the 4-week study period, suggesting there is not a 1:1 correlation with reduction in the number of bacteria in the wound and wound healing. Conclusion. Rapid removal of the nutrient source from wound biofilm, while not diminishing the number of bacteria, may suppress a biofilm’s negative effects on wound healing.

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Exudate, a fluid rich in protein and other nutrients that oozes out of blood vessels due to inflammation, is not well understood in the pathophysiology of chronic wounds. There is agreement as to how exudate forms, but a consensus does not exist as to why exudate occurs, and more importantly, what it means to the nonhealing wound.1 This study will attempt to explore possible roles that exudate may play as a barrier to wound healing.

Increased exudate has negative connotations for wound healing. Antibiotics and biocides can decrease exudate, as can the management of edema and inflammation, but most management strategies rely on methods to remove exudate locally after it has formed. Through trial and error, it has been firmly established that chronic wounds heal better if exudate is managed aggressively.2,3 Therefore, it becomes critical for clinicians to understand exudate at the cellular and biochemical level.
Chronic wounds are stuck in a chronic, hyper-inflammatory state. Biochemically, there is a 100- to 1000-fold increase in proinflammatory cytokines, such as gamma interferon, tumor necrosis factor-alpha (TNF-α), interleukin 1, interleukin 6, interleukin 8, and many others. At the cellular level, the most notable abnormality is an increase in polymorphonuclear cells, up to 1000 times greater than normal levels. Additionally, it is important to note that these neutrophils have lost their chemotactic abilities and cannot be cleared by macrophages. This results in retention of neutrophils in a wound bed that will eventually die and release elastase, metalloproteinase-8 (MMP-8), and other matrix metalloproteinases (MMPs) into the local wound milieu. There are many well-defined molecular mechanisms demonstrating that bacteria in biofilm phenotype can, and do, produce all of these biochemical and cellular effects in many host tissue environments.

Biofilm transformation begins when a free-floating bacterium (planktonic) attaches to a surface. The bacterium divides and quickly coats itself with a self-secreted matrix of sugary polymers (extracellular polymeric substance [EPS]). The EPS binds the group of bacteria to the host surface and protects the society from white blood cells, antibodies, and compliment, as well as antibiotics and biocides. These biofilm defenses allow bacteria to cause a persistent infection in a host environment.

Kim and colleagues reviewed the molecular pathways necessary to cause human infection by bacteria in biofilm phenotype. Some pathways are necessary for attachment and the secretion of EPS, but just as importantly, multiple pathways are hijacked, usurping the cellular functions of host immune cells, as well as wound bed cells. The bacteria possess specific effector proteins that block cell migration, manufacturing, mitosis, and most importantly, prevent cell death.

The biofilm must maintain a stable attachment in order to successfully infect the host, otherwise it will slough off. Biofilm does this by producing host cell senescence, but this also means that biofilm cannot use the local host tissue for nutrition. The biofilm community has solved this problem by developing multiple tools to increase proinflammatory cytokines and increase neutrophils to sustain a hyper inflammatory environment. It is this inflammation that causes plasma to leak from the local vascular bed, providing a sustainable source of nutrition for the biofilm. What clinicians see as exudate in the clinical setting is plasma percolating up through the biofilm.

Keypoints
- Through trial and error, it has been firmly established that chronic wounds heal better if exudate is managed aggressively. Therefore, it becomes critical for clinicians to understand exudate at the cellular and biochemical level.
- Multiple tools have been developed to increase pro-inflammatory cytokines and increase neutrophils to sustain a hyper inflammatory environment. It is this inflammation that causes plasma to leak from the local vascular bed, providing a sustainable source of nutrition for the biofilm. What clinicians see as exudate in the clinical setting is plasma percolating up through the biofilm.

Behavior of chronic infections, waxing and waning, incompletely responding to antibiotics, and persistence are best explained by the behavior of biofilm. Chronic infections demonstrate increased MMPs, increased proinflammatory cytokines, and increased neutrophils. Specifically targeting biofilm improves situations of chronic infection. Similarly, it has been shown that suppressing wound biofilm improves wound healing outcomes. Enhanced wound healing, as a result of suppressing wound biofilm, demonstrates that biofilm is indeed a barrier to wound healing.

It is necessary to address the infecting organisms with any infection. A prudent clinician will augment host factors (control blood sugar, improve nutrition, improve perfusion, offload), but the biofilm must be targeted. The most obvious impulse is to directly attack the microbes with antibiotics and biocides; however, biofilm possesses multiple effective countermeasures. Despite these countermeasures, a simple, well-established strategy is to deplete the biofilm’s nutritional source. Corticosteroids, TNF-α inhibitors, and immunosuppressants, can suppress exudate by reducing inflammation, but they are commonly associated with significant negative consequences to wound healing. A more readily available strategy is the rapid removal of exudate from the wound.

The purpose of this study was to determine if the rapid removal of exudate using a hydroconductive dressing has the ability to suppress wound biofilm, and thereby improve wound healing.

Methods
A total of 10 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 #20101569). The aver
The age of the study participants was 56.3 years (range, 42–68 years). There were 6 men and 4 women, and 4 of the patients were under management for diabetes. There were no other significant comorbidities.

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

All wounds were managed under a general treatment regimen, which included standard of care techniques. Measurements were obtained utilizing Aranz Silhouette™ (Aranz Medical Ltd, Christchurch, New Zealand) equipment adhering to the manufacturer’s recommendations. Venous leg ulcers were assessed clinically and then cleansed with a nontoxic, nonantimicrobial product. Low adherent, hydroconductive wound dressings (Drawtex®, BeierDrawtex Healthcare [Pty], Ltd, Pretoria, South Africa) were then applied. A multilayer compression wrap was then applied to manage lower limb edema. The dressings were changed on a Monday/Wednesday/Friday basis until the next clinic visit.

**Results**

An important metric in any clinical trial, even a pilot study, is wound healing outcome. In this small cohort, there was a 62% average reduction in wound volume over a 4-week period (Table 1). Only one wound showed a limited response. Nine of the 10 patients showed a significant reduction in wound volume in the 4 weeks of the study. Two patients’ wounds healed completely during the study period. Since the study regimen produced good clinical outcomes, the question was whether suppression of biofilm led to this favorable response.

Two different molecular methods were utilized to evaluate the wound bioburden. Polymerase chain reaction (PCR) methods were utilized for all samples, which were evaluated under exact conditions (ie, on the same plate), and therefore should be quantitative. Real time PCR is conducted with probes and primers that produce a quenchable light signal with each cycle. When enough of the amplified DNA is present, the light signal becomes detectable, which is called the cycle threshold (Ct). A higher Ct number means more amplification was necessary to obtain the light signal, thus, a smaller amount of initial DNA was present. Therefore, the greater the Ct number, the lesser the number of bacteria in the sample. Six of the 10 patients showed fewer numbers of bacteria at the end of the study, which means that 4 patients actually showed an increase in the number of bacteria within the sample taken (Table 2).

A second method exploiting pyrosequencing was also utilized. This provided the advantage of identifying

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**Keypoints**

- Venous leg ulcers were assessed clinically and then cleansed with a nontoxic, nonantimicrobial product. The wounds were sharply debrided to manage the surface accumulation of slough, devitalized tissue, and any other debris. Low adherent, hydroconductive wound dressings (Drawtex) were then applied. A multilayer compression wrap was then applied to manage lower limb edema.
- Nine of the 10 patients showed a significant reduction in wound volume in the 4 weeks of the study. Two patients’ wounds healed completely during the study period.

**Table 1.** A significant reduction in wound volume was seen in 9 of the 10 patients in the study. Two patients’ wounds healed completely within the 4-week study period.
all the microbes present in each sample. The initial and final samples were compared to assess their similarity using the dendrogram statistical methodology. It should be noted that in 4 patients the heat map evaluation could not be evaluated because 2 of the patients had healed, so there was no final sample. The 2 remaining samples had such low bacterial DNA in their final sample that a valid analysis could not be obtained. Of the remaining 6 subjects, 4 showed fewer bacteria and less diversity after treatment, and 2 patients showed more bacterial numbers at the end of treatment (Figure 1). There is a tendency (not statistically significant) for a reduction in microorganism diversity after treatment with hydroconductive dressings.

Figure 1. The first 4 subjects represented in this heat map did not have any final sample to compare against because of complete wound healing or very little bacterial DNA, which was used for the PCR testing. Of the remaining 6 subjects, 4 show less bacteria and less diversity after treatment. There is a tendency, but not a statistical significance, for a reduction of microorganism diversity after treatment with hydroconductive dressings.

Figure 2. Biopsies were taken at the first and final visits in an effort to qualitatively assess the wound bed surface, particularly wound biofilm pre- and post dressing. However, fibers and particulate matter from the dressing confounded this comparison. It is interesting to note that the biofilm, as well as white blood cells, is nestled in the residual dressing fibers.

The biopsies were also sent for scanning electron microscopy (Figure 2). It was clear from the initial biopsies that the dressing did leave residual fibers and particulate matter on the surface of the wounds. The residual material was often laden with well-developed biofilm, but it
from the clinical outcomes that this did not disrupt host healing. This paradoxical finding will require further investigation. Finally, the qualitative evaluation of each wound showed that there was less maceration, less inflammation, and more scaling (Figure 3A, B). The rapid removal of exudate had a positive effect on the clinical appearance of the wounds. There were no adverse events reported by any of the 10 patients for the duration of the study.

Discussion

The rapid removal of exudate utilizing a hydroconductive dressing did improve the clinical qualities of the wound bed. Most notably, there was less maceration and less erythema. Fluid removal may reduce inflammation, and therefore, improve wound healing by removing matrix metalloproteases and inflammatory cytokines. The decrease in erythema may indicate that the biofilm is producing less inflammation. Perhaps the most important observation is that 2 wounds healed rapidly within the 4 weeks of the study.

The hydroconductive dressings exert the physical property of a very strong capillary force upon fluids. This property can be utilized in wound care to decrease dwell time of nutrient rich plasma exudate within the wound bioburden. It seems reasonable to assume that decreasing the contact of plasma exudate with wound biofilm may suppress biofilm activity and improve wound healing. The hydroconductive dressings had a positive effect on wound healing by reducing wound volume, yet only 6 of the 10 patients also showed reduction in bacteria present (Table 2), and only 4 out of 6 wounds showed reduction.

Table 2. The initial cycle threshold (Ct) number versus the final Ct number for the 10 patients that were evaluable. The cycle threshold number indicates how many times the sample had to be doubled before a signal could be obtained. The more 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-week study period.

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Keypoints

• It was clear from the initial biopsies that the hydroconductive dressing did leave residual fibers and particulate matter on the surface of the wounds. The residual material was often laden with well-developed biofilm, but it seems from the clinical outcomes that this did not disrupt host healing. This paradoxical finding will require further investigation.

• These data clearly show an improvement in wound healing for 90% of the study subjects (2 completely healed over 4 weeks), although this healing is not a 1:1 correlation with reduction of wound biofilm.
in diversity of organisms (Figure 1). The molecular results failed to demonstrate any correlation between reduced bacterial numbers or reduced diversity with improved wound healing.

These data clearly show an improvement in wound healing for 90% of the study subjects (2 completely healed over 4 weeks), although this healing is not a 1:1 correlation with reduction of wound biofilm. This may mean that the hydroconductive dressing also impacted wound healing by mechanisms unrelated to biofilm. Confounding factors may include increasing the density of the wound biofilm through the dehydration caused by the dressing itself. Drying out the wound biofilm may spuriously increase bacterial density, which would yield a smaller Ct number, and is suggestive of more bacterial presence. A second caveat is that regardless of the quantity of bacteria present, the rapid removal of the nutrient source may suppress the activity of the remaining bacteria; thus, the positive healing effect may not be dependent upon a reduction of bacterial numbers, but rather suppressing their activity. Future research regarding the activities and interactions of individual bacteria will be necessary.

Conclusion

Hydroconductive fibers may suppress wound biofilm through the putative property of rapid exudate removal, resulting in nutrient depletion. This would lead to a less robust wound biofilm that would not be as capable of inhibiting host-healing pathways. A trial focusing on the microbial and host transcriptomes will be necessary in order to fully understand the mechanisms and importance of these possible mechanisms for suppressing wound biofilm.

Acknowledgement

Disclosure. Dr. Wolcott owns stock in PathoGenius Laboratories.

References