Disrupting the biofilm matrix improves wound healing outcomes

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**Objective:** The most unyielding molecular component of biofilm communities is the matrix structure that it can create around the individual microbes that constitute the biofilm. The type of polymeric substances (polymeric sugars, bacterial proteins, bacterial DNA and even co-opted host substances) are dependent on the microbial species present within the biofilm. The extracellular polymeric substances that make up the matrix give the wound biofilm incredible colony defences against host immunity, host healing and wound care treatments. This polymeric slime layer, which is secreted by bacteria, encases the population of microbes, creating a physical barrier that limits the ingress of treatment agents to the bacteria. The aim of this study was to determine if degrading the wound biofilm matrix would improve wound healing outcomes and if so, if there was a synergy between treating agents that disrupted biofilm defenses with Next Science Wound Gel (wound gel) and cidal agents (topical antibiotics).

**Method:** A three-armed randomised controlled trial was designed to determine if standard of care (SOC) was superior to SOC plus wound gel (SOC + gel) and wound gel alone. The wound gel used in this study contains components that directly attack the biofilm extracellular polymeric substance. The gel was applied directly to the wound bed on a Monday–Wednesday–Friday interval, either alone or with SOC topical antibiotics.

**Results:** Using a surrogate endpoint of 50% reduction in wound volume, the results showed that SOC healed at 53%, wound gel healed at 80%, while SOC plus wound gel showed 93% of wounds being successfully treated.

**Conclusion:** By directly targeting the wound biofilm matrix, wound healing outcomes are improved.

**Declaration of interest:** None declared

Chronic wounds, regardless of the aetiology, exhibit similar clinical behaviours, such as stalled healing and exudate production, which are directly related to microorganisms on the wound surface growing as a biofilm. It is now widely recognised that all chronic infections are produced by microorganisms in the biofilm mode of growth. How biofilm produces a host infection is now well defined at a molecular, cellular and clinical level.

There are countless molecular strategies used by bacteria, yeast and fungus to produce host infection, which we see clinically as chronic infection. Of the subcellular pathways employed by biofilm, four areas of research hold great promise for wound care. The first is how and why microbes express adhesins, surface complexes that target host tissues, to allow them to attach to a host environment. Second is the vast array of communication molecules (quorum sensing) produced by different species of microbes to organise the activity of the entire biofilm. Third is the amazing variety of ‘effectors’ (small proteins), which bacteria can secrete from a number of different secretory systems (for example, T3SS and T6SS), which take over the function of the wound bed cells. By producing cellular senescence, with these effectors, the biofilm establishes a stable attachment site from which to continue its persistent infection.

However, the most formidable molecular activity of biofilm communities is the matrix structure it can create around the individual microbes that constitute biofilm. The molecules used to make up this slimy covering are generally polymeric sugars (for example, poly-n-acetylglucosamine), microbial and/or host DNA, microbial proteins and host molecules (for wounds, primarily fibrinogen). The type of polymeric substances, polymeric sugars, proteins, DNA and even co-opted host molecules are dependent on the microbial species present within the biofilm. Whatever the source of molecules, the extracellular polymeric substances (EPS) that make up the biofilm matrix give the wound biofilm very strong defences against host immunity, host healing and wound care treatments.

The majority of the resistance of the bacteria in a biofilm population is conveyed by the EPS matrix. This polymeric slime layer creates a physical barrier that limits the entry of treatment chemicals to the bacteria. In addition, RNA, proteins, and waste products excreted by the bacteria contained within the EPS matrix react with active treatment chemicals,
Fig 1. Suspension time kill — bacterial counts after treatment

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Plate Counts (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0.0E+00</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1.2E+07</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>1.0E+07</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>6.0E+06</td>
</tr>
<tr>
<td>Enterobacter cloaceae</td>
<td>5.0E+06</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>4.0E+06</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>3.0E+06</td>
</tr>
</tbody>
</table>

The polyethylene glycol (PEG)-based hydrogel creates a moist environment that promotes granulation, epithelialisation, and autolytic debridement. It also prevents tissue dehydration and cell death (necrosis and apoptosis), increasing angiogenesis, and breakdown of dead tissue and fibrin.

Once the solution has accessed the bacteria within the biofilm, the high osmolarity solution creates an osmotic imbalance across the bacterial cell wall membrane, causing the cell wall to become more permeable and exposing proteins on the cell wall membrane to the surfactant. The surfactant molecule directly lyases the cell wall membrane by pulling these proteins into solution.

In a study by Miller et al., the antimicrobial components within the gel directly attack the biofilm EPS matrix and also lyse the bacteria contained within the matrix. The molecules of the EPS matrix can be ionically or covalently cross-linked. In the ionic case, the acid component within the gel chelates the metal ions which form the ionic cross-links. This allows the individual EPS molecules to go into solution, aided by the surfactant components. When covalent cross-linking takes place, the acid component hydrolyses the covalent bonds, although at a slower rate than for ionic cross-link dissolution. The high osmolarity solution at acidic pH additionally induces a great deal of swelling within the EPS matrix, facilitating access of the treatment chemicals to the bacteria within the biofilm.

The wound gel has been demonstrated to be antibacterial through a number of in vitro and in vivo efficacy tests, including suspension time kill, biofilm drip flow reactor, and in vivo chronic wound testing. Suspension time kill testing of the wound gel demonstrated broad-spectrum efficacy against a number of bacterial and fungal pathogens. As can be seen in Fig 1, the gel completely eliminated ~6 log bacterial inoculations of all of the tested planktonic bacteria and fungi in one hour.

To demonstrate efficacy against biofilm, drip flow reactor biofilm testing of a three-day biofilm were performed at the Montana State University Center for Biofilm Engineering, using this gel. The product was demonstrated to achieve a 5.8 log reduction in Pseudomonas aeruginosa and a 3.5 log reduction in Staphylococcus aureus in a 24-hour application (unpublished data). In vivo, the gel was tested in a murine model of wound biofilm infection at Texas
Tech University using the method of Miller et al. In this study, the wound gel was found to completely inhibit biofilm formation for 72 hours. After the 72-hour treatment, there were no bacteria in the treated wounds, and a higher percentage were healing compared with controls, which were highly inflamed and not closing.

The objective of this study was to determine if degrading the wound biofilm matrix would improve wound healing outcomes. A second aim was to see if there was a synergy between treating agents that disrupted biofilm defences (wound gel) and cidal agents (topical antibiotics).

In the study groups, the wound gel was used either alone on a Monday-Wednesday-Friday (MWF) basis or in conjunction with biofilm-based wound care. The hypothesis was that adding a chemical constituent to continuously degrade and suppress the extracellular matrix might be an important adjunct to manage wound biofilm and improve wound healing outcomes.

Methods
Ethics
This study was submitted to and approved by Western IRB (WIRB STUDY NUM: 1139777, WIRB PRO NUM: 20130982). The study was explained to patients, who gave informed consent before participating in the study.

Inclusion and treatment
Patients met the inclusion criteria if they had a full-thickness wound of any aetiology, for longer than 30 days, that required repetitive debridement.

Patients were randomised, using a locally written computer algorithm, into three study groups:
- SOC
- Wound gel only (gel)
- SOC plus wound gel (SOC + gel).

The control group received SOC biofilm-based wound care treatment. This included an initial evaluation, which identified and mitigated host barriers to healing, such as repetitive trauma, hyperglycaemia and poor perfusion. The wound was debrided and a sample obtained to identify and quantitate the microbes present. The first week an empiric gel (a high-tech drug delivery nanolipid gel named lipogel (Sanguitec gel, Southeast Medical Compounding Pharmacy) containing antibiotic agents (including hamameltannin, xylitol, gallium) as well as antibiotics chosen to cover the most common microbes we have identified in chronic wounds in our geographic region was applied MWF. Once the diagnostics returned the next week, the patient’s treatment consisted of a personalised topical gel covering the identified microbes applied MWF, weekly debridements, and continued management of host healing barriers. Each patient was evaluated and debrided weekly for four consecutive weeks (five visits). Wound measurements were obtained using an Aranz Silhouette device to calculate the wound volume reduction for each wound over the four weeks of treatment for each of the groups. The wound was considered ‘healed’ if there was a reduction in volume of 50% in the four weeks.

Statistical comparisons of the wound volume were obtained by students’ t-test comparisons of the treatments to the SOC control. Statistical comparison of the number of wounds healed by 50% were obtained by chi-square analysis.

Results
There were 45 patients consented as per the protocol. The demographics, of the population can be seen in Table 1. The patients ranged from 23–72 years of age, with the average age being 60, 57 and 63, in the SOC, gel and SOC plus gel groups respectively. The demographics, wound type and size of the initial wound were similar for all three groups.

The volume reduction over four weeks for these groups is shown in Fig 2. This data shows that the volume reduction in the wound gel samples in four weeks was 32% better than the SOC (62% and 47% respectively). The performance combining the SOC and wound gel was even better, with the combination having 53% better performance than the SOC alone (72% and 47% respectively). This improvement of efficacy was statistically significant (p<0.05).

The use of the wound gel improved the success rate for healing of chronic wounds. Using the surrogate end-point of 50% reduction in wound

### Table 1. Demographics and characterisation of each study cohort

<table>
<thead>
<tr>
<th></th>
<th>Standard of care</th>
<th>Wound gel</th>
<th>Standard of care plus wound gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (range)</td>
<td>60 (23–76)</td>
<td>57 (31–67)</td>
<td>63 (39–72)</td>
</tr>
<tr>
<td>Gender male:female</td>
<td>9:6</td>
<td>5:10</td>
<td>7:8</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>6</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Black</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Hispanic</td>
<td>7</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Initial wound size (average)</td>
<td>2.7cm²</td>
<td>2.3cm²</td>
<td>3.1cm²</td>
</tr>
<tr>
<td>Wound type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic foot ulcer</td>
<td>4</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Venous leg ulcer</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Pressure ulcer</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Other*</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

* includes non-healing surgical wound (7), arterial ulcer (1), burn (1) and trauma (4)
volume at four weeks, the percentage of wounds that were successfully healed was determined for each of the treatment groups. The percentage of wounds that were successfully healed shows that the percentage of wounds successfully healed in the wound gel samples in four weeks was 50% better than the standard of care (80% and 53% respectively; Fig 3). This improvement was statistically significant, at a p-value of 0.05. Again, the performance combining the SOC and wound gel was even better, with 93% of the wounds being successfully treated. The combination of the SOC plus the wound gel was 75% higher than the SOC alone (93% and 53% respectively). This improvement of efficacy was statistically significant at a p-value of 0.05.

Discussion

Combining the anti-biofilm strategies of wound gel to degrade the protective biofilm matrix with personalised antimicrobial treatment to target the biofilm constituents significantly improved wound healing outcomes. This should come as no surprise, as research has shown the very thing that makes chronic wounds chronic has been our inability to overcome the defences provided to the wound biofilm by the matrix molecules. Wound gel adds a valuable therapy that specifically targets the molecules of the biofilm matrix which, in turn, degrades the biofilm’s defences.

Wound biofilm generally demonstrates significant diversity of microbial species, including bacteria, yeast and fungus. Because wound biofilm is polymicrobial, along with the protection provided by its molecular shield, a single strategy for therapeutic intervention is often insufficient. The planktonic concept of a single antibiotic or a single biocide to eradicate the microbial pathogen is not valid for chronic infections produced by biofilm phenotype microorganisms. The results of this study confirm this general principle.

In managing wound biofilm, it becomes important to pursue multiple concurrent strategies. These include: physical and chemical means to disrupt wound biofilm supportive structures (matrix); disrupting and preventing attachment of microbial cells; disrupting synergies between different microbial species within the biofilm; disrupting communication language; and applying high continuous concentrations of cidal strategies to the individual microbial cells making up the biofilm.

It is vital that strategies used simultaneously do not interfere with one another. There is no question that the use of silver and iodine in the same wound bed at the same time neutralises the efficacy of both.23 The benzalkonium chloride in wound gel may react with true alginate (not microfibers) but is stable in contact with most other wound care products. This study clearly demonstrated that wound gel retains its efficacy and works synergistically with topical antibiotics and the other biocides used in this investigation.

Limitations

There was no effort to identify biofilm structures within any of the chronic wounds included in this study. Besides cost, the main reason is because the author felt this was unnecessary. The European Guidelines for management of chronic infections state that chronic infections are caused by biofilm phenotype bacteria.2 These guidelines also include chronic wounds as chronic infections and therefore possessing biofilm. Several articles have been published on the clinical indicators of biofilm
infection and each of these wounds exhibited the majority of the characteristics considered as indicators of the presence of biofilm.24,25

The study compared a number of different wound types, suggesting the method works on different chronic wounds; however, a larger, more comparative study is required to confirm the results we saw.

References


Conclusion

In this study of 45 patients, there was a clear pattern of synergy between wound gel and topical antibiotics. This demonstrates the value of multiple simultaneous strategies in the general management of the chronic wound—and that wound gel specifically might be a very effective constituent in wound healing.