Molecular diagnostics and personalised medicine in wound care: assessment of outcomes

• **Objective:** This large, level A, retrospective cohort study set out to compare healing outcomes in three large cohorts of wound patients managed universally for bioburden: standard of care group, who were prescribed systemic antibiotics on the basis of empiric and traditional culture-based methodologies; treatment group 1, who were prescribed an improved selection of systemic antibiotics based on the results of molecular diagnostics; treatment group 2 who received personalised topical therapeutics (including antibiotics) based on the results of molecular diagnostics.

• **Method:** Apart from the differences in diagnostic methods and antibiotic treatments described above, all three cohorts were subjected to the same biofilm-based wound care protocol, which included evaluation of the host and bioburden, frequent sharp debridement, use of wound dressings and comprehensive standard care (reperfusion therapy, nutritional support, offloading, compression and management of comorbidities).

• **Results:** In all, 1378 patients were recruited into the study. In the standard of care group 48.5% of patients (244/503) healed completely during the 7-month study period. This increased to 62.4% (298/479) in treatment group 1 and 90.4% (358/396) in treatment group 2. Cox proportional hazards analysis revealed the time to complete closure decreased by 26% in treatment group 1 ($p<0.001$) and 45.9% in treatment group 2 ($p<0.001$) compared with the standard of care group. Patients in treatment group 2 had >200% better odds of healing at any given time point compared with the other cohorts.

• **Conclusion:** Implementation of personalised topical therapeutics guided by molecular diagnosis resulted in statistically and clinically significant improvements in outcome. The integration of molecular diagnostics and personalised medicine provides a directed and targeted approach to wound care.

• **Conflict of interest:** SED and RDW are owners of PathoGenius Laboratories, a clinical diagnostic laboratory. SED and RDW are owners of Research and Testing Laboratory, which develops molecular diagnostics. CJ and JK are clinical advisors for PathoGenius. CJ and JK are owners of Southeastern Medical Compounding, Savannah, GA and Southeastern Medical Technologies, Savannah, GA.

chronic wounds; clinical culture; molecular diagnostics; bioburden; topical antibiotics

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Host factors that impair wound healing, such as diabetes, age, comorbidities, diet and poor perfusion, must be properly addressed or managed. However, individual host factors such as these are not universal barriers in all patients. In contrast, there is strong supporting evidence that bioburden and/or biofilm is an universal barrier to healing in all chronic wounds.[1-4] The vast majority all of chronic wounds (>3000) evaluated using molecular diagnostics have been found to be polymicrobial in nature.[5-9] There is accumulating evidence that the more diverse the microbial consortium within a wound, the more recalcitrant the wound is likely to be.

Early efforts to combat this universal barrier led to the paradigm of biofilm-based wound care (BBWC),[1–3,10,11] which is based on the premise that regular disruption of the biofilm (in the form of sharp debridement) realigns the process of healing in favour of the host. Use of this approach alone (in combination with standard wound care) significantly improved healing rates and outcomes ($p<0.05$).[1–3,10,11]

The concept of BBWC is based on the fact that biofilm phenotype bacteria are more resistant than planktonic phenotype bacteria to host defences and xenobiotic treatments.[1] Disruption of the biofilm structure forces the bacteria to remain in the heightened state of metabolic activity needed to reconstitute the tissue surface. However, at this point, the microbial census is more susceptible to antibiotics and antibiofilm agents.[1,11] A purported rationale for this is that most antimicrobials are primarily effective against bacteria in states of active metabolism and cellular reproduction, whereas inhabitants of mature biofilms have a reduced metabolic profile and so are more resistant to equivalent antimicrobial challenges.[1,12–18] In addition, the biofilm's structural barrier (the extracellular polymeric substance

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[EPS]) impedes both the host immune response and therapeutic access to the organisms.

Although use of BBWC alone significantly improved healing outcomes, it became evident that antimicrobial therapies were being prescribed empirically — that is, without full knowledge of which organisms were within the biofilm — due to the absence of tools that could comprehensively diagnose the constituents of the polymicrobial community.[2–4,11,19]

Until very recently, clinical cultures were the only diagnostic tool available for evaluating clinical pathogens in wound bioburden. Research soon revealed that most bacteria found in chronic wounds grow poorly, or not at all, in the growth media used in routine clinical culture methods. Most bacteria, yeast and fungi in a biofilm phenotype are typically viable, but not easily cultivable in these media.[20–25] As a result, traditional culture methods are unable to identify the majority of the microbial population found in chronic wounds.[23] The focus, therefore, has been on pathogens that grow well in culture but may represent only a relatively minor component of the polymicrobial community. In short, culture methods have a strong selective bias toward organisms that can grow on artificial laboratory media.

Comparison of modern molecular DNA-based polymerase chain reaction (PCR) and sequencing methods — which can accurately and objectively diagnose the true reality of the wound bioburden — with traditional, agar-based clinical cultures substantiated our hypothesis.[2-4,5-9,19,26] Results showed that by far the majority of chronic wounds tested were not 'infected' by a single microbe, but were composed of complex, well-coordinated communities of bacteria, yeast and/or fungi.

Ultimately, molecular methods elucidated distinct bacterial patterns for the major types of chronic wounds. Diabetic foot ulcers (DFUs),[9,23] venous leg ulcers (VLUs),[6,23] pressure ulcers (PUs),[7] and surgical site infections[5,26] were evaluated in a comprehensive protocol to reveal their complex microbial reality. The concept of 'functional equivalent pathogroups' (FEPs)[2,5,7,8,10] was developed to identify relationships within microbial populations in chronic wounds. Based on this concept, dozens of related microbial populations have been identified in wounds, allowing general distinctions about the character of these wounds to be drawn.[2–4,5–9,19,26] Common recurring patterns of microorganisms were also traced. We postulated that accurately characterising the bioburden (that is, identifying the FEP associated with each chronic wound) and reducing the empiric nature of treatment would further improve healing rates.

We therefore modified our treatment protocol to include regular sharp debridement plus comprehensive molecular diagnostic methods (PathoGenius Laboratories, Lubbock, TX) to determine which antibiotics and antibiofilm agents would be most effective for an individual wound. We then conducted a retrospective study to compare healing outcomes achieved with the two approaches: BBWC alone versus BBWC that included systemic antibiotic therapy determined by molecular diagnostics and the FEB.[4] The results found that the latter approach was associated with a significant improvement in healing rates and outcomes in the same chronic wound types (Fisher's exact test, $p<0.001$; OR=1.76, 95% CI=1.36–2.29).[4]

We then postulated on the feasibility of delivering a truly personalised or patient-specific therapy targeting the microbial population within a given biofilm. This led to the development of topical therapeutics that, when guided by molecular diagnostics, could be algorithmically tailored to provide an individualised, highly targeted treatment for each patient and his/her unique microbial sensus.[27] We implemented this personalised therapeutic approach in January 2010.

This retrospective study set out to compare the outcomes data achieved before and after the implementation of this fully integrated theranostic approach.

Materials and methods

The study centre was a community-based, independent wound care facility (Southwest Regional Wound Care Center, Lubbock, TX, USA) that managed all types of chronic wounds in an ample patient population. All patients were managed with comprehensive standard care including reperfusion therapy, nutritional support, offloading, compression and management of comorbid conditions. In addition, particular attention is given to wound bioburden through biofilm-based wound care (BBWC) in all cohorts, as described previously.[1,4,11] The retrospective cohort groups were as follows:

Treatment groups

- Standard of care group, recruited in 2007, was treated using BBWC and standard of care treatments. Traditional culture techniques undertaken by an external, independent clinical laboratory were used to assess each patient's microbial bioburden and guide the selection of antibiotic therapy. Primary treatments included commercially available antibiofilm agents such as lactoferrin, xylitol and hamamelitannin and empirically prescribed systemic antibiotics
- Treatment group 1, recruited in 2009, was treated using BBWC and systemic therapy guided by comprehensive molecular diagnostics (PathoGenius Laboratories, Lubbock, TX, US). As with the standard of care group, primary treatments included commercially available antibiofilm agents and systemic antibiotics. The only clinical difference was that these therapeutic agents were selected using comprehensive molecular diagnostics

• Treatment group 2, recruited in 2010, was treated using BBWC, comprehensive molecular pathogen diagnostics (PathoGenius Laboratories) and personalised topical therapy (Southeastern Medical Compounding, Savannah, GA, US). Each topical treatment (wound gel) was made to order in the compounding pharmacy based on proprietary algorithms such that each gel was specific to each patient's polymicrobial census as identified in the molecular diagnostic report. All topical preparations contained antibiofilm agents; most contained antibiotics and some contained antifungal agents.

Enrolment and endpoint criteria

For each retrospective cohort under study, patients with new, full-thickness wounds presenting to the clinic within a 3-month enrolment period were included. There were no exclusion criteria.

The patients' progress was followed for a maximum of 7 months, which included a 3-month enrolment period. This 3-month enrolment period allowed a large study population to be included into each cohort; the subsequent 4-month period provided a clinically relevant study duration, which was sufficient to monitor healing to complete closure. It should be noted that patients enrolled in the third month of the study were only followed for 4 months, whereas patients enrolled in the first month were followed for up to 7 months. However, as all three cohorts were subject to the same enrolment methodology, they were equitably evaluated and compared with limited bias.

Healing was defined as full closure (100% epithelialisation) of the wound, as determined by the clinician. Patients who presented with more than one wound were followed up until their primary wound (typically defined as the largest) healed, as recorded in their electronic medical records.

The vast majority of patients in each cohort were evaluated weekly for the first 8-12 weeks and every 2 weeks thereafter, with the extent of healing recorded on the day of their visit. Any patient who withdrew or who did not attend the clinic follow-up visits for any reason prior to being healed was counted as non-healed.

The same BBWC algorithm[1,4,11] was used for every patient in each of the cohorts. However, as with any longitudinal study, it is possible that subtle, unidentified or indiscernible changes in methods and daily standard of care protocols may have developed over the time periods evaluated. If present, variables of this type could potentially contribute as unidentified confounding factors to any identified changes in wound healing.

Unfortunately, it was not possible to use wound duration and wound size as study variables as these data were incomplete, particularly for the standard of care group and treatment group 1.

Table 1. Patient demographic data and wound characteristics

	Standard of care group	Treatment group 1	Treatment group 2
	(n=503)	(n=479)	(n=396)
Demographic data			
Hispanic	220 (44%)	207 (43%)	100 (25%)
Black	57 (11%)	77 (16%)	45 (11%)
Caucasian	197 (39%)	173 (36%)	238 (60%)
Other	29 (6%)	22 (5%)	13 (3%)
Female	236 (47%)	251 (52%)	196 (49%)
Male	267 (53%)	228 (48%)	200 (51%)
Age years (range)	64.3 (5–101)	59.8 (2–97)	60.5 (1–96)
Comorbidities			
Diabetes	233 (46%)	214 (45%)	182 (46%)
Cardio/heart	127 (25%)	129 (27%)	108 (27%)
Circulatory	160 (32%)	88 (18%)	67 (17%)
Immobility	63 (13%)	22 (5%)	21 (5%)
Hypertension	223 (44%)	232 (48%)	183 (46%)
Wound type			
Pressure ulcers	134 (27%)	112 (23%)	103 (26%)
Diabetic foot ulcer	138 (27%)	146 (30%)	140 (35%)
Surgical site	67 (13%)	67 (14%)	45 (11%)
Traumatic/abscess	50 (10%)	59 (12%)	57 (14%)
Venous leg ulcer	114 (23%)	95 (20%)	51 (13%)

Statistical analysis

An external, independent, academic biostatistician performed all of the statistical analyses. To limit the introduction of experimental bias, an information technology consultant was also recruited and was not informed about the goals of the study.

A retrospective chart review and analyses were performed on the 1378 patients under study in 2007 (the standard care group), 2009 (treatment group 1), and 2010 (treatment group 2). First, differences between the cohorts in potential categorical confounders (race and wound type) as well as comorbidities (diabetes, hypertension, venous insufficiency and heart disease) were evaluated using Fisher's exact or chi-squared tests. Then, logistic regression was used to compare the proportion of individuals who healed by the study end, controlling for age, confounders and comorbidities. Finally, a Cox pro-

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Table 2. Patient enrolment broken down into 7-day periods over the first 3 months. Results are presented as the total number of patients for each parameter

Days	Standard of care group		Total	Treatment group 1		Total	Treatment group 2		Total
	Healed	Non-healed		Healed	Non-healed		Healed	Non-healed	
1–7	26	15	**41**	17	16	**33**	22	3	**25**
8–14	18	24	**42**	27	12	**39**	34	3	**37**
15–21	25	18	**43**	16	18	**34**	30	2	**32**
22–27	18	21	**39**	30	12	**42**	30	4	**34**
28–34	13	15	**28**	17	12	**29**	20	0	**20**
35–41	23	21	**44**	27	13	**40**	22	6	**28**
42–48	19	23	**42**	24	11	**35**	22	4	**26**
49–55	23	22	**45**	23	14	**37**	34	2	**36**
56–62	12	16	**28**	23	18	**41**	31	2	**33**
63–69	21	20	**41**	28	15	**43**	32	1	**33**
70–77	18	26	44	22	13	**35**	32	2	**34**
78–84	15	18	**33**	22	16	**38**	23	3	**26**
85–91	13	20	**33**	22	11	**33**	26	6	**32**
Total	**244**	**259**	**503**	**298**	**181**	**479**	**358**	**38**	**396**

portional hazards model was used to test for differences in the number of days to complete healing, controlling for age, confounders, and comorbidities. To elucidate healing patterns with respect to wound types, the Cox proportional hazards model was evaluated for each wound type separately.

All analyses were performed using R (R Development Core Team 2010) and the R survival package (Therneau 2009; http://cran.r-project.org/web/packages/survival/survival.pdf).

Results

A summary of the demographic information for the three comparative groups is provided in Table 1. Caucasians made up a larger portion of the total in treatment group 2 than in the other cohorts (60% versus 39% or 36%, p<0.001). Moreover, DFUs comprised a slightly larger proportion (35% versus 27% or 30%) and VLUs a slightly lower proportion of the total (13% versus 23% or 20%) in treatment group 2 compared with the other cohorts (p=0.005). Of the comorbidities, circulatory (p<0.001) and immobility (p<0.001) differed statistically among cohorts.

Table 2 gives data on patient enrolment broken down by 7-day periods over the 3-month enrolment period. There was no significant difference in enrolment patterns between years. Enrolment was relatively consistent across each of the 7-day periods.

In the standard of care group 244/503 (48.5%) patients were identified as healed, compared with 298/479 (62.4%) in treatment group 1 (p<0.001) and 358/396 (90.4%) in treatment group 2 (p<0.001). Thus, over an equivalent time period, a statistically significant higher percentage of patients healed in treatment group 1 compared with the standard care group (OR=1.56, 95% CI=1.20–2.04) and in treatment group 2 compared with the standard of care group (OR=9.67, 95% CI=6.61–14.47).

Based on the Cox proportional hazards model, after controlling for confounders and comorbidities, the time to healing was significantly different between the cohorts. Specifically, the median time to heal improved from 177 days in the standard of care group, to 77 days in treatment group 1, and 28 days in treatment group 2.

Moreover, compared with the standard of care group at any given time, the hazard of healing for patients in treatment group 1 was 41.0% greater (relative hazard = 1.41; 95% CI=1.18–1.68) and for patients in treatment group 2 it was 3.17 times greater (relative hazard = 3.17; 95% CI=2.67–3.78).

In treatment group 1 the median healing time reduced significantly only for DFUs (Table 3); however, all other wound types except for traumatic/abscess wounds approached statistical significance. On the other hand, treatment group 2 significantly (p<0.001) reduced the median time to heal both for all wound types combined and for each individual wound type. A Kaplan-Meier plot (Fig 1) illustrates the healing times for a patient within each group.

In the standard of care group, systemic antibiotics were utilised in 29.4% of all patients. This increased to 50.7% in treatment group 1. This increase is directly attributable to the more comprehensive

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identification of pathogens afforded by the molecular diagnostic methods used. An expanded formulary of antibiotics was implemented (replacing empirical assignment) to specifically target the diverse microbial populations elucidated.

In treatment group 2, following the implementation of the personalised topical therapeutic gels (LipoGel base), the use of systemic antibiotics declined significantly (p<0.001) to only 5.5% (Table 4). In fact, systemic antibiotics were limited primarily to patients at risk of significant clinical peril (amputation). As anticipated, the use of personalised LipoGels increased from 0% (in both the standard of care group and treatment group 1), to 100% of subjects in treatment group 2 (p<0.001). The use of topical therapy did increase with progressive cohorts from the standard of care group to treatment group 1. However, in treatment group 2, 100% of patients received personalised topical therapeutic gels (non-empiric), accounting for the primary difference in treatment modality and consequent healing outcomes in this group.

Table 3. Median number of days to heal by wound type

Wound type	Standard of care group	Treatment group 1	Treatment group 2
Pressure ulcer	NA	107 (p=0.086)	28 (p<0.001)
Diabetic foot ulcer	168	84 (p=0.030)	32 (p<0.001)
Non-healing surgical wound	176	75 (p=0.071)	44 (p<0.001)
Traumatic/abscess	39	33 (p=0.400)	14 (p<0.001)
Venous leg ulcer	177	98 (p=0.101)	37 (p<0.001)
Total	**177**	**77 (p<0.001)**	**28 (p<0.001)**

Median estimates are from a Cox proportional hazards model with age as a covariate. P values denote significance of comparisons of treatments versus standard of care group in the Cox proportional hazards model, which included all confounders and comorbidities
NA indicates that the median time to heal was longer than the study duration

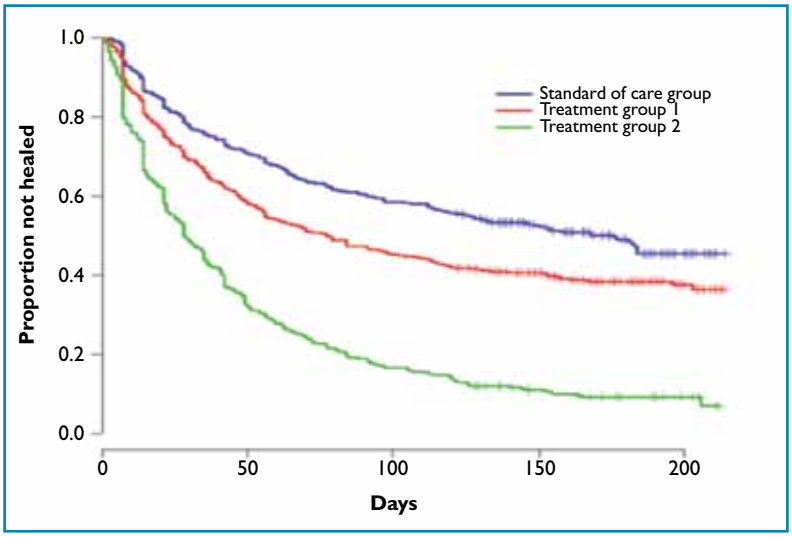

Fig 1. Kaplan Meier plot illustrating the time to heal for a patient of average age. Cross marks indicate censored observations.

Discussion

In patients who received personalised topical therapeutic gels, based on the results of molecular diagnostics, the average healing time and healing rate reduced significantly and reliance on systemic antibiotics was nearly eliminated. We believe these results will have significant ramifications for wound management. Modern medicine is founded on the premise of evaluation, diagnosis and then treatment. Previously, practitioners had few diagnostic tools to aid them in the management of chronic wound bioburden, biofilm and infections, resulting in reliance on a trial-and-error methodology, often founded on bioburden maintenance rather than objective and aggressive treatment.

Modern medicine demands that decisions be based on real information, preferably derived from methods that are measurable and reproducible. Relative to wound care, such diagnostics are available for blood flow, systemic diseases, nutritional status and even pressure damage, yet scientific analysis of the wound bioburden has been inadequate. The diagnostic and treatment protocols disclosed here empower practitioners to manage chronic wounds with DNA-level certainty.

To date, the wound bioburden has been mainly evaluated using basic clinical microbiology methods such as traditional clinical cultures, Gram staining, biochemical identifications and, occasionally, biopsies with special stains. All of these methods are ill suited to assess the polymicrobial microorganisms colonised in bioburden or that have progressed to a biofilm phenotype on the chronic wound surface. At their crux, the inadequacy of these methods is founded on:

- The inability of most organisms to grow in a laboratory environment with routine clinical procedures
- Inaccurate identification of the organisms through antiquated biochemical methods
- The inescapable limitation of these methods due to lack of absolute or reliable relative quantification.[23,28-30]

The failures of microbial culture-based diagnostics have led many practitioners to abandon evaluation of wound bioburden in most chronic wounds or, worse, to assume the bioburden is not a significant barrier to healing.[31]

Our findings validate recent research and emerging clinical evidence that bioburden is a clinically significant barrier to healing in *all* chronic wounds.[1,4,11] These findings indicate that we can increase the odds of a patient healing at any given

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Table 4. Total courses of systemic antibiotic used in each group

	Standard of care group	Treatment group 1	Treatment group 2
Doxycycline	73 (15%)	100 (21%)	10 (3%)
Ertapenem/Invanz	41 (8%)	77 (16%)	3 (1%)
Sulfamethoxazole/trimethoprim	6 (1%)	47 (10%)	1 (0.0%)
Ciprofloxacin	5 (1%)	31 (6%)	2 (1%)
Daptomycin	9 (2%)	21 (4%)	2 (1%)
Clarithromycin	0 (0%)	24 (5%)	0 (0%)
Levofloxacin	16 (3%)	19 (4%)	0 (0.0%)
Cefepime	1 (0%)	11 (2%)	1 (0.28%)
Clindamycin	7 (1%)	14 (3%)	2 (0.56%)
Imipenem/cilastatin	15 (3%)	17 (4%)	1 (0.28%)
Cefalexin	17 (3%)	10 (2%)	0 (0.0%)
Metronidazole	0 (0%)	11 (2%)	1 (0.28%)
Linezolid	10 (2%)	7 (2%)	0 (0.0%)
Amoxicillin/clavulanate	5 (1%)	16 (3%)	1 (0.28%)
Penicillin G benzathine	0 (0%)	4 (1%)	1 (0.28%)
Rifampin	0 (0%)	9 (2%)	0 (0.0%)
Ceftriaxone	2 (0%)	3 (1%)	0 (0.0%)
Amoxicillin	3 (0%)	1 (0%)	0 (0.28%)
Tigecycline	8 (1%)	2 (0%)	0 (0.0%)
Piperacillin/Tazobactam	0 (0%)	3 (1%)	0 (0%)
Vancomycin	0 (0%)	1 (0%)	0 (0%)
Dicloxacillin	1 (0%)	2 (0%)	0 (0.0%)
Bicillin DL	0 (0%)	1 (0%)	0 (0%)
Fluconazole	0 (0%)	2 (0%)	0 (0.0%)
No. of patients receiving systemic antibiotics	148	243	22
Total no. of patients	503	479	396

time by more than 200% if we address the bioburden of all wounds in a targeted approach.

Furthermore, these results are not biased by academic arguments about the relevance of microbial phenotypes (planktonic versus biofilm) or clinical arguments on how to determine 'infection'. We believe these arguments will become largely irrelevant if the principles of BBWC, comprehensive diagnostics and patient-specific therapeutics are applied.

Many professional associations have discouraged the use of topical antibiotics/antimicrobials, despite the absence of controlled scientific studies supporting this position. However, in our experience, a targeted, scientific approach in which molecular diagnostics guide the use of systemic antibiotics improves outcomes and healing rates in chronic wounds.[4]

Systemic (injectable and oral) delivery has significant clinical, pragmatic and often economic limitations for the treatment of chronic wounds. The innate antimicrobial resistance of biofilms, coupled with their inherent physical and metabolic defences, limit the penetration and effectiveness of agents delivered at relatively low concentrations systemically. This can result in the delivery of sub-therapeutic concentrations at the site of a biofilm infection, potentially increasing the risk of bacterial resistance.[32] Contrary to popular belief, there is both theory and evidence to support a lower risk of antibiotic resistance following high-dose topical antibiotics.[33–43] For example, topical antibiotics are the standard of care for ear, nose and throat infections such as otitis media, and for ocular infections.[42,44–49]

The diagnostic and treatment protocols used in this study rigorously applied the principles of sound infectious disease therapy and antibiotic stewardship, including the objective identification of the microorganisms present with greater than reasonable scientific certainty, and the administration of appropriate antibiotics far in excess of all potential minimum inhibitory or minimum bacteriocidal concentrations (MIC or MBC) of the organism(s) identified. Furthermore, by more efficiently removing the site of clinical or subclinical infection (by achieving wound closure), we reduced the potential for bacterial resistance and maintained antibiotic efficacy.[33–43]

The personalised topical therapy reported here involved the simultaneous use of three to five antibiotics, which were chosen algorithmically to optimise synergistic strategies and provide overlapping coverage for the majority bacterial census identified in the wound by molecular diagnostics. The therapeutics were then delivered at antibiotic concentrations of 5000–25,000ug/ml within the applied product. Although the MIC/MBC concentrations applied dramatically exceed those of oral or intravenous doses, each ounce of the individualised topical therapy contains, on average, only a fraction of a single oral or intravenous dose.

We hypothesise that systemic antibiotics distributed throughout the body can indiscriminately affect remote physiologic systems such as the skin, gastrointestinal and genitourinary tracts, and the oral cavity. In addition, we predict that systemic antibiotics reach the wound and external site of biofilm infection in, at best, minimally therapeutic doses, which rapidly become sub-therapeutic over the dosing interval.

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In contrast, when a comparable, focused and timed topical therapy is applied, the MIC or MBC of the organisms may be easily exceeded directly at the site of infection over the entire dosing interval without disrupting the commensal flora. If systemic antibiotics given for a chronic wound disrupt the normal commensal microflora throughout the body, the diverse effects on the gut, for instance, might include reduced nutritional uptake,[50] further complicating the patient's health.

We also propose that antibiotic resistance is probably triggered by the diverse pool of undiagnosed and untreated or undertreated bacteria not elucidated by traditional culture methods and which are then exposed to subtherapeutic doses of systemic antibiotics.[5–9,51] Therefore, the present strategy (comprehensive molecular diagnostics followed by personalised topical therapeutics) is an efficient and economical use of resources and a powerful modality for accurately diagnosing and treating non-healing wounds, particularly those containing difficult-to-treat bacteria such as meticillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* and multi-drug resistant *Pseudomonas*. Furthermore, we believe that, within the medical community, a common misperception has been perpetuated that the use of topical antibiotics may increase the risk of sensitisation. However, a thorough literature review[41] does not provide any evidence to support this. Finally, few cutaneous reactions were observed in our reasonably large cohort, and the attending clinicians did not observe any new sensitivities that could be attributed to the topical therapeutics.

Study limitations relate to the lack of baseline data on wound duration and wound size. These parameters were excluded because they were poorly documented or unreliable. Wound size, especially as it can now be measured objectively and reproducibly, is an important variable in studies of this kind. Its absence is therefore a notable limitation.

Conclusion

The agents and methods used to manage wound biofilm have been previously described.[4,10,11,52] In 2007, these methods were largely empiric due to the limitations of culture-based diagnosis. The improvement in healing rate achieved in 2009[4] represents the first step in a targeted therapy founded on DNA-based molecular diagnostics. The success of the empirically defined LipoGel-based TKS topical gel[4] inspired us to develop personalised topical gels that would individualise therapy to the microbial census of each wound. By 2010, a comprehensive system for compounding these gels had been achieved and was ready for clinical implementation. This constitutes a fully integrated system of BBWC, comprehensive bioburden/biofilm diagnostics and personalised topical therapy.

Based on our results, we conclude that bioburden is a significant barrier to healing in all chronic wounds, regardless of aetiology. We believe that BBWC, comprehensive bioburden pathogen diagnosis and personalised topical therapy that addresses these polymicrobial communities should therefore be an universal treatment strategy for all chronic wounds. This would improve overall healing rates, reduce healing times and enhance healing trajectories, regardless of the presence or absence of clinical signs of 'infection' or how recalcitrant they are. ■

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