New Molecular Techniques to Study the Skin Microbiota of Diabetic Foot Ulcers

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Significance: Diabetic foot ulcers (DFU) are a major and growing public health problem. They pose difficulties in clinical practice in both diagnosis and management. Bacterial interactions on the skin surface are important in the pathophysiology of DFU and may contribute to a delay in healing. Fully identifying bacteria present in these wounds is difficult with traditional culture methods. New molecular tools, however, have greatly contributed to our understanding of the role of the cutaneous microbiota in DFU.

Recent Advances: Molecular technologies revealed new information concerning how bacteria are organized in DFU. This has led to the concept of "functionally equivalent pathogroups," meaning that certain bacterial species which are usually nonpathogenic (or at least incapable of maintaining a chronic infection on their own) may coaggregate symbiotically in a pathogenic biofilm and act synergistically to cause a chronic infection. The distribution of pathogens in multispecies biofilms is nonrandom. The high bacterial diversity is probably related to the development of a microbial biofilm that is irreversibly attached to the wound matrix.

Critical Issues: Using molecular techniques requires a financial outlay for high-cost equipment. They are still too time-consuming to perform and reporting is too delayed for them to be used in routine practice. Finally, they do not differentiate live from dead or pathogenic from nonpathogenic microorganisms.

Future Directions: Molecular tools have better documented the composition and organization of the skin flora. Further advances are required to elucidate which among the many bacteria in the DFU flora are likely to be pathogens, rather than colonizers.

SCOPE AND SIGNIFICANCE

Foot ulcers are common in diabetic patients, with a lifetime prevalence as high as 25%.[1] Infection of diabetic foot ulcers (DFU) is a frequent (40–80%) and costly[2] complication, representing a major cause of morbidity and diabetes-related hospital admissions, as well as one of the major pathways to lower limb amputation.[3–5] The complete identification of bacteria present in wounds is difficult with traditional culture methods, as they do not fully reveal the bacterial diversity present, when compared to results using newer molecular techniques.[6] This review aims to assess the contribution of these molecular techniques to the understanding of the role of cutaneous microbiota in DFU.

TRANSLATIONAL RELEVANCE

The human–microbe interface is often the key point in the development of wound infections. However,

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at this interface, the number of pathogenic microbial species is small compared to the many commensal bacteria. The complete flora colonizing a mucosal or cutaneous surface constitutes the microbiota. Newer molecular tools allow the detection of microbiota on various colonized surfaces, including on skin.[7–9] Over 500 species have been identified in the various microbiota, which appear to comprise a host's individual microbial fingerprints.[10–12] Better defining skin microbiota is essential for understanding the host–bacterial interactions that lead to clinical evidence of infection and problems with wound healing.

CLINICAL RELEVANCE

Spectacular progress in sequencing bacterial genomes, coupled with the development of new molecular approaches, has enabled us to study the steps in the evolution of the complex flora of microbiota as well as the development of wound infections.[13–15] In parallel, recent studies showed that disruptions of the balance at the host–microbe interface in the gut may lead to severe chronic diseases, such as obesity or diabetes.[16–22] This suggests that our understanding of physiopathology of many diseases requires further exploration of this delicate balance between the host and microorganism. The greater understanding emerging from these studies raises hopes for finding improved methods to both prevent and treat DFU.

BACKGROUND

Metagenomics is a science that applies a suite of genomic technologies and bioinformatics tools to directly access the genetic content of entire communities of microrganisms.[19,23] In microbiology, this approach allows the study of genomes of the different bacterial populations in a given environment (*e.g.,* skin, gut).[23] While traditional microbiology, as well as microbial genome sequencing and genomics, rely upon clonal cultures, environmental gene sequencing using the 16S rRNA gene produces a profile of the genetic diversity in a specific sample. This has revealed that the vast majority of microbial biodiversity had been missed by cultivation-based methods.[6,8,24] Because of their ability to reveal the previously hidden diversity of microscopic life, metagenomic tools offer an opportunity to view the microbial world in a way that has the potential to revolutionize our understanding.

Another important finding concerning the pathophysiology of colonization and infection in DFU is the recognition that the bacteria are often found in biofilms. While bacteria may be planktonic (free floating), they commonly become sessile (attached to surfaces) and form biofilms, that is, composites of aggregated cells encased in the extracellular matrix of hydrated polymers and debris.[25] These may impair wound healing and protect the enmeshed bacteria from both host immune responses and antimicrobial treatments. Many biofilms, like those on teeth and mucous membranes, are not only harmless but also necessary for many normal functions. A fundamental yet still unanswered question therefore is, why are some biofilms benign and others able to induce clinically significant infection?

Regarding the pathogenic role of bacteria found in DFU, there are currently two main hypotheses under discussion.[26] The specific bacteria hypothesis suggests that only a few species of bacteria within the heterogeneous polymicrobial biofilm are involved in the infectious process. Conversely, the nonspecific bacteria hypothesis (or community hypothesis) considers the bacterial composition of biofilm as a whole to constitute a functional unit, and does not examine the role of individual pathogenic bacteria alone. This concept has led to use of the term "functionally equivalent pathogroups" (FEP).[24] This postulates that certain bacterial species that usually behave in a nonpathogenic manner, or at least are not capable of maintaining a chronic infection when present on their own, may coaggregate symbiotically in a pathogenic biofilm and act synergistically to cause a chronic infection.[27]

Molecular microbiological methods have uniformly demonstrated that most DFU play host to many more bacterial species than were previously appreciated, based on the results of standard microbiological cultures.[24] Which among these organisms, acting alone or in combinations, are most likely to cause clinical manifestations of infection is the subject of considerable speculation. Furthermore, when the host has diabetes or has been exposed to antimicrobial therapy, this can alter the community structure of the bacterial flora in the foot ulcer, reducing some species while enhancing growth of others.[28] Defining the full microbiota of both intact and wounded skin should improve our understanding of the microbial nature of DFU.[29]

DISCUSSION

Problems regarding the microbiology of DFU

With the increasing prevalence of diabetes worldwide (now affecting nearly 6.5% of the population), diabetic foot complications are a growing problem (www.idf.org/diabetesatlas). In one study from the United States, the risk among diabetic

persons of hospitalization and lower extremity amputation were ≈ 56 and 155 times greater, respectively, for those who had a foot infection than for those without.[30] In the European Eurodiale study, 58% of diabetic patients from 14 foot clinics in ten European countries with a new foot ulcer had a wound that was clinically infected.[2] In our own experience, like that of many others, the prognosis for patients with a diabetic foot infection (DFI) remains poor; in one study, nearly half of those admitted in specialized French foot clinics for DFI had some form of lower limb amputation and 8% of patients died during the 1-year study period.[31]

At presentation, about half of the DFU are clinically infected, that is, they have classical signs or symptoms of inflammation. These DFI pose difficult problems in clinical practice, not only in terms of their management but also their diagnosis. The frequent presence in persons with diabetes of peripheral arterial disease, sensory neuropathy, or impaired immunological functions may reduce the local inflammatory response and thereby the evidence of local infection.[32,33] Moreover, systemic signs of toxicity, such as leukocytosis or fever, are often lacking or appear late, even in severe DFI cases.[34–36] Thus, neither local nor systemic inflammatory signs or symptoms, or even biological markers, should be regarded as reliable for diagnosing foot infection in diabetic individuals.

For many decades, a culture of a wound specimen was the only way to determine the causative pathogen(s) in a DFI. In the past few years, however, molecular microbiology techniques have demonstrated the presence of greater numbers and varieties of species in various types of wounds than had previously been recognized.[24,28,29,37–39] The goal now is to better understand the role of the many organisms we now know that comprise the cutaneous microbiota in DFU.

New molecular techniques for better understanding DFU

Most new techniques for studying DFU are based on the amplification of the 16S rRNA gene. This highly conserved gene is present in the genome of all prokaryotes, but it contains hypervariable regions that can be used for identifying specific bacterial species. The different techniques currently available include denatured gradient gel electrophoresis (DGGE); temperature gradient gel electrophoresis (TGGE); pyrosequencing; and multitarget polymerase chain reactions (PCRs) (Table 1 and Fig. 1). These tools are useful for both identifying and monitoring of microbial populations. DGGE and TGGE are closely related technologies. They allow identification of different bacterial species by separating the 16S rRNA amplicons using denaturation (DGGE) or temperature (TGGE). After the separation of the amplicons, the identification of bacteria requires sequencing the different bands present on the gel. Metagenomics tools are the most recent development in the study of microbiota. They use massive parallel sequencing of the partial 16S rRNA amplicons or a whole genome from a cutaneous biopsy. All these technologies now permit description of the microbiota, a collective community of bacteria, as well as their total genome capacity in a given environment, such as a DFU.

Advantages and drawbacks of molecular tools

Molecular tools help expand our knowledge of the full range of bacterial species present on a chronic wound, the association between wound microbiota, and the development and outcome of a DFI. The key point is that we must reconsider the ways we identify microorganisms in a DFU, now that we understand the relative weakness of culture-based results (Table 1). Metagenomics studies have certainly made a major breakthrough in the analysis of microbial flora, detecting a greater complexity of flora and revealing the presence of previously unknown or uncultivated microorganisms. It is also important to consider that molecular microbiology methods, especially pyrosequencing techniques, do have some drawbacks.[40] First, there is the high cost of acquiring molecular technology equipment and the substantial demand on the microbiology technician's time to assess and compare the sequences provided. Then, there is the problem that this technology amplifies not only the living but also the dormant or dead bacteria in a sample. Additional problems include the fact that these techniques estimate most, but not all, of a microbial population. Indeed, the quality of DNA extraction varies according to the species.[41] The 16S rRNA primers lack universality and neglect some microbial populations such as viruses, *Archae*, and members of the divergent superphylum *Planctomycetes-Verrucomicrobia-Chlamydiae* that may be responsible for pathological conditions.[42] Interestingly, some viruses and fungi have been identified in various types of chronic wounds,[43] but the clinical significance of these isolates is unclear. Finally, these technologies are unable to display the microorganisms' metabolic activity, as they only detect the presence of their genes.

Molecular tools and management of DFU

While recognizing that these new methods have limitations, the availability of molecular microbiology technologies has brought to light new

Table 1. *Comparison between the different technologies used to study the skin microbiota*

	Culture	*DGGE/TGGE*	*Metagenomic*	*Culturomic*	*Metatranscriptomic*	*Metaproteomic*	*Metabolomic*
Principle	Isolation of bacteria on different selective media	Gel separation of 16S rRNA amplicons using denaturant or temperature	Massive parallel sequencing of partial 16S rRNA amplicons or whole genome	Cultures of direct samples (>50) followed by massive parallel sequencing of partial 16S or 18S rRNA amplicons	mRNA products by bacteria detected by metagnomic	All protein products by bacteria detected by 2D electrophoresis and LC-MS/MS	HPLC or LC-MS
Strengths	Cheap, semiquantitative	Detection of a great part of complex flora, best than cultures	Detection of collective assembly of genomes from an environment, phylogenetic identification, quantitative, identification of unknown bacteria	Phylogenetic identification, quantitative, identification of unknown bacteria, virus or parasites, Isolation of live bacteria	Detection of collective repertoire of mRNA molecules, Detection of a panel of gene expression	Detection of collective set of proteins expressed by or present	Detection of collective low molecular-weight compounds (volatile metabolites) produced or present in bacteria
Limits	Fastidious, isolation of a small part of the bacteria present, difficult to obtain fastidious bacteria or bacteria in biofilm	Fastidious, isolation of a part of the bacteria present, no difference between live or dead bacteria	PCR bias, laborious	Laborious	RNA extraction, polymicrobial infections	Laborious, low reproducibility of 2D, many proteins detected and difficulties to interpret results	Polymicrobial infection
Complexity of data analysis	Very easy	Easy	High	High	Very High	High	High
Time to results	1–15 days	48 h with scale 2 weeks without	Hours	Hours	48 h	5–7 days	24–48 h
Time to analysis	Minutes	Minutes	Week(s)	Months	Months	Weeks	Weeks
Detection of new microorganisms	No	Possible, but limited	Yes	Yes, including viruses	Possible	No	No
Running costs	Low	Low	High	High	High	High	High
Application in DFU (PubMed)	Yes	Yes	Yes	No	No	No	No

2D, two dimensional; DFU, diabetic foot ulcer; DGGE/TGGE, denatured gradient gel electrophoresis/temperature gradient gel electrophoresis; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography-mass spectrometry/mass spectrometry; PCR, polymerase chain reaction.

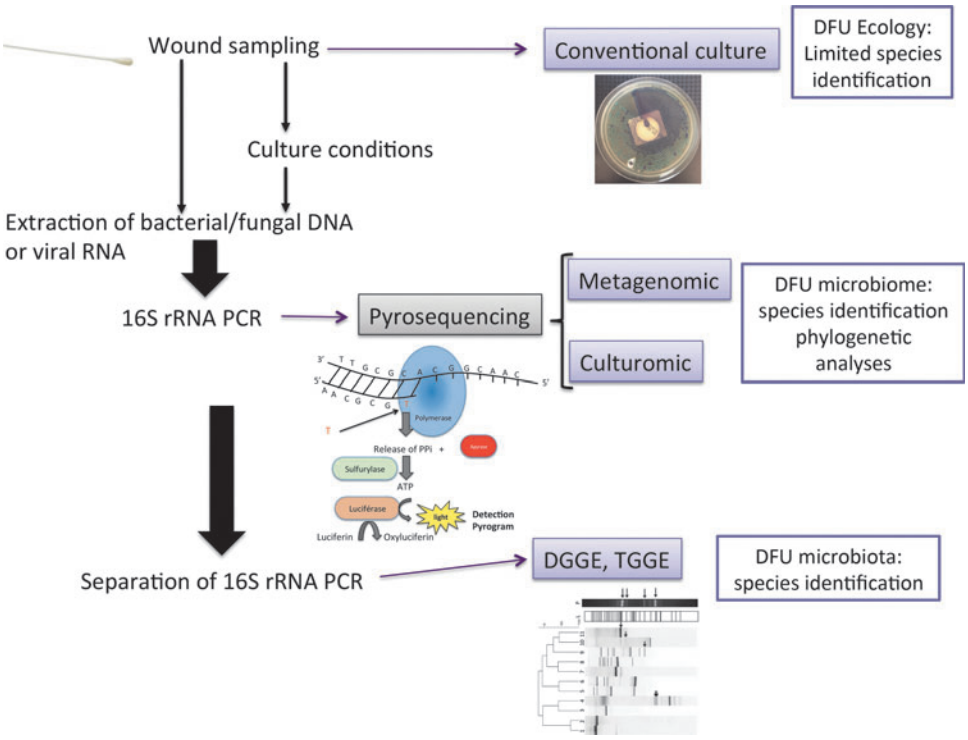

Figure 1. Overview of currently available techniques to characterize the skin microbiota in diabetic foot ulcers. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

information concerning the bacterial populations in DFU. Taken together, available data suggest that infections of DFU more often arise from the presence of specific combinations of pathogens, rather than a simple increase in the microbial load of any one opportunistic microbe. Furthermore, using DGGE to separate the different amplicons generated after the 16S rRNA PCR (Table 1 and Fig. 1), we have confirmed that the deep flora of diabetic foot wounds is more diverse and complex than the superficial flora. Moreover, we noted that there was a predominance of pathogenic bacteria localized in the deepest portion of the wound, confirming the need to collect deep tissue samples to accurately define the microbiology of a DFU.[44]

Other studies have shown that using the techniques of sequencing the 16S rRNA gene and DGGE reveals much more complex bacterial communities in DFU than those identified by culture.[37,38] This high bacterial diversity is probably related to the development of biofilm, a (usually polymicrobial) sessile community made of microorganisms that are irreversibly attached to a surface and encased in an extracellular polymeric matrix that they produce.[45] Indeed, it has been reported that 60% of chronic wounds (and 77% of DFU) exhibit biofilms.[46] When enmeshed in biofilm, the bacteria demonstrate greater resistance to the various host immune mechanisms as well as to antibiotics. Biofilm bacteria also appear to have a greater virulence than the planktonic (free-floating) cells, which are the ones mainly identified by standard wound culture techniques. A complex sequence of events takes place during the formation of these biofilms: it begins with a random bacterial settlement of early colonizers, followed by increased competition among the various species present, and then a niche differentiation resulting in a heterogeneous biofilm (Fig. 2).[47] The presence of organisms in biofilm may also account for the resistance of some DFI to a single antibiotic agent. In sum, these observations corroborate that the skin microbiota of a DFU is organized in a pathogenic biofilm FEP that acts synergistically to cause a chronic infection.[24,27] This understanding reinforces the need for vigorous mechanical debridement of chronic wounds before sampling in the aim to reduce, if not eliminate, the FEP.

We also now understand that the distribution of various pathogens in multispecies pathogenic biofilms is nonrandom.[48] For example, a quantitative analysis of the distance of bacterial aggregates from the wound surface showed that those composed of *Pseudomonas aeruginosa* were located significantly deeper in the wound bed than those composed of *Staphylococcus aureus*.[49] This distribution of

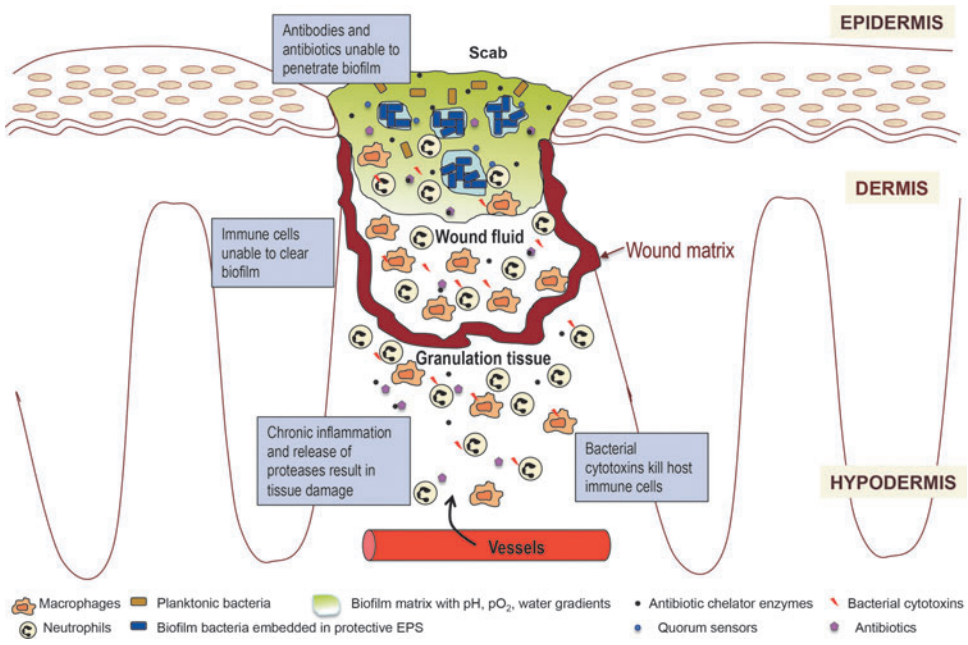

Figure 2. Factors influencing delayed wound healing. The presence of biofilm and abundant leukocytes surrounding the biofilm prevent the healing of the wounds. Moreover, the balance of proteases produced by inflammatory cells also damage normal and healing tissues and immune cells, adversely affecting healing (adapted from Phillips *et al.*[47]). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

aggregates could explain the fact that *S. aureus* at the skin surface forms a biofilm specifically inhibiting wound healing mechanisms, thereby averting the effects of localized immunity and enabling other microorganisms to colonize and infect the wound.[50] Moreover, it may explain the underrepresentation of *P. aeruginosa* and overrepresentation of *S. aureus* noted with conventional culturing of swab samples from chronic wounds. The recognition of this organization of organisms further corroborates the need for debridement of the wound to reduce the number of bacteria of relatively low pathogenicity, such as *P. aeruginosa*, *Stenotrophomonas maltophilia*, and *Enterococcus* spp. Of importance is that even if these bacteria are not mixed they can collaborate; for example, waste products from *P. aeruginosa* may serve a protective role for *S. aureus*.[51] Similarly, in a rat model, it was observed that the presence of low levels of *P. aeruginosa* associated with *S. aureus* increased the infection rates.[52]

Molecular techniques have also revealed that obligate anaerobes are far more prevalent in wounds than had been suggested by the results of standard cultures, perhaps because they are often present in FEPs.[24,39] Because molecular tools are not yet available in most clinical settings, identifying obligate anaerobes in pathological biofilm requires using suitable culture methods. These include optimal methods of wound sampling, specimen transport, and selection of culture media. Furthermore, we observed that aerobic bacteria, such as corynebacteria, are localized in the upper portions of the wound, where the oxygen content is relatively high, while anaerobes are localized in deeper hypoxic niches created by consumption of oxygen by overlying aerobes. Results using the accurate and robust technique of the 16S rRNA pyrosequencing,[53,54] underscore the high bacterial density and diversity of species found in various clinical settings. It has also revealed the relative presence of various organisms in DFU, for example, the overrepresentation of *Staphylococcus* spp. and underrepresentation of anaerobes in neuropathic DFU.[39] These findings were not unexpected, because staphylococci grow more easily than exigent bacteria, like anaerobes.

The 16S rRNA technique has also demonstrated that standard wound cultures underestimated the presence of *S. aureus*, when it was in low quantities. Interestingly, *Staphylococcus* species account for 70% of the normal skin microbiome on the plantar zone of the foot.[9,55] Most of these are coagulase-negative staphylococci and are in competition with *S. aureus*.[56] In contrast, *Staphylococcus* spp. constitute only 7% of the microbiota on the intact skin or contralateral limb of patients with DFU, suggesting either a loss of these protective bacteria or their replacement by other species (Fig. 3).[57] Finally, molecular microbiology tools have shown that microorganisms in DFU can be partitioned

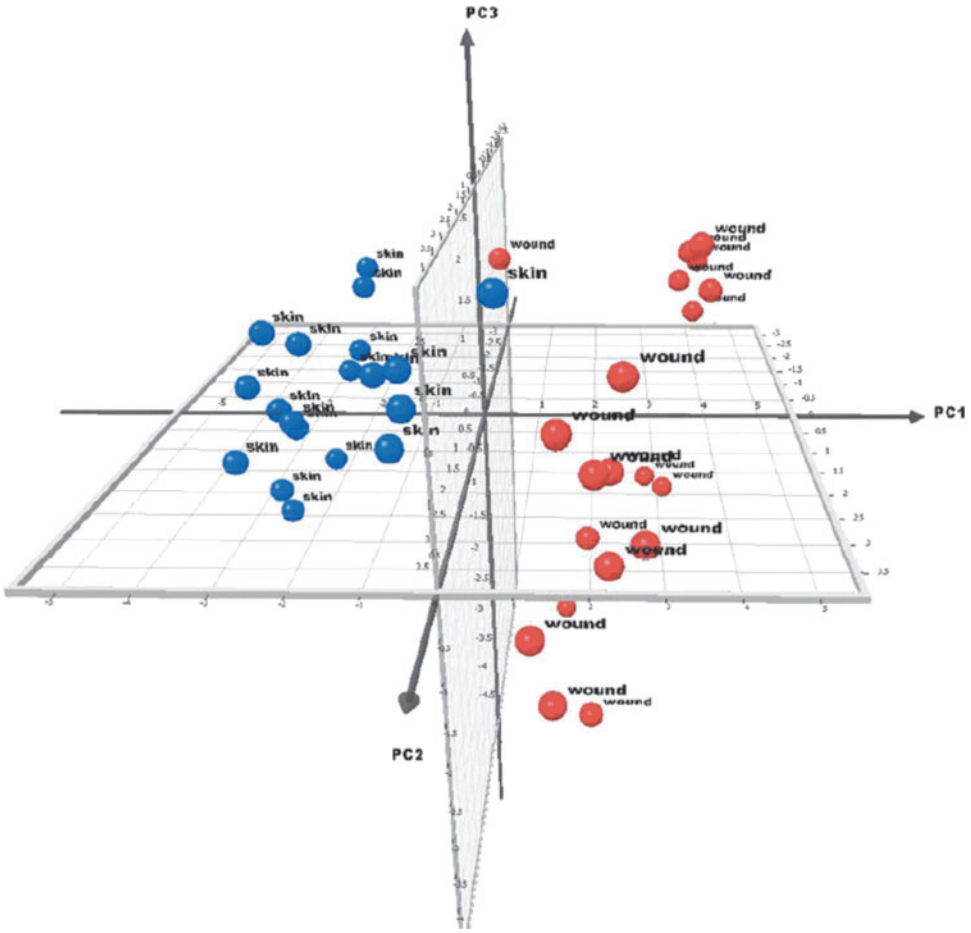

Figure 3. Displayed are the three main groups of microorganism comprising the skin flora, as demonstrated by PCA. The axes represent the values for principal components 1, 2, and 3. Points lying in the negative portion of an axis indicate a negative correlation between the principal component and the sample. Organisms found on intact skin are shown in blue, while those from wounds are shown in red. The ability to linearly separate the classes within the PCA figures indicates that bacteriology of intact skin differs from that of wounds (adapted from Gontcharova *et al.*[57]). PCA, principal component analysis. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

into three so-called Euclidean ulcer clusters (EUCs): EUC1, with a high proportion of anaerobes and *Proteobacteria*; EUC2, with a high proportion of *Staphylococcus*; and EUC3, with a high proportion of *Streptococcus*.[39] Similarly, a recent study found that the intact skin microbiota of diabetic patients is more diverse and has a different composition than the microbiota of nondiabetic patients.[58] These ecologic changes are consistent with the decrease of coagulase-negative staphylococci and the increase of *Corynebacterium* spp. and *S. aureus* described above. This enrichment of the number of *S. aureus* organisms may be a precursor to developing clinically apparent infection in a DFU.

There are many possible reasons for the changes seen in the flora in wounds, including alterations in sweat glands, sebaceous glands, or hair follicles. Each of these skin structures contributes to specific microenvironments and each has its own microbiota.[55] Diabetic patients are known to have altered sweat and thermoregulatory responses, even before they develop clinical neuropathy.[59] The glucose concentration of sweat is also elevated in persons with diabetes.[60] Autonomic neuropathy induced altered thermoregulation of the skin on the foot could partly explain why the microbiota in that region is more diverse than at other skin sites. Another possible explanation is that sweat glands have a role in innate immunity, mediated by their secretion of antimicrobial peptides, such as dermcidin.[61] This peptide has activity against various bacteria, including *S. aureus*. The fact that these glands are less prevalent in the lower extremities of diabetic patients could explain some differences in the microbiota.

Another common cause of changes in wound flora is the use of antimicrobials, either topical or systemic. These typically alter the bacterial community structure, often by reducing *Streptococcaceae* and allowing an increase in *Pseudomonadaceae* abundance

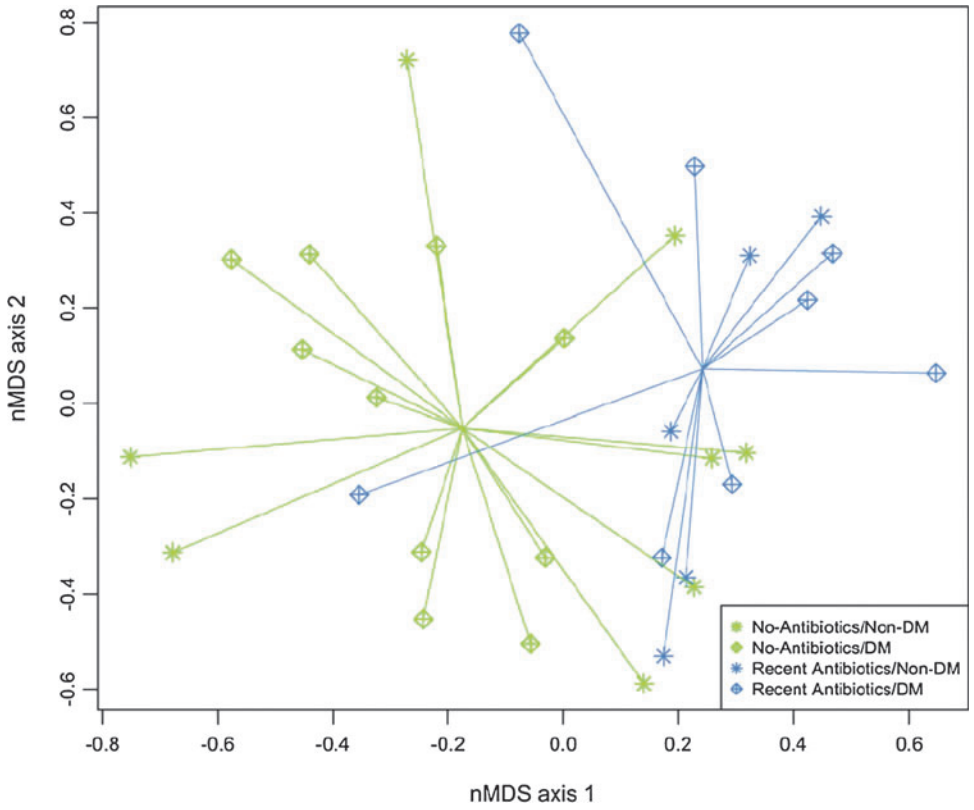

Figure 4. The nMDS ordination plot comparing wound bacterial communities from antibiotic-treated versus untreated participants. Each data point represents the bacterial community identified from a single wound specimen. Comparison by the multiresponse permutation procedure demonstrated a significant difference in wound microbiota between antibiotic-treated and untreated patients ($p=0.0069$) (adapted from Price *et al.*[28]). DM, diabetes mellitus; nMDS, nonmetric multidimensional scaling. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

(Fig. 4).[28] These findings, along with those demonstrating the nonrandom distribution pattern of bacteria, when combined with the FEP concept can provide a fuller microbial picture. From a clinical point of view, microbiota studies have shown that aspects of the complex skin and wound flora are associated with several specific characteristics of the DFU, including ulcer depth (a surrogate for wound severity) and duration (which may be a surrogate for delayed healing).[39]

In summary, new data derived from molecular tools suggest that chronic wounds contain consortia of microorganisms coexisting as combinations of highly structured communities. This includes not only bacteria but also viruses, protozoans, and fungi attached to biotic surfaces[43,62] that display specific intermicrobial and host interactions.[62] By including multiple bacterial and fungal species in a single community, microbes can potentially reap advantages, such as passive resistance, metabolic cooperation, quorum sensing systems, and DNA sharing. Biofilms composed of mixtures of bacteria with fungi or viruses may be common, and these interactions are highly complex.[63] Further research could uncover the specific roles of these pathogens in chronic wounds.

Molecular tools and local treatment of DFU

It is clear that the use of topical antibiotics and antiseptics is not recommended for treating clinically uninfected wounds, regardless of the results of any cultures obtained.[64–66] To date, available studies show no benefit in either healing the wounds or preventing overt infection. By understanding the concepts of pathogenic biofilm and the organization of microorganisms into FEP, clinicians may better understand the delayed healing that characterizes chronic wounds, as well as why cultures from chronically infected DFU are often polymicrobial (Fig. 2). They should bear in mind that bacterial species traditionally considered to be relatively nonvirulent can be pathogenic if they are part of an FEP. Recognizing these points, the fact that the mere presence of microorganisms is not necessarily harmful and that topical antimicrobials may be associated with adverse effects (especially generating multidrug resistant bacteria) makes it clear why it is usually best to withhold

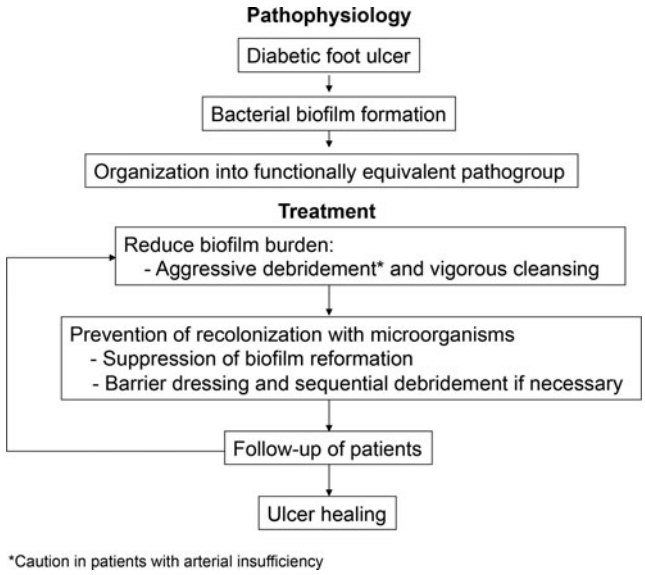

Figure 5. Principles of wound biofilm formation and management.

these agents. Moreover, findings derived from analyses of the skin microbiota emphasize the importance of sharp debridement in treating DFU to reduce any adverse effects of bacterial bioburden (Fig. 5). The hope is that this will help turn a chronic wound into a more acute one, and that reduction of excessive bacterial populations may help the wounds to heal.

It is possible that some types of topical antimicrobials (preferably antiseptic rather than antibiotic) may prove useful in removal of biofilms, but this area requires further investigations. Other alternate treatment strategies include the use of negative pressure wound therapy, particularly combined with timed cyclical instillation of topical wound solutions. The application of negative pressure across a wound surface leads to removal of interstitial fluid from the wound site, favor the debridement and increase vascularity for enhanced tissue repair and microbial clearing.[67,68] Instillation therapy provides, at a minimum, irrigation of the wound, but agents that have antibacterial or antibiofilm effects may be even more efficacious.

Future perspectives

Metagenomic approaches have vastly increased our knowledge on the genomes, activity, and functionality of the complex ecosystem residing within DFU. This information is highly relevant to both the microbiologist and the clinician involved in the management of DFU. Undoubtedly, future developments will deliver new technologies that will help overcome some of the limitations of the current techniques that we have noted (Table 1).[69,70]

One example of such a new technology is the culturomic approach.[71] This technique uses first more than 50 different culture conditions (using variable physicochemical conditions, preincubation, antibiotics, or bacteriophages). All the microorganisms obtained after this treatment were identified by the use of mass spectrometry or the 16S rRNA amplification (targeting the V6 region) and sequencing. The culturomic complements the metagenomic by overcoming the previously noted bias. Moreover, we can use this technique, after separation of viral RNA/DNA from bacterial DNA, to more efficiently identify viruses present.

To optimally treat DFU, we must elucidate which bacteria among the complex flora in a wound are actually pathogenic. Several new methods hold promise: metatranscriptomic (detection of mRNA by metagenomic), proteomic (detection of all proteins by mass spectrometry), and metabolomics (detection of volatile metabolites by bacteria by mass spectrometry) approaches could help us understand the symbiotic relationship between skin microbiota and the human host, and to differentiate between the pathogenic and commensal bacteria (Table 1). These methods could also help the clinician better understand bacterial interactions, such as cooperation, competition, and inhibition. Finally, it is likely that some factors make certain that DFU are more prone to develop clinically apparent infection than others. It would be interesting to know if analysis of the microbiome can elucidate these. If so, then two main dogmas in the management of DFU may need to be reappraised: (1) should we continue to advice against wound sampling in wounds lacking clinical signs or symptoms of infection? and (2) should we continue to argue against antimicrobial treatment for clinically uninfected DFU?

In conclusion, the development of molecular microbiological technologies has been a promising tool to better understand the local ecology of chronic wounds, especially of DFU. These methods have allowed us to better differentiate colonization from infection and to more effectively select the most appropriate therapy, especially concerning antibiotic agents. Nevertheless, these techniques are still too time-consuming and reporting is too delayed for them to be used in routine practice. In the near term, our efforts should be focused on the development of more rapid molecular technologies.

SUMMARY

Fully identifying bacteria present in DFU is difficult with traditional culture methods. New

molecular tools have contributed to our understanding of the role of the cutaneous microbiota in these wounds.

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TAKE-HOME MESSAGES

- DFU are a major public health problem affecting nearly 6.5% of the population worldwide.
- DFI is a frequent and costly complication of DFU, often leading to substantial morbidity and sometimes lower limb amputation. Optimal treatment of DFI requires knowing the causative pathogens and their antibiotic susceptibilities.
- New molecular techniques for studying the skin microbiota have recently been developed. They are based on the amplification of the 16S rRNA genes, highly conserved genetic sequences among bacterial species that vary in a manner that allows genus and species identification.
- Molecular tools have helped expand our knowledge of microorganisms present on DFU, the association between the wound microbiota and the development and outcome of a DFI. They also provide new information concerning the best management of DFU.
- Currently, the available methods are mostly useful for investigations. They are limited by cost and processing times and not yet generally available for clinical use.

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Abbreviations and Acronyms

DFI = diabetic foot infection
DFU = diabetic foot ulcer
DGGE = denatured gradient gel electrophoresis
DM = diabetes mellitus
DNA = deoxyribonucleic acid
EUC = euclidean ulcer clusters
FEP = functionally equivalent pathogroups
HPLC = high-performance liquid chromatography
LC-MS/MS = liquid chromatography-mass spectrometry/mass spectrometry
nMDS = nonmetric multidimensional scaling
PCA = principal component analysis
PCR = polymerase chain reaction
RNA = ribonucleic acid