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ORIGINAL ARTICLE

Diabetic foot infection: A critical complication

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The number of people in the world with diabetes has nearly quadrupled in the past 40 years. Current data show that 25% of these diabetics will develop a foot ulcer in their lifetime and that the cost of care for a diabetic foot ulcer (DFU) is over twice that of any other chronic ulcer aetiology. Microbial biofilm has been linked to both wound chronicity and infection. Close to 1 in 2 diabetics with a DFU are predicted to go on to develop a diabetic foot infection (DFI). The majority of these DFIs have been found to evolve even before the diabetic individual has received an initial referral for expert DFU management. Of these infected DFUs, less than half have been shown to heal over the next year; many of these individuals will require costly hospitalisation, and current data show that far too many DFIs will require extremity amputation to achieve infection resolution. The development of an infection in a DFU is critical at least in part because paradigms of infection prevention and management are evolving. The effectiveness of our current practice standards is being challenged by a growing body of research related to the prevalence and recalcitrance of the microbes in biofilm to topical and systemic antimicrobials. This article will review the magnitude of current challenges related to DFI prevention and management along with what is currently considered to be standard of care. These ideas will be compared and contrasted with what is known about the biofilm phenotype; then, considerations to support progress towards the development of more cost-effective protocols of care are highlighted.

KEYWORDS
antimicrobials, biofilm, diabetic, foot, infection

1 | INTRODUCTION

Diabetes is a costly health issue for both the patient with diabetes and for the health care system. Unfortunately, both the prevalence of diabetes and the related costs are continuing to rise. The estimated number of people globally with diabetes has almost quadrupled in the past 40 years, rising from 108 million in 1980 to 422 million in 2014.[1] The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014.[1] Up to 25% of diabetics will develop a diabetic foot ulcer (DFU) in their lifetime.[2] Furthermore, a meta-analysis[3] revealed a higher all-cause mortality rate in diabetics with a DFU than those without a DFU, showing a mortality rate of 99.9 per 1000 person-years in the DFU population compared with 41.6 per 1000 in the diabetes-only population. People with diabetes are more likely to be hospitalised for complications associated with a DFU than any other complication of diabetes,[4] and hospitalisation is the costliest aspect of DFU management.[5] The mean annual global health care cost of managing a DFU has been found to be $44 200 (adjusted 2015 US dollars), over twice that of any other chronic ulcer aetiology.[6]

2 | DIABETIC FOOT INFECTION

A wound infection results from microbial invasion into the tissue in adequate numbers to elicit a host response, which will then result in impaired wound healing.[7] An individual's risk for infection is multifactorial, resulting from interactions

between numbers and virulence of microbes present at the wound site and the host's ability to resist infection. Elevated blood sugar, such as seen with diabetes, even in the short term, can significantly alter innate immune function, therefore increasing susceptibility.[8,9] Sustaining a foot wound is the leading risk factor for the development of a diabetic foot infection (DFI).[10–13] Approximately 9.1% of South Texas diabetics enrolled in a programme designed to prevent and treat diabetic foot complications went on to develop ulceration and infection over the subsequent 2-year period.[11] The global prevalence of DFI has been reported to range between 25.2% and 58%.[12–15] Jai[13] reported that 40.1% of 853 non-infected DFUs went on to develop infection. Based on this, close to 1 in 2 diabetics with a DFU will develop a DFI, which is a critical development because DFI is the last clinical state prior to limb loss.[16] A recently published 12-month prospective observational study of clinically infected DFUs revealed that healing incidence at 1 year was only 44.5% once wound infection developed.[17] This healing rate is considerably lower than has been reported in earlier studies, 68.3%[15] and 77%,[18] which did not focus on infection status. During this 12-month period, DFI resulted in 23.4% of patients requiring surgical interventions (revascularisation or amputation), 4.3% primary ulcer recurrence possibly indicating incomplete infection resolution, and a patient death rate of 15.1%.

A recent study evaluated the healing response of wounds on diabetic and non-diabetic mice to the introduction of biofilm.[19] After induction of Type I diabetes in 8-week-old male mice, a mature bioluminescent *Pseudomonas aeruginosa* biofilm was transferred to the right side of two surgically created, full-thickness shoulder wounds on both the diabetic and non-diabetic mice. No biofilm was introduced to the contralateral control. In this study, biofilm was found to significantly impair the rate of healing in non-diabetic mice ($P < 0.0001$), yet bioluminescent imaging indicated that non-diabetic mice were eventually able to effectively clear this biofilm load. The biofilm-treated wounds on the diabetic mice also demonstrated a statistically significant slower rate of closure than control ($P = 0.0234$). In addition, the authors found statistically significant differences in biofilm-related complications in the diabetic group relating to wound depth and presence of granulation/fibrosis ($P = 0.0123$, $P = 0.0041$ respectively). In the next phase of this study, a mature biofilm was grown using a mutant of the original bacterium from which a gene was deleted to induce more persistent biofilm formation. This biofilm was again introduced into both diabetic and non-diabetic wounds. This resulted in the death of one of the non-diabetic mice from sepsis compared with 9 of the 14 diabetic mice. The authors concluded that diabetic mice are less capable of dealing with biofilm-related infection than non-diabetic mice.

Peripheral arterial disease (PAD) is an underlying factor in approximately half of all DFUs[13,14,16] and has been shown to double the risk of developing DFI.[11] Both PAD and elevated glycosylated haemoglobin (HbA1C) are specific risk factors for DFU chronicity,[11,20] and ulcer chronicity along with PAD are specific risk factors for developing a DFI.[11,13] The risk for hospitalisation, the costliest aspect of DFU management, has been estimated to be 55.7 times greater for diabetics who develop a DFI.[11] Diabetics who develop a DFI reportedly have a 155-fold increased risk for amputation than those who do not.[11] Of even greater potential concern, however, is the fact that a DFI with underlying PAD has the greatest risk for amputation, increasing by up to 90%.[14,21,22] Unfortunately, up to 58% of DFUs are already infected at initial presentation to a diabetic foot clinic (Figures 1 and 2), and one-third of these present with both DFI and PAD.[14] This leads to suspicion that wound infection, rather than initial ulceration, may be the driver of patient and/or non-expert provider concern and may also

Key Messages

- optimal management of diabetic foot infection is particularly critical to controlling the cost of care and maintaining quality of life for a growing global diabetic population
- this challenge is now confronting a growing body of research related to prevalence and recalcitrance of microbes in biofilm, which questions the efficacy of current practice standards

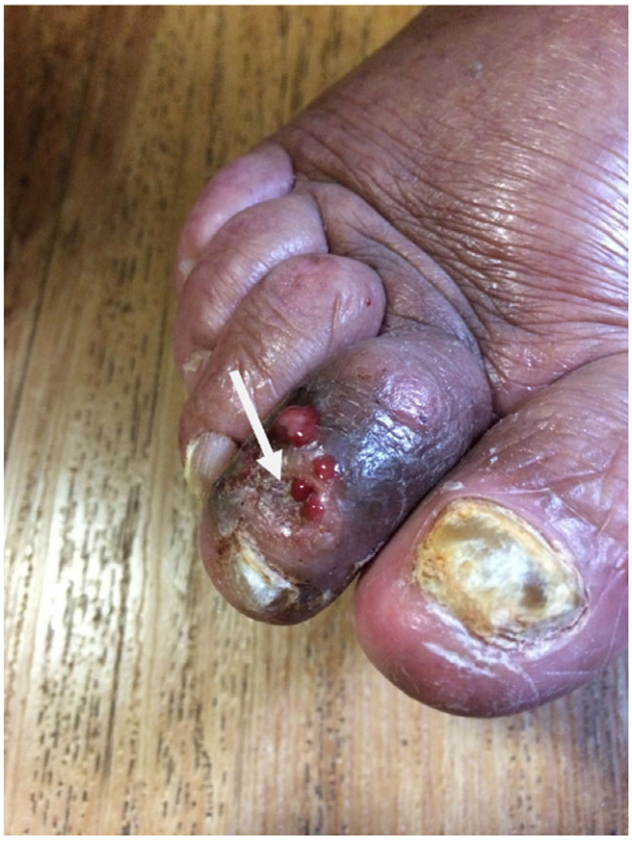

FIGURE 1 Diabetic foot ulcer pictured at presentation for specialist care related to concern that small wound on dorsal second toe keeps reopening

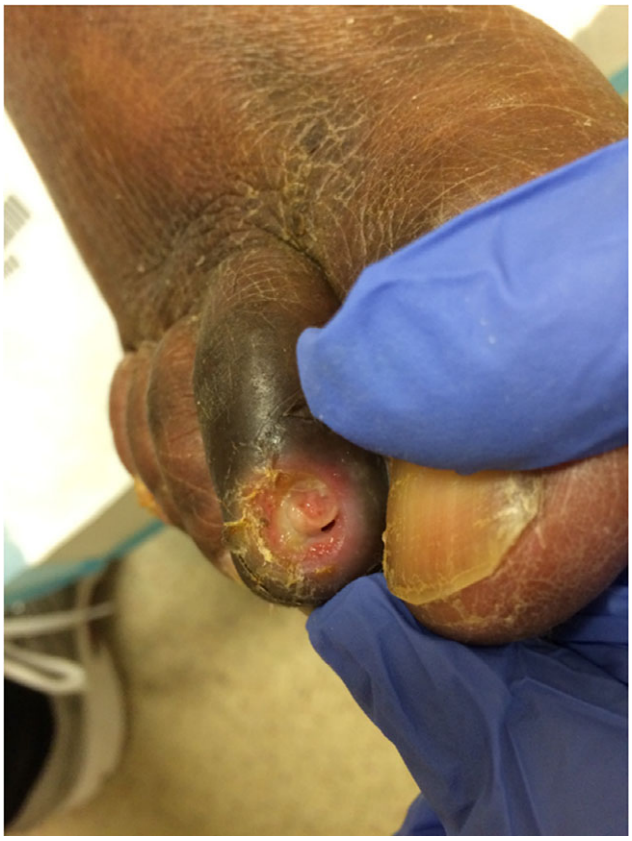

FIGURE 2 Diabetic foot ulcer pictured at presentation for specialist care because of concern that wound healing is stalled

indicate that referral sources are not testing for, or perhaps do not fully understand, the risks associated with PAD in diabetics. More than 135 amputations are carried out each week and up to 20 per day in England, their highest recorded rate.[23] Amputation has been directly linked to reduced quality of life, patient mortality, and a 100% increase in total cost of care.[5,16,24]

By the very nature of their role, health care providers should seek to achieve optimal disease reversal with maximal preservation of patient autonomy. To this end, the goal of every provider should be to work towards the prevention of amputation. This goal faces its first challenge with the development of a DFU, after which the goal must change focus to healing and, most crucially, to successful DFI prevention. As with any critical health care issue, these goals are best achieved by immediate referral to an expert in DFU management.

3 | INFECTION MANAGEMENT

DFI can be a particularly difficult health care issue to manage. It is currently recommended that DFIs should be diagnosed clinically because of a lack of evidence that the microbiological measurement of bacterial load is a valid marker for infection in a typically colonised wound.[25] Clinical diagnosis relies on markers of inflammation, such as periulcer redness or induration, increased or purulent drainage, and increased pain related to tissue congestion. Early infection diagnosis and management are important for diabetics, yet diagnosis may be delayed because local signs and symptoms of infection are often diminished in a DFI because of concomitant peripheral neuropathy and PAD.[11,26] However, a close correlation between neutrophil-derived enzymes and wound infection status was reported in a clinical study involving 81 patients, of which 42% were diabetic.[27]

4 | SYSTEMIC ANTIBIOTICS: CURRENT PRACTICE AND THE EVIDENCE

Once diagnosis of DFI is made, current clinical practice guidelines agree that virtually all clinically infected DFUs require systemic antimicrobial therapy.[25,28] However, a recent meta-analysis has suggested the presence of biofilms in most chronic wounds.[29] In another recent study, biofilm was additionally confirmed to be present in most chronic and acutely infected DFUs.[30] Biofilm is a tertiary architectural structure of cells interacting with a surface, either abiotic or biotic.[31] Subsequently, these cells adhere intimately to each other and secrete a matrix that facilitates their ability to circumnavigate nutritional challenges and antimicrobial onslaught. It is also purported that bacterial biofilms are protective against phagocytosis by innate immune cells. Not only has the presence of wound biofilm been shown to impair healing,[32–35] but it has been known for decades that microbes in the biofilm phenotype are highly tolerant of the action of systemic antibiotics compared with planktonic microbes.[36,37] In many cases, antibiotic concentrations adequate to achieve antimicrobial efficacy would risk toxicity to the host.[38–45] The tolerance of biofilms towards antimicrobials has received a considerable amount of research attention. Whilst it is a multifactorial process, the immobilisation of microbial cells within the biofilm matrix is believed to be particularly important. Stewart[42] conducted a quantitative investigation into the biofilm tolerance through a meta-analysis of published data. A numerical tolerance factor was used to compare inactivation rates of bacteria in both planktonic and biofilm states.[42] Considerable variation in tolerance factors (three orders of magnitude) was observed, which could not be explained solely based on the chemistry or mass of the antimicrobial, the substratum, or the identity of the microorganisms. Rather, regional cell density and biofilm age were important determinants, suggesting that the physiological status of microorganisms contributes to the antimicrobial tolerance associated with biofilms. This agrees with the current consensus that biofilm tolerance is driven by physiological differentiation, including dormancy and the persister phenotype together with metabolic and taxonomic diversity. These biofilm characteristics are facilitated by immobilisation with the matrix as outlined in Figure 3.

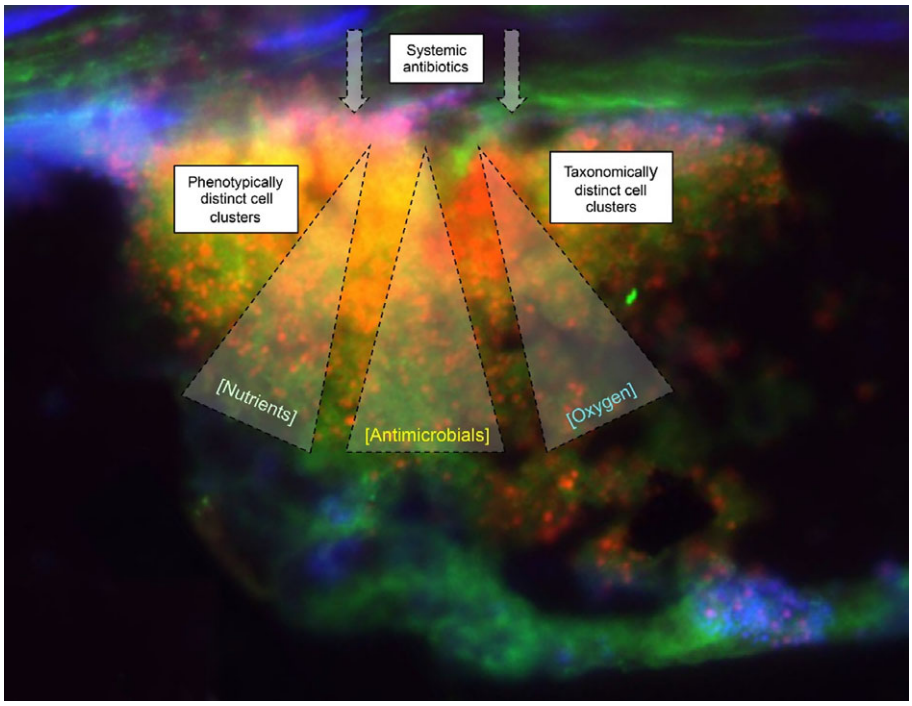

FIGURE 3 Diagrammatical representation of some of the major mechanism that complicate the clinical treatment of biofilms within chronic wounds. These include reduced access for systemic antibiotics because of impaired blood supply and the tolerance to antimicrobials of biofilms. This occurs through several mechanisms, including phenotypic and taxonomic heterogeneity of microorganisms and impaired penetration of antimicrobials. Source of background image: Reference 92

Although retarded antimicrobial penetration is unlikely to be the primary determinant of antimicrobial tolerance in treatment regimens of adequate duration, it could contribute in sub-therapeutic antimicrobial dose durations and concentrations. Adding to this challenge of biofilm-related tolerance, delivery of systemic antibiotics into the tissue to adequately treat DFI may be further inhibited by the presence of lower limb PAD.[46,47] The fact that microbes within biofilms are inherently tolerant to antimicrobial action and that PAD is an underlying factor in approximately one-half of all DFUs leads to concern about the true benefit of using systemic antibiotics to treat DFI, especially when used at doses that can be sustained systemically without toxicity (Figure 4).

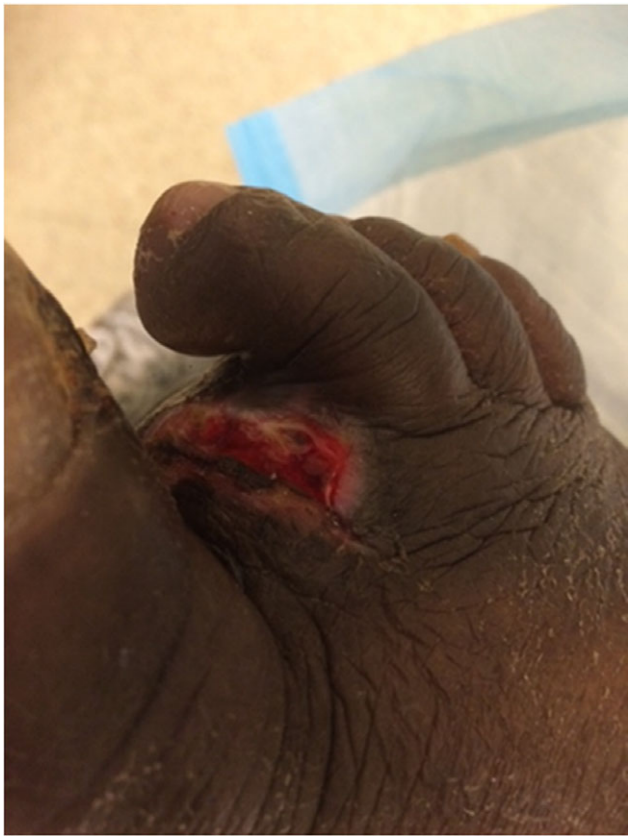

FIGURE 4 Dehisced incision at site of recent gangrenous toe amputation. Remains recalcitrant after standard dosing of systemic antibiotic

Wound biofilm has been linked to wound infection.[19,48] This link was demonstrated in a clinical investigation involving 16 individuals with chronic wounds, predominantly of venous stasis aetiology.[49] A recurring macroscopic material removed from the surface of the wounds was landmarked using histological techniques, and selected portions of samples were investigated using scanning electron microscopy (SEM). Dense accumulations of bacteria were apparent in SEM indicative of biofilm. Of the 16 non-healing wound samples collected, 12 were microscopically confirmed to contain a tertiary biofilm structure, 1 sample showed an immature biofilm, and 1 sample collected from a patient receiving chemotherapy for ovarian cancer showed lysed microbial cells. SEM, histological methods, and macroscopic visualisation indicated that biofilm-associated material recurred within 2 to 4 weeks of completing standard doses of oral or intravenous antibiotic treatments and despite the use of topical antiseptic wound dressings.

5 | ANTIMICROBIAL RESISTANCE VERSES TOLERANCE

Antimicrobial tolerance associated with biofilm is distinct from genetically mediated antimicrobial resistance (AMR). As outlined above, biofilm-related antimicrobial tolerance involves mechanisms that inhibit the ability of antimicrobials, including both antibiotics and antiseptics, to inflict their action on a susceptible microbe.[40,41,50] On the other hand, AMR results from a fundamental change in the microbe itself, leading to the loss of antibiotic effectiveness against a previously susceptible microbe. The exposure of microorganisms to antibiotics promotes the potential for a selective pressure to develop AMR.[43,51] This change in susceptibility can evolve via protective mutation and through the acquisition of genes encoding resistance transferred from other resistant organisms, potentially existing within the same biofilm.[40,50] The inappropriate use of less than optimally effective doses of antimicrobial agents, as may occur during treatment of a DFI, may accelerate this process.

AMR is currently responsible for an estimated worldwide annual mortality of 700 000 deaths[52] and leads to higher health care costs associated with treatment and economic losses because of reduced productivity caused by sickness. In the EU alone, it is estimated that AMR annually costs EUR 1.5 billion in health care costs and productivity losses.[53] This is predicted to increase to as high as US$100 trillion worldwide by 2050[52] unless we can begin to effectively address this problem. Because of what we are learning about the role of biofilm in both DFI and AMR, adherence to the goals of antimicrobial stewardship mandate that protocols for use of antimicrobials to manage DFI should be reconsidered. This evidence supports the need for a more reliable wound management protocol if cost-effective care promoting DFI prevention and management is to be achieved. Optimal vigilance requires that any such protocol should address biofilm tolerance as well as our global challenges with antibiotic resistance.

6 | BIOFILM IN WOUNDS

Intact epidermis provides a protective barrier to microbial invasion. Wounds involve a break in the protective epidermal barrier, which allows microbial invasion into deeper, normally non-colonised tissues. Advanced wound dressings were developed with the aim of limiting microbial colonisation and ultimately reducing infection risk,[54] yet standard modern antiseptic agents (eg, silver, iodine) have been shown to be unreliable in controlling wound biofilm maturation and risk of infection..[32,55–58] Hurlow et al[49] used scanning electron microscopy techniques to look for the presence of biofilm within a specifically described, reoccurring, macroscopic wound bed substance. This substance, which contained biofilm, was found to reform despite the use of standard antiseptic wound dressings. Interestingly, in one instance, this reforming substance was found to be completely composed of a macroscopic biofilm. In this particular case, wound biofilm could be seen by the naked eye, but this is not enough evidence to support an assumption that all wound biofilms can be identified in this manner.

Effective management of biofilm in wounds has been shown to require a series of consecutive and concurrent strategies referred to as biofilm-based wound care (BBWC). This protocol involves strategies believed to disrupt and control the redevelopment of biofilm on a wound.[59] Biofilm maturity studies[32,60,61] have shown that sharp debridement will effectively disrupt the protective structure of biofilm to expose more susceptible planktonic microbes to the action of an antimicrobial.[32,61,62] However, the therapeutic window promoted by sharp debridement has been reported as being limited to 2 to 3 days,[60] a much shorter time frame than can typically be addressed throughout the wound-healing process. Because of challenges associated with cost, specialist provider access, and transportation, standard wound care protocols tend to involve weekly visits, especially in the outpatient setting. Sharp debridement protocols have been confirmed to aid healing,[63,64] perhaps as a result of biofilm disruption, yet a large retrospective cohort study of 312 744 chronic wounds revealed that 30% did not adequately respond even to sharp debridement.[65] Conversely, an anti-biofilm protocol of care has been proposed, which involves aggressive sharp debridement along with the complimentary use of the anti-biofilm agents lactoferrin and xylitol.[66] This protocol was reported to promote healing of ischaemic wounds that were otherwise considered unlikely to heal.

Anti-biofilm substances have been proposed as a mechanism for reducing biofilm tolerance, thus potentially enhancing the efficacy of both debridement and antimicrobial dressings.[38,59,67] These proposed anti-biofilm pathways include strategies to inhibit biofilm formation or to chemically disrupt extant biofilm.[44,68–70] As with other environments where biofilms present a continuous challenge to effective microbial control (eg, the oral cavity), the combination of physical disruption with anti-biofilm treatment is generally associated with enhanced efficacy.[44,71] Proteolytic enzymes, commonly referred to as enzymatic debriders, which have been used for decades to remove necrotic wound bed tissue, may be ineffective in dispersing the biofilm structure that is not primarily comprised of extracellular proteins.[72]

A limited number of compounds for use in the treatment of wounds have been proposed to exhibit a chemically related anti-biofilm activity, including lactoferrin, an important constituent of the innate immune system with a high affinity for iron. The potential anti-biofilm activity of lactoferrin was originally reported in a letter to the *Nature* journal[73] where it was hypothesised that it serves the innate immune system by specifically inhibiting biofilm formation

on mucosal surfaces. The authors reported that lactoferrin demonstrates a concentration-specific ability to prevent *P. aeruginosa* biofilm formation by stimulating bacterial twitching motility, a mechanism by which the bacterium can spread rapidly across a surface. Promotion of twitching motility was found to lead to bacterial dissipation, therefore limiting the tendency for surface attachment and subsequent biofilm formation.[67,69,74] It should, however, be noted in terms of the clinical interpretation of these observations that not all wound pathogens are motile and will therefore not necessarily respond to lactoferrin in this manner. Iron sequestration is, however, an important mechanism by which microbial growth can be controlled, irrespective of the biofilm phenotype. Xylitol, also with proposed anti-biofilm activity, is a sugar alcohol that occurs naturally but in low concentrations in fruits and can be used as a dietary sweetener with purported oral health benefits. Combinatorial antibacterial activity of xylitol has been reported with Farnesol, a naturally occurring acyclic sesquiterpene alcohol,[75] and more recently, it has been claimed to weaken biofilm matrix structure when used in combination with lactoferrin.[68] Ethylenediaminetetraacetic acid (EDTA) is a substance that has been and is used in a range of formulations. EDTA sequesters stabilising metal ions and, in so doing, can reduce the physical integrity of biofilms.[38,67,76–78] Surfactants, such as those used in cleaning products, have been utilised for many years for their antimicrobial activity and may partly target the protective biofilm matrix[79,80] as well as enhance the potency of antimicrobial compounds. A combination technology involving EDTA, a surfactant, and ionic silver has demonstrated synergy in disrupting biofilm and killing associated microorganisms[81] The action of surfactants is concentration-dependent and can be enhanced by physical disruption.[70,78,79,82] Povidone iodine 10%, an iodophor of elemental iodine patented in 1956 (U.S. Patent 2739922), has been found to have cidal activity against microbes protected in mature biofilm,[83] yet this concentration has also been reported to be toxic to wound fibroblasts.[84,85] Cadexomer iodine (CI), an iodophor of 0.9% elemental iodine, has been shown to have in vitro biofilm suppressive activity.[86] More recently, CI has been shown to have some ability to decrease microbial load in the clinical wound setting over a 7-day period;[87] however, this data is based on the use of a quantitative polymerase chain reaction (PCR) method directed towards bacteria regardless of phenotype. Recently, some concern has been expressed about the therapeutic longevity of any potential CI anti-biofilm activity.[49,88] In essence, it is reasonable that the inclusion of anti-biofilm action will be necessary to achieve most cost-effective wound care protocols of care, but reliable use of anti-biofilm substances in the delicate environment of an exudating wound will require an improved understanding of the practical balance between anti-biofilm substance concentration efficacy and substance toxicity, as well as attention to length of clinical efficacy.

7 | FUTURE DIRECTIONS

There is cause for cautious optimism when it comes to achieving the goal of improved foot and overall care for individuals with diabetes. A 20-year evaluation of hospitalisation related to non-traumatic lower-extremity amputations (NLEA) revealed that, despite increasing prevalence of diabetes, the NLEA rates in diabetics decreased at an annual percentage change of −8.6% going from 11.2 per 1000 to 3.9 per 1000.[89] Yet only 30% to 50% of patients still receive adequate and timely care after the development of a DFU.[16] If such statistics are to further improve, it is paramount that all providers embrace the reality that these high-risk individuals should be referred for expert care before the critical development of a DFI. Furthermore, it is essential that all providers, generalists, and experts alike become receptive to the possibility that a protocol for expert prevention and treatment of a DFI is still evolving. A recent analysis looking at the effectiveness of interventions to enhance healing of chronic ulcers on the diabetic foot revealed that controlled studies continue to remain few, and those that do exist continue to be of poor methodological quality.[90] Even the most basic intervention, cost-effective DFU prevention remains a challenge.[91] Considering our growing global challenges with diabetes, higher quality, controlled studies are warranted. Improving the outcome of a DFU also requires consideration of an alternative to reliance on clinical signs of infection in this population known to exhibit delays in presentation and known to be less able to mount adequate infection response. Furthermore, treatment of DFI must move beyond current protocols of care with systemic antibiotics. Microbial biofilm tolerance to both systemic antibiotics and topical antiseptics as well as growing global challenges associated with AMR mandate consideration of the addition of anti-biofilm strategies to any accepted protocol of care for both DFUs and DFIs. There is increasing evidence to support the opinion that any significant future improvements in DFU and DFI outcome will not evolve without serious consideration of the biofilm paradigm.

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