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The Effects of Debridement and Secondary Dressing on Planktonic and Biofilm Protected Bacteria: An Analysis of 3 Studies

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Abstract

Introduction: It has been speculated that bacteria colonizing chronic wounds exist as persistent biofilms and characterized microorganisms. Their presence is often thought to contribute to the poor healing that is seen in clinical practice. Treating infection and reducing microbial colonization with sharp debridement has been the standard of treatment. However, little empiric evidence exists to support these assumptions.

Methods: We combined data points from three separate studies looking at clinically critically colonized wounds; analysing the actual prevalence of biofilm and planktonic bacteria; and the effects of debridement and secondary treatment on this microbiome, over a four week period. Weekly biopsies from 50 subjects were performed to test for quantitative planktonic and biofilm bacteria.

Results: A total of 190 wound biopsies were performed, 90% had identifiable planktonic and 59% had identifiable biofilm bacteria. All biopsies had a diverse polymicrobial community. The most prominent microorganism in planktonic group was *streptococcus* and anaerobic (96%), and *streptococcus* (68%) in biofilm biopsies. We found a significant decrease in both planktonic and biofilm protected bacteria with sharp debridement ($p=0.03$ and $p<0.05$, respectively). Secondary dressing type did not impact the rate of bacterial growth significantly.

Conclusion: Serial debridement with secondary dressing is an effective way to reduce wound bioburden. While identifying bacteria has technical difficulties at times, we believe this to be one of the largest human samplings to date; it gives us an idea that not all chronic lower extremity wounds have a biofilm present. In addition we see the limited effects some therapies have on the microbes.

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Introduction

Microbial bioburden can play a significant role in the persistence of chronic wounds. In a patient with an aberrant or impaired immune system, such as diabetes, the presence of microbes in a chronic wound initiates an immune response intended to destroy the invaders, but, instead, inevitably harms the host and causes further propagation of the wound [1,2]. Historically, the medical community has only evaluated the level of planktonic, or "circulating" bacteria; while more recently focus has moved to evaluating the slow growing but exquisitely hard to eradicate biofilm protected bacteria [Schultz].

The presence of bacteria shifts the keratinocyte to apoptosis [3], and causes local hypoxia by stimulating the release of vasoconstrictive leukotrienes and other cytokines [1,4] while developing an inflammatory state through the development of oxygen free radicals and serine proteases. Acknowledging that in many wounds the balance between the bacteria and the host has shifted in favor of the bacteria, deleterious effects microbial bioburden has on a chronic wound, we look to evaluate the efficacy of debridement and various secondary dressing applications on microbes in both biofilm and planktonic form in chronic wounds.

During the course of three pilot studies we set out to answer two questions: the first question was how big an effect debridement alone has on planktonic bacteria, and how big an effect does it have on biofilm. The second question: are specific dressings or topical therapies alone able to reduce biofilm in the actual chronic wound bed. Within those two questions we asked how different bacteria responded to different therapies.

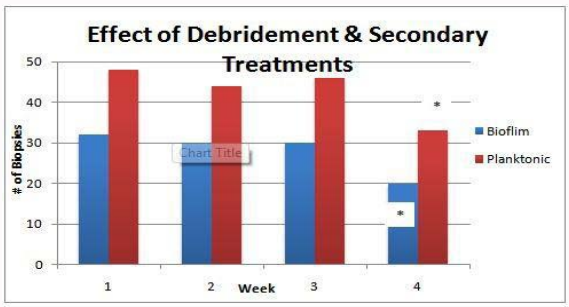

Figure 1: Presence of bacteria in wound biopsies separated by biofilm and planktonic.

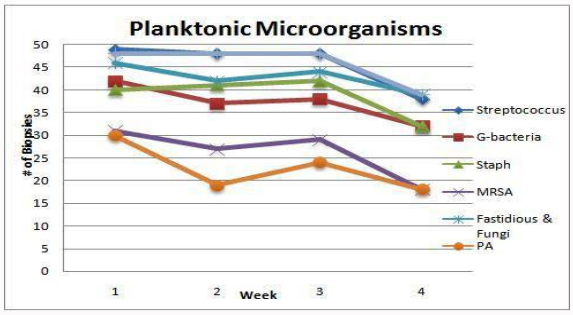

Figure 2: Specific microorganisms in planktonic positive wound biopsies.

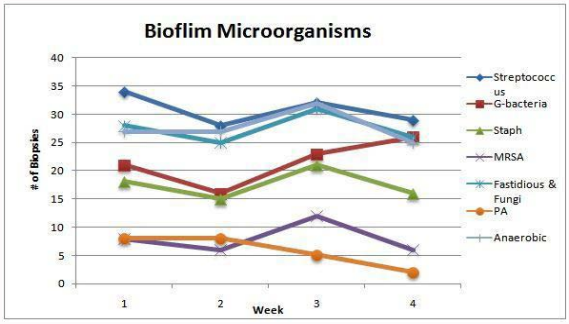

Figure 3: Specific microorganism in biofilm positive wound biopsies.

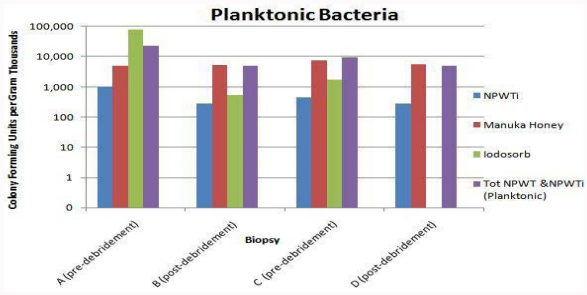

Figure 4: Planktonic Bacterial load stratified by secondary dressing type.

Methods

Over a span of three years data from three different prospective trials with fifty subjects were combined and analyzed to measure the effects of various secondary dressings on wound bioburden after debridement. All patients had before and after debridement wound biopsies performed, as well as after one week of additional topical therapy a third biopsy was performed. Tissue samples for planktonic bacteria were serially diluted, plated on tryptic soy agar media and allow to incubate (proprietary information to the University of Florida, Gainesville, FL), while the tissue sample for biofilm analysis were shipped a sterile 15 mL tube containing Phosphate-Buggered Saline (PBS) and Tween-80 (Sigma-Aldrich, St. Louis, MO) and then vortexed for 30 seconds, sonicated for 5 cycles of 90 seconds, and vortexed again for 30 seconds followed by seral dilution with PBS, plating in triplicate on tryptic soy agar and incubated at 37°C for 24 hours to 48 hours.

Twenty patients were randomized to either 1 week of negative pressure wound with 0.125% sodium hypochlorite solution instillation (NPWTi) (n=11) or NPWT without instillation (NPWT) (n=9). Fifteen patients with Venous Leg Ulcers (VLU) were treated with weekly Manuka honey, sharp debridement, and multilayer compression. Fifteen patients with chronic diabetic foot ulcers were randomized to either topical cadexomer iodine gel with foam dressing or hydrogel with foam dressing.

Wounds were assessed weekly for size and evidence of infection prior to sharp debridement. Serial wound biopsies were performed to test for quantitative biofilm and planktonic bacteria during the first four weeks in each clinical trial.

Results

We looked at bacteria in two forms: planktonic and biofilm. Each patient followed up weekly for a total of 4 weeks, which are listed as visits A, B, C, and D. Visits A and C were biopsies taken pre-debridement, and then visits B and D were biopsies taken post-debridement. A total of 190 biopsies were performed over the 4 weeks in the 3 studies.

Of the total biopsies, 90% of the wound biopsies had identifiable planktonic bacteria and 59% had identifiable biofilm bacteria.

The trend of biofilm and planktonic bacterial presence was charted, and an overall reduction of both planktonic and biofilm bacteria is seen. By the end of the 4 weeks, there was a 37% decrease in biopsies with identifiable biofilm bacteria and 31% decrease in those with identifiable planktonic bacteria. Biopsies were analyzed for presence or absence of specific microorganisms and, by the end of 4 weeks, there was a decrease in all strains of planktonic bacteria with an average 20% decrease amongst specific planktonic bacteria. *Streptococcus* and *Pseudomonas aeruginosa* (PA) planktonic species were seen to have had the largest reductions with 24% each at the end of 4 weeks while Fastidious organisms and Fungi (FF) with the least with 14% decrease (Figures 1 and 2).

A similar overall reduction in biofilm bacterial presence was seen after 4 weeks of debridement with secondary dressing; although, it was not a uniform decrease in all microbial species like that in planktonic bacteria. An average reduction of 1.3% of specific biofilm bacteria was noted after 4 weeks. Greatest reductions were seen in *Streptococcus* species with a 13% overall reduction while both gram negative (G-) and FF had a 13% increase of wound presence by the end of the 4 weeks (Figure 3).

Side by side comparison of the various secondary dressings (NPWTi, NPWT, Manuka Honey, and Cadexomer starch and Iodine) was assessed to identify a more efficacious treatment in terms of sustained decrease in bioburden load after debridement (Figures 4 and 5).

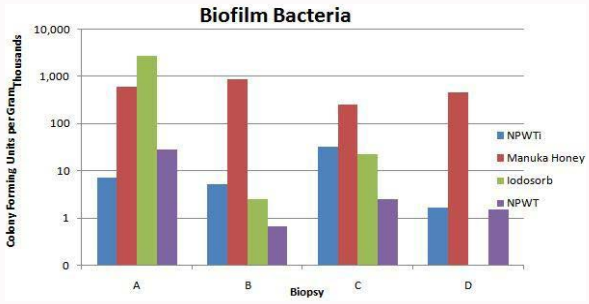

Figure 5: Biofilm Bacterial load stratified by secondary dressing type.

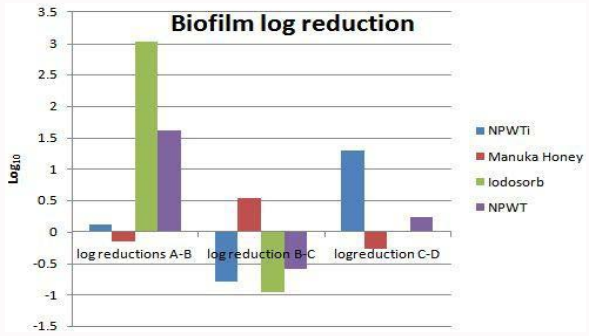

Figure 6: Logarithmic reduction in Biofilm Bacteria.

Cadexomer starch and 0.9% Elemental Iodine had a 99% decrease in both planktonic and biofilm bacterial load after one debridement. Between debridements (visit B and C) there was an increase in bacterial load, but when compared to the initial bioburden, Iodine therapy patients maintained a 97% decrease in planktonic bacteria and a 99% decrease in biofilm bacteria. Unfortunately, there was the no 4th biopsy in the Iodine therapy patients to observe the effects after a second debridement (between visits C and D).

Both NPWTi and NPWT saw similar sustained decreased bioburden. After one debridement NPTWi and NPWT had a 78% decrease in planktonic bacteria along with a 26% and 97% decrease in biofilm bacteria for NPWTi and NPWT, respectively. Similarly, there was an increase of bioburden between debridement and then a stark decrease after the second debridement with an overall 95% and 77% decrease of biofilm bacteria after 2 debridements in NPWT and NPWTi patients respectively. An overall total 79% decrease in planktonic bacterial load was observed from visit A to visit D.

Manuka Honey did not see such a robust response as the other secondary dressing types. For planktonic bacteria, there was a 5% increase after one debridement and overall 11% increase after two. This increase is negligible after logarithmic transformation.

When log reductions in between visits are calculated, it is seen that there is an overall reduction in bacterial load after debridement. However, in between debridements (between visits B and C), Iodine therapy was seen to have the greatest increase in bacterial bioburden with close to 1 log increase in biofilm bacteria and half a log increase in planktonic bacteria. ManukaMed Honey had a reduction in biofilm bacteria during this period. NPWT and Manuka Honey prevented significant logarithmic increases in bacterial load between debridements. After the second debridement the NPWTi group had the 1.3 log reduction in biofilm bacteria, which is close to the 1.6 log reduction seen in NPTW after the first debridement.

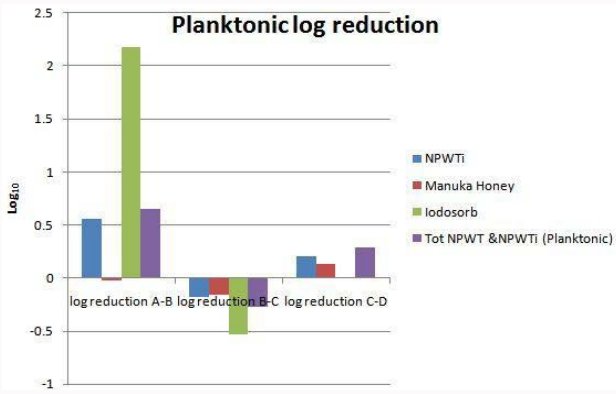

Figure 7: Logarithmic reduction in Planktonic Bacteria.

The other modalities did not have a significant change, while no data was collected in visit D for the iodine treated patient subset (Figures 6 and 7).

Our data shows the debridement can effectively reduce both planktonic bacteria and biofilm. When evaluating the log reductions of microbes between visits A-B and B-C, the effects of debridement on the reduction of bioburden is significant in both planktonic bacteria and biofilm ($p=0.03$ and $p<0.05$, respectively) regardless of ulcer type.

Discussion

Microbial inhabitation of cell migration and proliferation has been theorized to play a vital role in the propagation of chronic wounds. Normal wound repair follows a precise sequence of events (coagulation, hemostasis, inflammation, cell proliferation, cell migration, and tissue remodeling) that chronic wounds fail to undergo, and, instead, are in a state of persistent inflammation [1,5]. Most chronic wounds occur in those patients with underlying pathology or systemic disease that impairs the immune response [1,6]. Microbial invasion and colonization causes further inflammation by inducing the neutrophil and macrophage infiltration, which subsequently results in the release of free oxygen species, cytotoxic enzymes and proteases [1,6]. This inflammatory reaction in the setting of an already impaired immune systems leads to poor healing outcomes.

It is thought there are three aspects of wound microbiology that can be predictors of poor wound healing: microbial load, presence of pathogenic organisms and microbial diversity [2]. Our study looks at all three of these aspects, and then evaluates whether debridement supplemented with various secondary dressing types can influence the microbial load, diversity, and presence of certain pathogenic organisms.

Our data shows that sharp debridement can potentially have a significant impact on both planktonic and biofilm bacteria; although the level of bioburden reduction does tend to vary. One reason for the difference in reduction amongst the patient populations could be due to the type of wounds evaluated in each study with neuropathic wound allowing for a more aggressive debridement compared to painful venous wounds. If one looks at the rate of bacterial growth between planktonic and biofilm bacteria, biofilm bacteria seem to increase at a faster rate than the planktonic bacteria, which can speak to the resilience of bacteria in biofilm [7,8], or potentially a greater effect of the secondary dressing on the planktonic bacteria. Although speaks against the slow growth that some cite as an "in bred" protective quality of biofilms.

A set of longer studies may have elucidated a stronger relationship between secondary dressing and their effects on microbial bioburden, but this study shows that regardless of the type of secondary dressing, sharp debridement seems to consistently reduce the bacterial load; although the extent tends to vary.

Many commercial entities wish to show that they have a topical agent which will limit the growth of bacteria-both planktonic and biofilm. Data has already shown that repeated regular debridement is the most effective way to physically reduce the biofilm load due to the resistant nature of the beast [9]. Many different therapies have been studied in the effort to find a topical treatment modality that significantly decrease biofilm burden. Some data has shown the NPWT and topical antibiotics can potentially reduce biofilm, but to date their remains limited *in vitro* work in this area and topical therapy should not be used a sole treatment modality [10]. That said the combination of topical therapy with debridement is the most effective way to reduce bioburden, especially in the discussion surround biofilm, because debridement physically disrupts the biofilm, thus allowing for better antibiotic agent penetration and uptake when the bacteria are forced to become more metabolically active in effort to reattach to the host and reconstruct the disrupted extracellular matrix. Regular implementation of this strategy can keep the wound bed environment in favor of the host rather than the microbes colonizing it [8,9,11,12]. At this time, there is no secondary dressing that is deemed the champion of microbial management in the chronic wound, so the choice of secondary dressing is left up to the clinician based on the wound type, exudative amount, and experience.

Conclusion

Based on this analysis at present the main effect that one can ascribe to topical therapies is the stabilization of the bioburden in a wound between episodes of debridement. The episodes of debridement's efficacy may be enhanced by certain types of topical therapy; however, the absolute reduction in planktonic or biofilm protected bacteria in the real wound environment may not be easily facilitated by agents which to not have a true mechanical component.

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