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Disrupting the biofilm matrix improves wound healing outcomes

R. Wolcott, M.D., Medical Director of Southwest Regional Wound Care Center; Southwest Regional Wound Care Center, Lubbock, Texas.

Email: Randy@randallwolcott.com

Disrupting the biofilm matrix improves wound healing outcomes

• **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

biofilm; extracellular matrix; Next Science Wound Gel; chronic infection; ulcer; chronic wound

R. Wolcott, M.D., Medical Director of Southwest Regional Wound Care Center; Southwest Regional Wound Care Center, Lubbock, Texas.

Email: Randy@randallwolcott.com

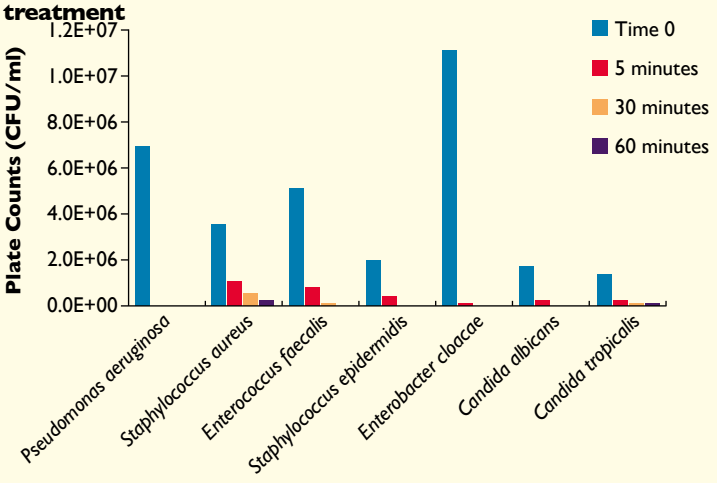
Chronic wounds, regardless of the aetiology, exhibit similar clinical behaviours, such as stalled healing and exudate production, which are directly related to microorganisms on the wound surface growing as a biofilm.[1] It is now widely recognised that all chronic infections are produced by microorganisms in the biofilm mode of growth.[2,3] How biofilm produces a host infection is now well defined at a molecular, cellular and clinical level.[4]

There are countless molecular strategies used by bacteria, yeast and fungus to produce host infection, which we see clinically as chronic infection.[5,6] 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.[7] Second is the vast array of communication molecules (quorum sensing) produced by different species of microbes to organise the activity of the entire biofilm.[8,9] 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.[6,10] 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).[11–13] 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.[14] 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



preventing them from interacting with the bacteria. In addition, the bacteria within a biofilm have developed a number of phenotypic resistance mechanisms. The sessile (attached) bacteria within a biofilm are not actively dividing, conferring resistance to a number of antibiotics. Another unique resistant phenotype are the persistor cells, which are capable of recreating the biofilm after any treatment application that is not completely effective.

Since this pleomorphic matrix confers so much protection to the individual microbial cells, it becomes very important in wound care to include strategies that degrade this protective shield. Debridement has been a mainstay in disrupting biofilm and forcing biofilm to reconstitute itself.[15] Post debridement, when the biofilm microbes are disorganised and inadequately protected by the disrupted matrix, they are forced to become metabolically active to reconstitute the matrix, becoming much more vulnerable to standard treatments such as antiseptics, biocides and antibiotics.[16] These principles anchored our standard of care (SOC) management of the control group of chronic wounds.

Technology background

Existing technologies directed to the treatment of biofilms are intended to either penetrate the EPS that encapsulates the bacteria in a biofilm, or use dispersing agents, which typically target a narrow range of bacterial biofilms.

Next Science wound gel is a wound healing and disinfection technology that has been developed to target biofilms in wounds. The gel both improves wound healing by providing a moist healing environment through the activity of the gel components and it treats biofilms by degrading the EPS. The gel is composed of a number of ingredients; a pH buffer system of an acid and its conjugate base, which is included at a high osmolarity within the aqueous phase of the gel (2,330mOsm/l), and a surfactant (benzalkonium chloride) at a concentration of 0.13% within the gel (w/v).[17] The antimicrobial activity of the gel is directed against the phenotypic form of resistance, primarily by degrading the biofilm matrix, and will not contribute to the growing problem of genotypic resistance.

The polyethylene glycol (PEG)-based hydrogel creates a moist environment that promotes granulation, epithelialisation, and autolytic debridement.[18] It also prevents tissue dehydration and cell death (necrosis and apoptosis), increasing angiogenesis, and breakdown of dead tissue and fibrin.[19,20]

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 lyses the cell wall membrane by pulling these proteins into solution.

In a study by Miller et al,[17] 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 testings 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.[17] 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.[21] 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 antibiofilm agents (including hammamelitannin, 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.[22]

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 ($p<0.05$).

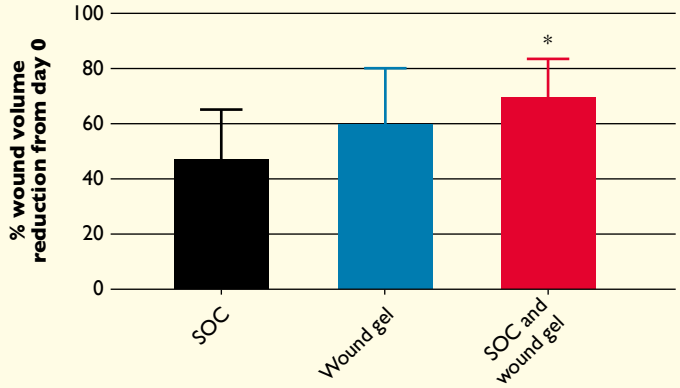
The use of the wound gel improved the success rate for healing of chronic wounds. Using the surrogate end-point of 50% reduction in wound

Table 1. Demographics and characterisation of each study cohort

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

* includes non-healing surgical wound (7), arterial ulcer (1), burn (1) and trauma (4)

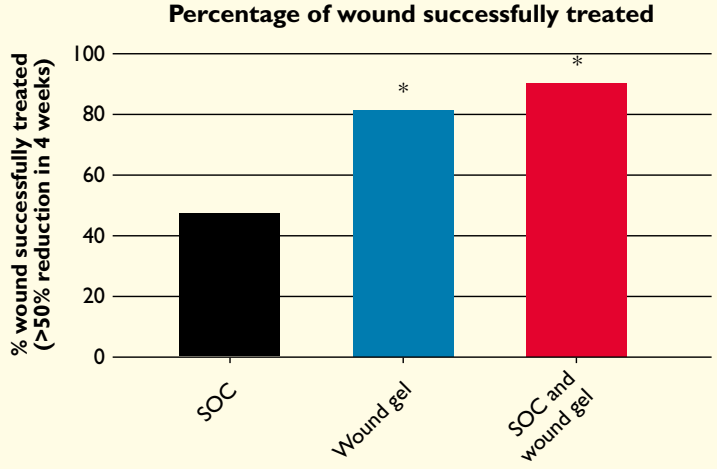
Fig 2. Percentage wound volume reduction in 4 weeks



SOC – standard of care; *denotes p<0.05

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.

Fig 3. Percentage of wounds from each group that healed by more than 50% in four weeks



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 use 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.[3] 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.

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. ■

References

1 GA, J., et al., Biofilms in Chronic Wounds. Wound Repair Regen, 2007. In Press.

2 del Pozo, J.L. and R. Patel, The challenge of treating biofilm-associated bacterial infections. Clin.Pharmacol.Ther., 2007. 82(2): p. 204-209.

3 Hoiby, N., et al., ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. Clin Microbiol Infect, 2015.

4 Wolcott, R.D. and G.D. Ehrlich, Biofilms and chronic infections. JAMA, 2008. 299(22): p. 2682-2684.

5 Kim, M., et al., Bacterial interactions with the host epithelium. Cell Host.Microbe, 2010. 8(1): p. 20-35.

6 Raymond, B., et al., Subversion of trafficking, apoptosis, and innate immunity by type III secretion system effectors. Trends Microbiol, 2013. 21(8): p. 430-41.

7 Bartlett, A.H. and K.G. Hulten, Staphylococcus aureus pathogenesis: secretion systems, adhesins, and invasins. Pediatr Infect Dis J, 2010. 29(9): p. 860-1.

8 Rickard, A.H., et al., Production of cell-cell signalling molecules by bacteria isolated from human chronic wounds. J.Appl.Microbiol., 2010. 108(5): p. 1509-1522.

9 Wolcott, R., Clinical Wound Healing Using Signal Inhibitors, in Control of Biofilm Infections by Signal Manipulation, N. Balaban, Editor. 2008, Springer. p. 157-170.

10 Ding, Z., K. Atmakuri, and P.J. Christie, The outs and ins of bacterial type IV secretion substrates. Trends Microbiol, 2003. 11(11): p. 527-35.

11 Branda, S.S., et al., Biofilms: the matrix revisited. Trends Microbiol., 2005. 13(1): p. 20-26.

12 Hall-Stoodley, L. and P. Stoodley, Evolving concepts in biofilm infections. Cell Microbiol, 2009. 11(7): p. 1034-43.

13 Stewart, P.S. and J.W. Costerton, Antibiotic resistance of bacteria in biofilms. Lancet, 2001. 358(9276): p. 135-138.

14 Whitfield, G.B., L.S. Marmont, and P.L. Howell, Enzymatic modifications of exopolysaccharides enhance bacterial persistence. Front Microbiol, 2015. 6: p. 471.

15 Wolcott, R.D., J.P. Kennedy, and S.E. Dowd, Regular debridement is the main tool for maintaining a healthy wound bed in most chronic wounds. J Wound Care, 2009. 18(2): p. 54-6.

16 Wolcott, R.D., et al., Biofilm maturity studies indicate sharp debridement opens a time-dependent therapeutic window. J Wound Care, 2010. 19(8): p. 320-8.

17 Miller, K.G., et al., Next science wound gel technology, a novel agent that inhibits biofilm development by gram-positive and gram-negative wound pathogens. Antimicrob Agents Chemother, 2014. 58(6): p. 3060-72.

18 Field, F.K. and M.D. Kerstein, Overview of wound healing in a moist environment. Am J Surg, 1994. 167(1A): p. 2S-6S.

19 Velnar, T., T. Bailey, and V. Smrkolj, The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res, 2009. 37(5): p. 1528-42.

20 Diegelmann, R.F. and M.C. Evans, Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci, 2004. 9: p. 283-9.

21 Rhoads, D.D., R.D. Wolcott, and S.L. Percival, Biofilms in wounds: management strategies. J Wound Care, 2008. 17(11): p. 502-8.

22 Margolis, D.J., et al., Surrogate end points for the treatment of diabetic neuropathic foot ulcers. Diabetes Care, 2003. 26(6): p. 1696-700.

23 Cowan, L., Phillips, P., Liesenfeld, B. et al. Caution: when combining topical wound treatments, more is not always better. Wound Practice and Research 2011; 19: 2, 60–64.

24 Wolcott, R.D., et al., Chronic wounds and the medical biofilm paradigm. J.Wound.Care, 2010. 19(2): p. 45-50, 52.

25 Parsek, M.R. and P.K. Singh, Bacterial biofilms: an emerging link to disease pathogenesis. Annu Rev Microbiol, 2003. 57: p. 677-701.