Healing and healing rates of chronic wounds in the age of molecular pathogen diagnostics

• **Objective:** To compare healing outcomes at a wound healing centre both before and after the introduction of molecular pathogen diagnostics.
• **Method:** An IT consultant was recruited to analyse the medical records of patients at a wound healing centre, comparing patient groups from 2007 and 2009 — before and after the introduction of comprehensive molecular pathogen diagnostic methods.
• **Results:** Before the implementation of molecular diagnostics, 244/503 patients (48.5%) healed completely, while after implementation 298/479 patients (62.4%) healed. Furthermore, based on survival analysis and after controlling for potential confounding factors, time to healing was significantly shorter in 2009 than 2007 ($p<0.05$). Specifically, biofilm-based wound care, along with the implementation of comprehensive molecular diagnostics for venous leg ulcers, pressure ulcers and diabetic foot ulcers and all wounds combined showed, respectively, 21%, 23%, 25% and 22% reductions in the time to healing. In addition, after implementing molecular diagnostics, the use of expensive first-line antibiotics also declined in 2009, while a broader range of targeted antibiotics was used.
• **Conclusion:** The results of modern molecular pathogen diagnostic applications allow comprehensive evaluation of the microbial bioburden in chronic wounds. This comprehensive diagnostic in turn has led to a more precise and targeted therapeutic approach to wound care. With the comprehensive nature of molecular diagnostics future advances in topical patient specific therapeutics are now possible.
• **Declaration of interest:** SED and RDW are owners of Pathogenius diagnostics, a clinical diagnostic facility. SED is director of Research and Testing Laboratory, which develops molecular diagnostics. Analysis was performed and approved by outside consultant SBC, who indicates no conflict of interest.

clinical culture; molecular diagnostics; time to healing; antibiotics

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We propose that all cutaneous lesions classified as 'chronic wounds' possess surface-associated bacteria, regardless of host impairments. Clearly, any host factors that impair healing must be managed, but host factors are not universal. The microbial bioburden, however, can be considered a therapeutic target in all chronic wounds.

The microorganisms attached to the surfaces of chronic wounds have been shown to be predominantly organised in a biofilm phenotype.[1,2] This microbial bioburden is usually polymicrobial[3-7] and seems to impair host healing. Indeed, most chronic wounds are host to an incredibly diverse array of bacterial and fungal species — their community structures, combinations and synergies seem infinite.[3-7]

To simplify this concept, bioinformatic analyses of wound biodiversity data have been used to identify dozens of co-occurring populations of microorganisms, termed functional equivalent pathogroups (FEPs),[8] which appear to form common and somewhat recurring groups on chronic wound surfaces. The diversity of biofilms, along with their intrinsic resistance to antibiotics, biocides and host immunity, makes wound bioburden a notable (and increasingly appreciated) potentially universal barrier to the healing of chronic wounds.

Although advances are being made rapidly, biofilm-specific therapies have remained elusive. Targeting the microorganisms that comprise a particular biofilm is very difficult, due to a lack of sufficiently comprehensive clinical diagnostic tools; if we are to enable patient-specific therapies, then we must be able to diagnose these polymicrobial infections.

Clinical cultures (agar-based cultivation methods) are currently the main clinical pathogen diagnostic tool available for the evaluation of wound bioburden. It is well understood that most bacteria grow poorly or not at all in common clinical cultures. Anaerobes, yeast and biofilm phenotypes, for instance, are viable but non-culturable.[9] Multiple species within a biofilm phenotype remain difficult to diagnose in an economical manner using routine clinical culture.[10,11]

Agar-based cultures are a traditional method designed to try and find the 'one organism' causing an infection, through pure culture. A number of properties render their use irrelevant to biofilm analysis, including the selection bias for microorganisms that grow easily in artificial laboratory media and the fact that the vast majority of bacteria scientifically identified in human infections, especially in wounds, cannot grow in routine clinical cultures.[3-7]

Clinical culture methods have the advantage of providing resistance and sensitivity information, but these sensitivities are of limited use in chronic wound management as bacteria and yeast exist mainly in polymicrobial communities.[3-7] The sensitivities obtained from laboratory cultivation methods are relevant only to planktonic phenotypes and do not account for biofilm phenotype bacteria. Moreover, clinical cultures provide information on only those few bacteria that can be propagated efficiently in a laboratory.

Many other significant limitations related to the use of clinical culture methods have been reviewed in more detail throughout the scientific literature.[12]

The main factor that led our group to seek out alternative diagnostic tools was the vast number of clinical cultures that we have sent out over the years to be returned as 'no growth'. In a wound with obvious signs of infection, a diagnostic tool that returns a negative result is of limited use. The inability to correctly assess wound bacteria and fungi may have contributed to the current recommendations for limited and empiric antibiotic and biocide use in chronic wound management.[13,14]

Now there is evidence that by specifically targeting wound biofilms, healing outcomes are improved.[15] Molecular methods have proven valuable in evaluating the bioburden of chronic wounds.[5-7,16] These powerful research tools have evolved into validated clinical diagnostic tests that have been enormously useful at our clinic at the Southwest Regional Wound Care Center. They were developed, validated and put into clinical practice for the management of chronic wounds early in 2009. Therefore, we decided to examine the outcome data before and after their implementation in order to determine any differences that molecular diagnostics may have exerted on healing outcomes and antibiotic strategies.

The Southwest Regional Wound Care Center is a community-based, freestanding, comprehensive centre involved in the management of all types of

References

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Table 1. Demographic information for patients in 2007 and 2009

	2007 (n=503)	2009 (n=479)
Patient demographics		
Hispanic	113 (46%)	118 (40%)
Black	34 (14%)	54 (18%)
White	84 (34%	111 (37%)
Other	8 (5%)	11 (5%)
Female	106 (44%)	166 (56%)
Male	137 (56%)	132 (44%)
Age (years) Mean (range)	61.9 (5–97)	59.4 (2–97)
Comorbidities		
Diabetes	98 (40%)	122 (41%)
Heart disease	56 (23%)	55 (18%)
Peripheral vascular disease	40 (16%)	33 (11%)
Spinal cord impairment	25 (10%)	11 (4%)
Immune suppression	4 (2%)	6 (2%)

Table 2. Clinical microbiology culture results obtained during the selected period of 2007

Microorganisms	No. of patients	No. of occurrences
No growth	15	15
Staphylococcus aureus (MRSA)	10	10
Group D *Enterococcus*	8	8
Coagulase-negative *Staphylococcus*	6	6
Group B *Streptococcus*	5	5
Serratia marcescens	5	5
Proteus mirabilis	3	3
Pseudomonas spp.	3	3
Escherichia coli	2	2
Klebsiella pneumoniae	2	2
Yeast (not identified)	2	2
Bacillus spp.	1	1
Morganella morganii	1	1
Streptococcus viridans	1	1
Kluyvera spp.	1	1

chronic wounds. Patients are managed with what is widely accepted throughout the wound care community as 'standard care', which includes reperfusion, nutritional support, offloading, compression and management of systemic disease.

In addition to this, particular attention is given to biofilms, through a proven clinical regimen termed 'biofilm-based wound care'.[15] Biofilm-based wound care is predicated on frequent debridement, with suppression of wound biofilm by use of selective biocides, antibiofilm agents and the targeted use of antibiotics.

Although the same, unchanged algorithm was used for every patient in both of the study groups, it must be stressed that subtle, unidentified changes may have developed between the before and after time periods. Unidentified variables could very easily contribute to any identified changes in wound healing. In 2008, basic molecular diagnostic methods became available and were introduced as a part of the genaral management of chronic wounds. In 2009, comprehensive molecular diagnostics became available. We examined comparable patient groups from 2007 and 2009.

Materials and method

To limit the introduction of experimental bias, we recruited an information technology consultant, who was not given any guidance as to the goals of the study. This consultant used electronic medical records to identify groups of patients from common periods both in 2007, before molecular diagnostics was introduced, and in 2009, after the full implementation of clinical molecular diagnostics (Pathogenius Diagnostics, Lubbock, TX).

The consultant was asked to identify all patients with new full-thickness wounds who presented to the practice from June 1 to August 31 of the years in question. The consultant was then asked to identify the patients from these groups who had a healed wound by December 31 of the year of admission. This analysis period was chosen to give a comparable seven-month block from admission in both groups. Healing was defined as full epithelialisation of the wound. Patients who presented with more than one wound were documented until healing of their first healed wound.

It was noted that patients admitted in August were only followed for four months, whereas patients admitted in June were followed for up to seven months. However, since both groups were similarly treated, this seems to be an acceptable method whereby both groups can be consistently and equally evaluated and compared without bias.

Both groups were treated in accordance with a published biofilm-based wound care protocol.[17] No notable changes in clinical management were apparent or identified by the consultant, except for

the implementation of molecular diagnostics. Each component of biofilm-based wound care (a simple algorithm) was considered, including: host evaluation, bioburden evaluation, debridement methods, topicals, dressings and antibiotics. Components showing any significant changes were evaluated and have been included as data in this article. Evaluations included checking invoices for the quantities and types of dressings supplied during the two periods and checking billing records for non-invasive vascular testing (TCOMs and ABPI) and venous evaluation during both periods. Nutritional support, offloading and medical management of comorbidities were checked and found to have been managed using the same algorithm during both periods.

Western Institutional Review Board reviewed the proposed retrospective study and approved the design and patient safeguards (IRB number 20100213).

Data were then populated for each patient identified in each group. The patient record included demographics, comorbidities, culture methods and results, antibiotic use, wound dressings used and days to healing.

Statistical analysis

Cox proportional hazards model was used to test for differences in time to healing between years with age included as a covariate. Prior to this analysis we tested for differences in potential categorical confounders (race and wound type) as well as comorbidities (diabetes, hypertension, venous insufficiency and heart disease) between groups, using Fisher's exact test. If these results were significant ($p<0.05$), confounders were included in the Cox proportional hazards model. All analyses were performed using R (R development core team 2010) and the R survival package (Therneau 2009).

Results

In all, 246 patients were identified for the 2007 group and 307 patients for the 2009 group. A comprehensive manual review of these records was then conducted and it was found that two patients had been inadvertently included in the 2007 group, bringing the total healed down to 244. In the 2009 group, eight patients were identified as being inadvertently included, bringing the total to 298 healed patients. Of the 10 patients deselected from analysis, all showed healing times of less than three days,

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Table 3. Results of comprehensive molecular diagnostic tests undertaken in 2009

Bacterial species	No. of patients	Bacterial species	No. of patients
*Finegoldia magna**	75	*Candidatus Peptoniphilus**	11
Pseudomonas aeruginosa	74	*Clostridium hiranonis**	11
Staphylococcus aureus	73	*Fusobacterium nucleatum**	11
Staphylococcus epidermidis	71	*Parvimonas micra**	11
*Anaerococcus vaginalis**	45	*Prevotella buccalis**	11
Corynebacterium striatum	36	*S. piscifermentans**	11
Enterococcus faecalis	36	*Terrimonas ferruginea**	11
Serratia marcescens	34	*Burkholderia ambifaria**	10
*Anaerococcus lactolyticus**	33	*Corynebacterium jeikeium*	10
*Propionibacterium acnes**	28	*Peptoniphilus lacrimalis**	10
*C. tuberculostearicum**	27	*Staphylococcus capitis*	10
*Pelomonas saccharophila**	26	*Staphylococcus hominis*	10
*Peptoniphilus indolicus**	24	*Prevotella melaninogenica**	9
Streptococcus agalactiae	23	*Acinetobacter baumannii*	9
Escherichia coli	19	*Staphylococcus caprae*	9
*Peptoniphilus ivorii**	19	*Bacteroides fragilis**	8
*Anaerococcus octavius**	17	*C. aurimucosum**	8
*Ralstonia pickettii**	17	*Porphyromonas levii**	8
Streptococcus mitis	17	*Prevotella bivia**	8
*Porphyromonas somerae**	16	*Acinetobacter junii*	7
*Anaerococcus prevotii**	14	*Bacteroides thetaiotaomicron**	7
*Peptoniphilus harei**	13	*Candida albicans**	7
*Anaerococcus hydrogenalis**	12	*Staphylococcus lugdunensis*	7
Corynebacterium xerosis	12	*Streptococcus parasanguinis*	7
Pseudomonas hibiscicola	12	*Streptococcus sanguinis*	7
*Ruminococcus obeum**	12	*Streptococcus thermophilus*	7
Staphylococcus haemolyticus	12	*Veillonella parvula**	7
Stenotrophomonas maltophilia	12	*Actinomyces europaeus**	6

* Highly fastidious bacteria
The top 56 microorganisms out of the 584 species of bacteria and yeast identified are given here. Thirty-three organisms (over half) were difficult to culture or nearly impossible to diagnose as part of common clinical diagnostic procedures. It should be noted that while many advanced research laboratories can often culture these microorganisms, most clinical microbiology cannot

Table 4. Topical treatments used in 2007

2007 (n=244 patients)		2009 (n=299 patients)	
ABBA	203 (83%)	ABBA	158 (53%)
Iodosorb	14 (6%)	APG	57 (19%)
Panafil	13 (5%)	Iodosorb	27 (9.0%)
Bactroban	4 (2%)	TKS	25 (8%)
Hydroferra	2 (1%)	Santyl	12 (4%)
Triclosan	2 (1%)	Xenaderm	4 (1%)
Gladase	1 (0.4%)	Hydroferra	3 (1%)
		Bactroban	1 (0.3%)
		Prisma	1 (0.3%)
		Regranex	1 (0.3%)
		Silvadene	1 (0.3%)

ABBA = antibacterial biofilm agents: lactoferrin, xylitol farnasol and galium
APG = All-Purpose Gel: amikacin, triclosan and metronidazole in methylcellulose
TKS (anti-MRSA gel): triclosan, amikacin, kammamelatannin in Sanguitec Gel

which would have skewed the data towards shorter durations for healing in the 2009 group.

Table 1 provides a summary of patients' demographic information for the two comparative year groups (2007 and 2009). In 2007, 244 (48.5%) of the 503 patients admitted with a chronic wound healed completely, while in 2009, 298 (62.4%) of the 479 patients healed (Fisher's exact test, $p<0.001$; OR=1.76, 95% CI=1.36–2.29). Thus, over an equal period of time, a significantly higher percentage of patients healed in 2009 compared with 2007.

Of the potential confounders that were examined, only peripheral vascular disease (PVD) and spinal cord impairment differed between the two year groups (Fisher's exact test, $p<0.05$). Distributions of race, wound type, presence of diabetes, hypertension, and heart disease were consistent between the two groups.

The 2007 clinical culture results are summarised in Table 2. Fewer patients were sampled for clinical cultures in 2009. From a practice standpoint, both sampling and the use of culture results had become selective and limited in 2009, largely because of a growing distrust in the results (or lack of results) among staff. A significant number (Fisher's exact test, $p<0.05$) of diagnostic reports returned from the clinical culture laboratory reported 'no growth'.

Table 3 provides a summary of results from comprehensive molecular diagnostic testing in the 2009 group. There is a significant increase in diagnoses (Fisher's exact test, $p<0.05$) when compared with the 2007 group, and significantly more species

(Fisher's exact test, $p<0.00001$) were identified. The rapid uptake of these testing methods has been attributed to clinicians obtaining actionable diagnostic results.

Table 4 provides a breakdown of topical dressings used in 2007 and 2009. The numbers of hydrocelluoid, foam and other primary dressings were very comparable (within 10% for each group), although there was a 15% decrease in silver-impregnated dressings. Changes were noted in non-invasive vascular testing and venous evaluation between year-groups. In 2007, 56% of patients (137/244) had non-invasive vascular studies and 24% (59/244) had venous exams, whereas in 2009, 49% of patients (147/299) had non-invasive vascular studies and 25% (75/299) had venous exams.

Fig 1 provides the most important data related to patient outcome. Based on the Cox proportional hazards model, after controlling for age, PVD and spinal cord impairments, the time to healing was significantly shorter in 2009 ($p<0.05$). In fact, on average patients in 2009 were 22.9% more likely to have healed at any given time ($e^{\beta}=1.229$; 95% CI=1.032–1.463). Venous leg ulcers showed a 13.1 day or 21% reduction in time to heal, pressure ulcers showed an 11.7 day, 23% decrease in time to healing, diabetic foot ulcers showed a 14.2 day, 23.8% improvement in healing rate, and all wounds combined showed an 11.8 day (22%) decrease in time to healing when compared with 2007 rates.

Interestingly, very few cultures (16%) were conducted in the 2007 group and of these results, 23% reported 'no growth' or negative results. By June 2009, molecular diagnostics were being used in 68% of patients.

Discussion

The Southwest Regional Wound Care Center introduced molecular pathogen diagnostics in 2008. The improved diagnostic ability observed by clinicians led to their rapid uptake, and the use of these tests is now common practice at the Center in the management of many wound types. We largely attribute the decrease in time to healing and the significantly enhanced wound healing rates between our 2007 and 2009 year groups to the vast improvements in this diagnostic technology.

Molecular pathogen diagnostics provide clinicians with the ability to use specific and appropriately tar-

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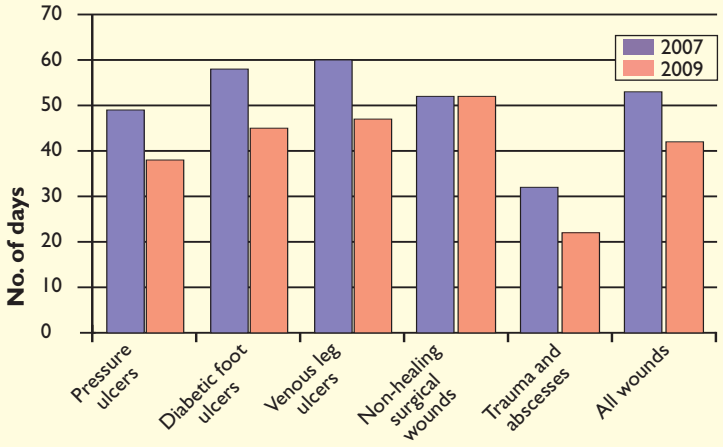
Fig 1. Reduction in days to heal



geted antibiotics and topical treatments. The reduction in associated medical costs, together with the humane and ethical considerations that accompany a decrease in overall healing times, now prevent us from denying any patient such diagnostics.

Table 5 summarises antibiotic use in the different year groups, illustrating how comprehensive molecular diagnostics have resulted in patient-specific, targeted treatments.

In 2007, very few bacterial species were identified from positive cultures, and 'no growth' was a common result of testing. In addition, some positive results were non-specific; for instance, 'yeast', with no further identifying information, was identified in two of the cultures.

The data obtained through molecular diagnostics is much more detailed. The results of such testing in the 2009 group gained no negative results — overall, 584 species of bacteria and yeast were identified, the most ubiquitous organism being an anaerobic bacterium, *Finegoldia magna*. A further benefit of molecular diagnostics is that results are obtained more quickly (four days for a full, comprehensive analysis compared with five days for culture-based diagnostics) and can be readily translated to clinical application.

Compared with molecular diagnostics, clinical cultures showed significantly fewer organisms (Fisher's exact test, $p<0.00001$) and did not yield a detailed enough analysis of wounds to allow for effective antimicrobial treatment. Therefore, the use of systemic, largely empiric, antibiotics in 2007 was sparse and anecdotally considered ineffective. Although healing rates in this group were acceptable by national standards, most of the improvement compared with previous years (data not shown) was achieved through a regimen of debridement, antibiofilm agents and broad spectrum biocides.[15] By 2009, systemic antibiotics were used only when diagnostic results were available. The use of systemic antibiotics increased by 16%, from 47% (115/244) in 2007 to 63% (188/299) in 2009. The other significant change in antibiotic use between year groups was the employment of five new antibiotics for specific microbes.

An example of the improvement in antibiotic therapy is *Staphylococcus* treatment, which was more focused and effective in 2009. In 2007, 10 cultures showed meticillin-resistant *Staphylococcus aureus* (MRSA), which accounted for 15% of all the results obtained from culture-based diagnostics and 100% of all samples demonstrating *Staphylococcus aureus*. For the 10 patients identified with MRSA, 20 courses of expensive first-line MRSA antibiotics (linezolid, daptomycin, tigecycline) were used, accounting for 12% of all antibiotics given to the 2007 group. In 2009, 41 patients (20% of all samples) were shown to have MRSA, an increase attributed to the improvement in sensitivity of molecular diagnostics. Yet because of the quantitative component of molecular diagnostics, only 26 courses of first-line antibiotics were given (8% of all antibiotics in this group), gaining significantly better results than the 2007 group. The use of second-line MRSA antibiotics, including doxycycline, trimethoprim/sulfa and clindamycin was the same in both groups, in spite of the increased detection. Because these changes in antibiotic therapy are solely a consequence of molecular diagnostic results, these data demonstrate that molecular diagnostics can allow more precise antibiotic use with greater efficacy.

Molecular diagnostics also revealed several organisms which, although prevalent, are seldom successfully cultured; their discovery requires that a broader selection of antibiotics be available to clinicians. These organisms include yeast, anaerobes, and difficult-to-treat organisms such as *Corynebacterium striatum*.

Five new antibiotics were used in 2009 to target the more complex microbial reality. For example, *Corynebacterium* as a genus is heterogeneous in its response to different types of antibiotics. But the more prevalent species, especially *C. striatum*, are known to be susceptible to clarithromycin, which accounted for 4.6% of all antibiotic use in the 2009 group. Other new antibiotics used included metronidazole (2.2%) for anaerobes, fluconazole (0.3%) for yeast, and procaine penicillin (1.2%) for dual coverage of *Streptococcus* spp. and anaerobes.

The one wound aetiology that did not show a significant decrease in time to healing was non-healing surgical wounds. This was the only category of wounds treated differently from the rest. Referring surgeons requested that skin edges remain approximated, and so these dehiscing wounds were not subject to the principles of biofilm-based wound

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Table 5. Summary of antibiotic use in 2007 versus 2009

Systemic antibiotics used	2007 (n=244 patients)	2009 (n=299 patients)
No. of antibiotic courses used	164	323
Average antibiotic courses per patient	1.4	1.7
Patients treated with antibiotics	115 (47%)	188 (63%)
Doxycycline	59 (36%)	85 (26%)
Ertapenem	30 (18%)	62 (19%)
Sulfamethoxazole/Trimethoprim	5 (3%)	43 (13%)
Ciprofloxin	4 (2%)	18 (6%)
Daptomycin	4 (2%)	18 (6%)
Clarithromycin	0 (0%)	15 (5%)
Levofloxacin	8 (5%)	14 (4%)
Cefepime	1 (1%)	10 (3%)
Clindamycin	5 (3%)	8 (3%)
Imipenem/Cilastatin	7 (4%)	8 (3%)
Cephalexin	18 (11%)	7 (2%)
Metronidazole	0 (0%)	7 (2%)
Linezolid	9 (6%)	6 (2%)
Amoxicillin/Clavulanate	3 (2%)	5 (2%)
Penicillin G Benzathine	0 (0%)	4 (1%)
Rifampin	0 (0%)	4 (1%)
Ceftriaxone	2 (1%)	3 (1%)
Amoxicillin	2 (1%)	2 (1%)
Tigecycline	6 (4%)	2 (1%)
Dicloxacillin	1 (0.6%)	1 (0.3%)
Fluconazole	0 (0.0%)	1 (0.3%)

The number of prescriptions for expensive first-line drugs decreased notably in 2009, while the use of antibiotics that specifically target bacterial populations increased (e.g. metronidazole for anaerobes)

care. For the first one to three weeks of treatment, it was not possible to combine aggressive debridement with targeted therapies based on molecular diagnostics, thus treatment was much less effective.

Future directions and topics for discussion

Topical antibiotics have often been discouraged in wound care, even though their effectiveness has not been disproven. The most readily available and mature tools for targeting specific bacteria are antibiotics, yet the increased use of systemic (injectable and oral) empiric antibiotic therapy risks producing resistant microorganisms. There also exists the possibility that systemic antibiotics will reach the biofilm bacteria in subtherapeutic doses, increasing the risk of resistance.[17,18] A review of the literature reveals no significant increase in the risk of allergy (sensitisation)[19] or antibiotic resistance with high-dose topical use.[19-23] Indeed, since topical antibiotics can be applied at 500 to 1,000 times minimum inhibitory concentration (MIC), resistance may be less likely.[24] It is consistent with infectious disease principles that if the microorganisms present in wound biofilms are diagnosed with scientific certainty and appropriate antibiotics are applied topically at several times MIC, then some of the risk of resistance will be reduced and antibiotic therapy will be more efficacious.[19-23]

When topical antibiotics have previously been used in chronic wounds, clinicians have noted that wound healing stops and the secondary signs of infection, such as increased drainage, increased slough and friability re-emerge. This has led to the mistaken assumption that wound bacteria develop resistance to topical antibiotics. However, using comprehensive molecular pathogen diagnostics, we have found wound biofilms to be truly polymicrobial. It is rarely possible to apply topical antibiotics that will be highly effective against all of the microorganisms present, either alone or in combination. Therefore, after a short period of time (2–4 weeks) the bacteria that are sensitive to treatment become suppressed and the remaining microorganisms, which were never sensitive to the treatment, take over the wound (soon to be published findings). This scenario is not the development of antibiotic resistance; rather, this is succession or replacement of one microbial population with another. It could well be true that remnants of the originally-targeted population may continue to exist within the biofilm as persister cells. But the main mechanism of biofilm response to specific antibiotic therapy is not one of 'resistance' but rather 'population adaptation'. Data is currently being accumulated to support this hypothesis; the strategy to overcome this scenario is, logically, to re-diagnose the new pathogen population and modify therapy accordingly.

Originally, biofilms have been viewed as being comprised mainly of known pathogens, such as *S. aureus* and *P. aeruginosa*, that are contaminated by minor populations. This view was fostered by data from agar cultures, which yielded a manageable number of different bacterial species. Clinically, culturing wounds seemed to confirm the precept of one organism per one infection. However, clinical responses to treatment based on these findings have

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been confusing. Molecular methods have been used to survey most aetiologies of chronic wound, including venous leg ulcers,[6] pressure ulcers (in press), diabetic foot ulcers[5,25] and non-healing surgical wounds.[7] These surveys depict a new reality — that most chronic wound biofilms comprise polymicrobial communities.

In the clinical setting, comprehensive molecular diagnostics have the ability to define and monitor the microbial composition of each specific wound biofilm. Each wound can benefit from diagnostics and personalised treatment. Although no approved clinical strategies currently exist to directly attack the synergies or other quorum-sensing activities within wound biofilms, some early efforts appear to be clinically effective against biofilm defences.[26] This has led to our concept of multiple simultaneous strategies to combat biofilm defences (attachment, extracellular polymeric substance formation, phenotypic diversity and slow growth/no growth states). By targeting these defences, more traditional treatments such as selective biocides and antibiotics become more effective.

Physical disruption of the biofilm (debridement) is one strategy; it works in synergy with others by promoting a window of therapeutic opportunity — by removing an established biofilm, the bacterial community is forced to reconstitute itself. Thus, the bacterial communities must increase their metabolism, theoretically making them more susceptible to antibiotics than stationary phage or biofilm phenotype bacteria. Hence, during re-growth, different bactericidal strategies should be more effective. The concomitant use of antibiofilm agents (which prevent reattachment, block EPS formation etc.) immediately post-debridement makes selective biocides (such as silver, cadexomer iodine) and targeted antibiotics more effective, slowing the re-accumulation of bioburden. We term the concomitant use of such strategies 'biofilm-based wound care'.[8,15,26]

By 2007, Southwest Regional Wound Care Center was practising biofilm-based wound care on all patients. Host barriers to healing were addressed by commercially available dressings and durable medical equipment, as well as nationally recognised protocols. The specific agents and methods used to manage wound biofilm have been previously described.[15] It is important to note that these methods were generic in 2007 and driven by culture-based diagnosis of the microbes present in wound biofilm. The impressive improvement in healing rate seen in 2009 represents the first step in a sniper approach (personalised or individualised therapy) to targeted treatment, which relies on information obtained through these comprehensive molecular diagnostic methods (Pathogenius Diagnostics, Lubbock, TX www.pathogenius.com). Early in 2009, comprehensive molecular diagnostics developed by the Research and Testing Laboratory were fully integrated into our wound care centre and by June molecular diagnosis was the only test used to characterise wound bioburden. Agar cultures were no longer used in the management of wounds.

Conclusion

The decrease in time to healing for venous leg ulcers, pressure ulcers and diabetic foot ulcers suggests that wound bioburden may be a barrier to wound healing. In fact, the improvement of wounds with specific appropriate antibiotics raises the possibility that, in these patients, wound biofilm may have been a true infection. We conclude that chronic wounds, regardless of their aetiology, progress into chronic infections, as evidenced by their persistent chronic inflammatory state,[27-30] their failure to progress through a normal wound-healing trajectory[31] and their response to appropriate antimicrobial strategies, including antibiotics.

These data also suggest that modern molecular methods are possibly more robust and clinically relevant than routine clinical cultures. The advantages of molecular cultures ware myriad, yielding very rapid, specific, sensitive, quantitative and comprehensive results, with little selection bias.

There exists the possibility that chronic wounds are chronic polymicrobial infections; we must be able to fully diagnose these microbial communities that work together. ■

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