JOURNAL OF PLASTIC SURGERY AND HAND SURGERY
2020, VOL. 54, NO. 1, 47–54
https://doi.org/10.1080/2000656X.2019.1673170

ARTICLE

Clinical experience with surgical debridement and simultaneous meshed skin grafts in treating biofilm-associated infection: an exploratory retrospective pilot study

Sik Namgoong[a]*, Su-Young Jung[b]*, Seung-Kyu Han[a], Ae-Ree Kim[c] and Eun-Sang Dhong[a]

[a]Department of Plastic Surgery, Korea University College of Medicine, Seoul, Republic of Korea; [b]Department of Plastic Surgery, Armed Forces Capital Hospital, Seongnam, Republic of Korea; [c]Department of Pathology, Korea University College of Medicine, Seoul, Republic of Korea

ABSTRACT

Current treatment guidelines for biofilm-associated infections (BAI) recommend repeated sharp/surgical debridement followed by treatment with antimicrobial agents until the wound becomes self-sustaining in terms of a positive wound-healing trajectory. However, complete removal of a biofilm is unlikely, and biofilms reform rapidly. We have treated BAI in patients with chronic diabetic ulcers using a meshed skin graft combined with negative pressure wound therapy (NPWT) immediately after surgical debridement, rather than waiting until the development of clean and healthy granulation tissue; the purpose of this exploratory study was to report the clinical results of this treatment strategy. This retrospective study included 75 patients with chronic diabetic ulcers who were treated for BAI by using surgical debridement, simultaneous meshed skin grafts, and NPWT. Healing time along with the percentage of complete wound closure within 12 weeks were evaluated; bacteria isolated from the wounds and their relation to the wound healing rate were investigated. All 75 wounds healed successfully, and the mean time for complete wound healing was 3.5 ± 1.8 weeks. In particular, 76% of wounds healed uneventfully without graft loss. A mean of 3.3 bacterial colonies/wound were isolated; however, no significant difference in wound healing was observed between the monomicrobial and polymicrobial groups. This exploratory study suggests that surgical debridement and simultaneous meshed skin grafts combined with NPWT may be successfully used to combat BAI in patients with chronic diabetic ulcers. We look forward to larger pivotal studies to confirm or refute these initially promising findings.

ARTICLE HISTORY
Received 14 December 2018
Revised 28 August 2019
Accepted 16 September 2019

KEYWORDS
Biofilm; diabetic ulcer; negative pressure wound therapy; skin graft; surgical debridement

Introduction

Biofilms in wounds are a form of infection comprised of living microbes within a three-dimensional matrix of extracellular polymeric substance (EPS) produced by sessile bacteria that can form colonies. The concept of biofilms was first described in detail in 1978 [1]. Although bacteria are perhaps most widely thought of as free-living or floating single cells (planktonic), the most natural environment for bacteria involves attaching to a surface and existing within a community of bacterial cells. In particular, most bacteria grow attached to a wound surface rather than exist as free-floating planktonic cells in chronic wounds [2].

Biofilms are difficult to eradicate with conventional treatments, as they are firmly attached to the surrounding tissue and are both resistant to and poorly penetrated by antimicrobial agents [3]. Antimicrobial agents are designed to attack bacteria but may only partially eradicate the bacteria contained within a biofilm. Bacteria in biofilms can be nearly 1000-fold more resistant to antibiotics than planktonic, free-floating cells [3]. Hence, biofilms constitute a major obstacle to wound healing.

Multiple strategies are used concurrently to suppress biofilm activity in wounds. Although there is some evidence that hydrotherapy, shockwave therapy, negative pressure wound therapy (NPWT) with fluid instillation, cadexomar iodine, and biofilm-dissolving agents such as lactoferrin, can be used to eradicate a biofilm [4], current treatment guidelines recommend repeated sharp or surgical debridement followed by topical antimicrobial agents and systemic antibiotics until the wound becomes self-sustaining in terms of a positive wound-healing trajectory [5]. It has been thought that placing skin grafts or replacements on wounds should be avoided until a biofilm is completely eradicated [6]. However, complete removal of a wound-associated biofilm is unlikely due to its durability, and biofilms are also able to reform very rapidly; although a wound bed may appear clean immediately after surgical debridement, a biofilm can redevelop and reach the mature stage within 72 h [7]. Therefore, repeated and regular treatment of the biofilm before it reaches the stage of maturity is recommended for facilitating wound healing. However, despite this approach, it remains challenging to completely remove a biofilm; rates of successful wound healing for biofilm-associated infections (BAI) are 16.7–77% [4,8,9].

Considering the window of the first 72 h after debridement, we hypothesized that a mesh skin graft and the use of NPWT as a bolster dressing immediately after surgical debridement, rather than waiting until clean healthy granulation tissue suitable for skin grafting develops or leaving the wound to heal by repeated debridement, could reduce the chance of bacterial regrowth and biofilm formation on the wound surface. This method serves two purposes, the most important of which is that an immediate skin graft obliterates the space for a biofilm to form due to the tight

CONTACT Seung-Kyu Han pshan@kumc.or.kr Department of Plastic Surgery, Korea University Guro Hospital, 148 Guro-Dong, Guro-Ku, Seoul 08308, Korea.
*Sik Namgoong and Su-Young Jung Contributed Equally to This Work and Should Be Considered as co-First Authors

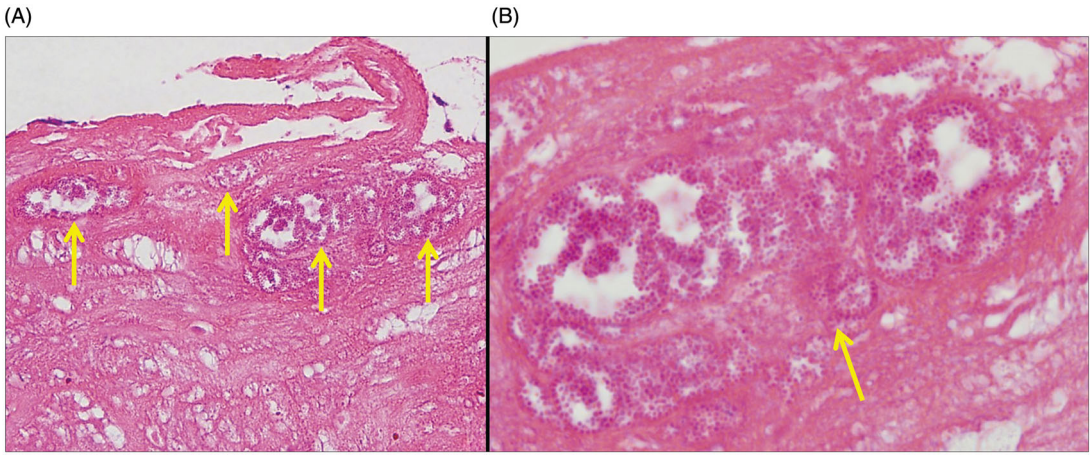

Figure 1. Hematoxylin and eosin staining of debrided biofilm structure shows the presence of bacterial clusters or microcolonies (arrows) (A) ×400; (B) ×1000.

contact between the graft and the recipient wound bed. Only 12 h are required for capillary buds to grow through a thin fibrin network on the undersurface of grafted skin [10], and a capillary network connects the skin graft to the recipient wound bed so that blood flow begins in the graft 48 h after grafting [11], which helps prevent reformation of the biofilm. In addition, meshing the graft skin and using NPWT as a bolster dressing facilitates effective removal of wound exudates, and the bioburden decreases significantly when the bacteria are in a low exudate environment. Meshing also allows better graft application to irregularly contoured surfaces.

We have treated BAI in patients with chronic diabetic ulcers by surgical debridement and a simultaneous meshed skin graft combined with NPWT and achieved favorable results. The purpose of this exploratory retrospective study was to report the clinical results of this treatment strategy.

Materials and methods

Management protocol in brief

A complete medical history was obtained from each patient at admission. General serologic tests, including those for blood glucose and other inflammatory markers, were performed. To evaluate the vascularity of diabetic foot, transcutaneous partial oxygen tension, Doppler wave, and toe pressure were measured. Patients with peripheral arterial disease received percutaneous transluminal angioplasty from an interventional cardiologist. For the management of wound bioburden, deep tissue culture was performed. When necessary, intravenous antibiotics were administered empirically and were then changed according to the results of culture and sensitivity tests. To evaluate neuropathy, a Semmes–Weinstein monofilament test, pin prick test, temperature test, electromyography, and nerve conduction velocity test were conducted. Appropriate off-loadings were provided according to ulcer locations.

Brief description of the surgical technique

Surgical debridement was performed on the wound bed until a healthy bleeding base was reached. Skin from the lateral thigh area of the patient was harvested using a Zimmer dermatome set to 0.012 inches in thickness. The harvested skin was meshed by passing the skin through a 1:1.5 mesher. The meshed skin was tailored to the size and shape of the defect and transferred to the recipient site. NPWT was applied as a bolster dressing to allow exudate to escape and was maintained for 3 days. Afterwards, a compressive dressing was applied daily using saline gauze until complete re-epithelization was achieved. Systemic antibiotics were administered for 2–6 weeks according to the results of deep tissue culture and sensitivity tests.

Materials

Medical records of 1005 patients with diabetic foot ulcers who were admitted and treated at the Diabetic Wound Center of the author's institution between January 2010 and December 2014 were reviewed. Of these patients, 75 patients who had BAI and were treated by surgical debridement, simultaneous meshed skin graft, and NPWT were included in the study.

Our clinical diagnostic criteria for BAI included the following: presence of a wound for >6 weeks, highly persistent slough, positive tissue biopsy culture microbiological result, and presence of bacterial microcolonies within wound tissue on microscopic examination (Figure 1).

Evaluation

The percentage of patients who achieved complete wound closure within 12 weeks and the mean time required for complete healing were evaluated. Complete wound healing was defined as a completely epithelialized state; no discharge was present and the patient was permitted to shower. Bacteria isolated from the wounds and their relation to wound healing rate were also investigated. For this purpose, bacteria were isolated within the wound through tissue or bone biopsy procedures to confirm the presence or absence of infection. Here, tissue or bone biopsy samples were obtained from a deep tissue and/or bone during surgical procedures, since the microbiology of the superficial and the deep tissues are known to differ in chronic wounds. The intraoperative samples were immediately stored in aseptic tubes for culture. The specimens were sent to a microbiology laboratory in the same hospital and incubated at 35 °C for 24–48 h on Sheep blood agar plates. Furthermore, MacConkey II agar plates were used to culture aerobes, and Chocolate agar plates were used to culture anaerobes. In addition, the recurrence of wound infection after healing was examined. The recurrence of wound infection was defined clinically when the following inflammatory signs were present: aberrant discharges from the margin or surrounding area where the wound was completely covered and healed; and

Table 1. Patient demographics ($n = 75$).

Variables	
Age, years	59.3 ± 11.5
Sex, *n* (%)	
Male	59 (78.7%)
Female	16 (21.3%)
HbA1C, %	8.6 ± 2.3
Dialysis, *n* (%)	13 (17.3%)
Baseline $TcPO_2$, mmHg	33.7 ± 22.1
Wound duration, weeks	12.4 ± 6.7
Wound area, cm^2	34.0 ± 55.8
Location, *n* (%)	
Dorsum	34 (45.3%)
Plantar	23 (30.6%)
Border	18 (24.0%)
Presence of osteomyelitis, *n* (%)	28 (37.3%)
Total number isolated bacteria, *n* (%)	
1	8 (10.7%)
2	16 (21.3%)
3	22 (29.3%)
4	14 (18.7%)
5	9 (12.0%)
6	3 (4.0%)
7	1 (1.3%)
8	2 (2.7%)
Follow-up duration, weeks	57.4 ± 44.5

HbA1C: hemoglobin A1c; $TcPO_2$: transcutaneous partial oxygen tension. Values were reported as means ± standard deviations (SDs) for continuous variables and proportions or percentages for categorical variables.

sudden development of swelling or induration around the wound after complete healing.

This study protocol was approved by the Institutional Review Board of the authors' institution (# KUGH 15092–001).

Statistical analysis

Study variables were summarized as means ± standard deviations (SDs) for continuous variables and proportions or percentages for categorical variables. Statistical comparisons were performed using the Chi-squared test and Mann-Whitney U test, as appropriate. The mean time to complete closure was also estimated using the Kaplan-Meier method. A p values < 0.05 was considered statistically significant. The statistical analysis was performed using SAS 9.4 (SAS institute, Inc., Cary, NC).

Results

Of the 1005 patients with diabetic foot ulcers, 75 met the inclusion criteria. The demographics and clinical characteristics of patients are shown in Table 1. All 75 wounds healed successfully in a mean of 3.5 ± 1.8 weeks without further radical surgical debridement. In particular, 57 of the 75 patients (76%) healed uneventfully after the skin graft. The mean time for complete wound healing was 2.8 ± 0.7 weeks (Figures 2–4). Partial graft loss occurred in 18 patients (24%); among the 18 grafts, 15 (20%) wounds healed by secondary intention without additional surgery in a mean of 5.2 ± 1.4 weeks, and three (4%) wounds healed after an additional skin graft surgery was performed 6.0 ± 1.7 weeks after the first skin graft. The time to complete wound closure in the three additional skin graft cases was 9.6 ± 1.6 weeks after the first skin graft. There were no cases of wounds that failed to heal.

A total of 248 bacterial colonies were isolated, with a mean of 3.3 colonies/wound (Table 2). Eighty-nine percent of the patients had polymicrobial infections, and monomicrobial etiology was observed in 11%. Gram-positive cocci constituted 52% of the bacteria isolated, while Gram-negative bacilli constituted 45%. The most frequently cultured bacteria were methicillin-resistant *Staphylococcus aureus* (MRSA; 52.0%) and *Acinetobacter baumannii* (42.7%).

Based on the Kaplan-Meier analysis of healing time in patients that showed complete healing, the estimated mean time for healing was 21.26 ± 5.53 days in patients with monomicrobial infections compared with 24.86 ± 1.54 days in patients with polymicrobial infections (Figure 5; $p = 0.627$, Log Rank test). No significant differences were observed between the monomicrobial and polymicrobial groups in the numbers of uneventfully healed patients, patients that showed healing by secondary intention, and patients in whom healing occurred by additional graft surgery (Table 3; $p = 0.483$, Chi-squared test).

The mean follow-up period was 57.4 ± 44.5 weeks (range: 27.6–243.0 weeks). Ulcer recurrence was observed in five patients (6.7%) 7.6 ± 3.0 weeks after healing. Of these five, recurrence associated with BAI was observed in two patients, 4 and 8 weeks after healing. In the other three patients, the ulcers recurred due to pressure injury caused by inappropriate off-loading.

Discussion

It is now recognized that the physical and behavioral characteristics of bacteria within a surface-attached biofilm community are very different from those exhibited by free-living bacteria. Free-living bacteria are metabolically active and often highly susceptible to antimicrobial agents and attack by immune cells. In contrast, bacteria in a biofilm often adopt a sessile behavior with a significantly reduced growth rate, resulting in slower uptake of antimicrobial agents and therefore lower susceptibility. Additionally, previous studies have shown that once attached to a surface, biofilm bacteria produce an outer protective matrix (EPS) that acts as a physical barrier to permeation and to the effects of antimicrobial agents [12].

The biofilm environment not only provides physical protection to bacteria from a potentially hostile external environment, but also provides a habitat where bacteria communicate with each other (through mechanisms such as quorum sensing), which may lead to increased virulence and propensity to cause infection [13]. Previous studies have shown that an elevated and persistent inflammatory response, such as that observed with biofilm infection, may lead to over-production of potentially destructive enzymes (e.g., matrix metalloproteinases and pro-inflammatory cytokines) and oxygen metabolites that promote tissue destruction, finally resulting in chronic infections and non-healing wounds [14].

The polymicrobial nature of BAI in chronic diabetic ulcers is well known [15]. In our series, 89% of patients also had a polymicrobial infection, while monomicrobial etiology was observed in 11%. These polymicrobial biofilms may contribute to the chronicity of diabetic foot ulcers. The most frequently cultured bacteria in this study (MRSA and *A. baumannii*) are both multi-drug resistant.

Biofilms begin to form after free-floating microorganisms attach to the wound surface. If the attached microorganisms are not immediately separated from the surface, they anchor themselves more permanently using cell adhesion structures, such as pili [2]. Some species are unable to attach to the surface on their own, but can anchor themselves to the extracellular matrix, or even attach directly to earlier colonists via quorum sensing [13]. Once colonization has begun, the microorganisms actively produce EPS, and the EPS typically encloses the colonized bacteria. The EPS may also contain materials from the surrounding

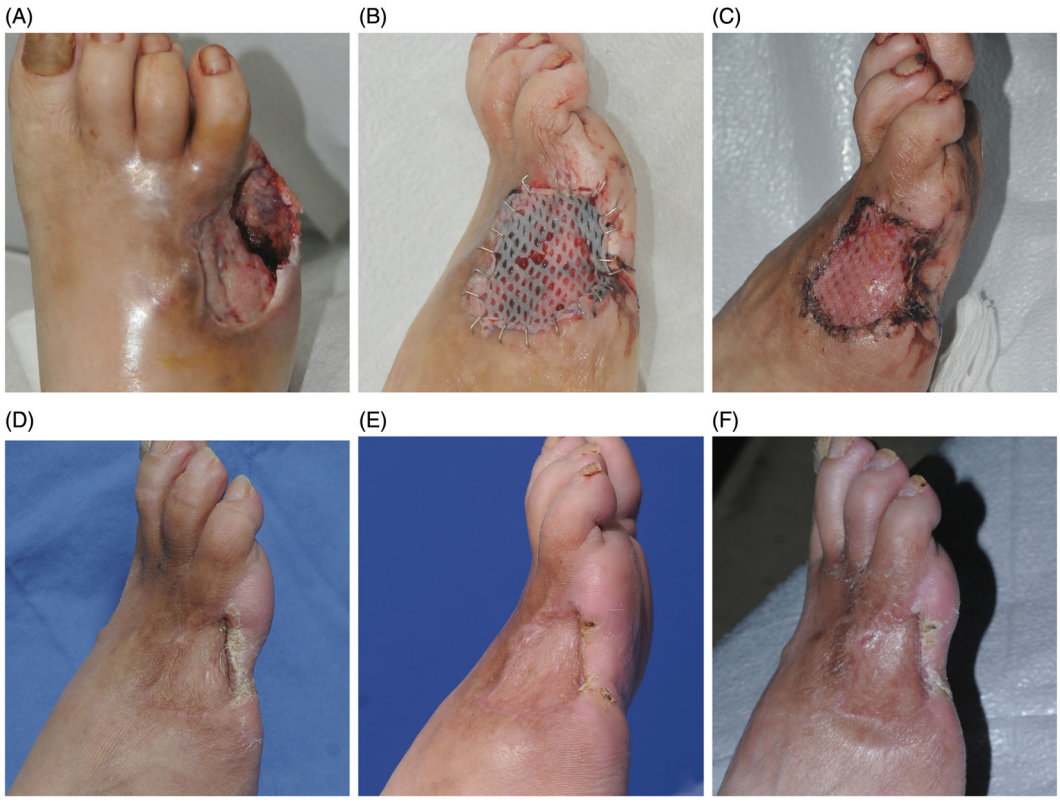

Figure 2. A 71-year-old man with a chronic non-healing diabetic ulcer due to 10-week long biofilm associated infection (A) Preoperative view; (B) The mesh split-thickness skin graft applied immediately after surgical debridement; (C) Two weeks after the graft; (D–F) Three, 6, and 12 months after the graft.

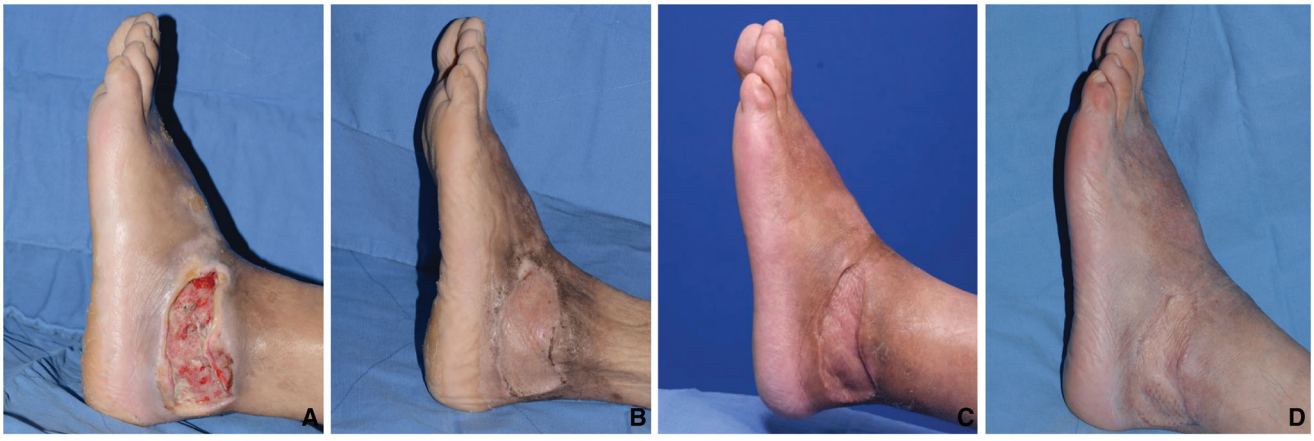

Figure 3. A 70-year-old man with a 15-week-old biofilm associated infection on the ankle area (A) Preoperative view; (B) One month after the mesh split-thickness skin graft applied immediately after the surgical debridement; (C and D) Three and 12 months after the graft.

environment, including minerals, soil particles, and blood components such as erythrocytes and fibrin. The biofilm grows through a combination of cell division and recruitment. In the final stage of biofilm formation, the biofilm structures detach as clumps of cells that move and may attach to another surface and propagate further [16]. These complex structures are resistant to the defense mechanisms of the immune system and to antimicrobial agents. Therefore, the primary and most effective treatment of biofilm infections is frequent debridement to eradicate the biofilm matrix, followed by the application of topical antimicrobial agents and systemic antibiotics to destroy the biofilm microbes and prevent reseeding of bacteria on the wound surface. However, complete removal of a biofilm is unlikely, as they tend to spread perivascularly below the surface of the wound and reform very rapidly. A clean, well-vascularized wound bed is established immediately after surgical debridement of a biofilm. This occurs when the biofilm is immature and the bacteria are more susceptible to antibiotics, biocides, and host immune mediators as they are in a more active phenotypic stage and the matrix is less developed. However, a biofilm will develop again over time and reach maturity within 72 h [7]. Therefore, the first 72-h period after debridement is the most effective therapeutic window. Repeated treatment of the biofilm on a regular schedule before it reaches the mature stage forces it to reattach and reform, during which time it is susceptible to antibiotics and host defenses. Several authors have reported that therapeutic strategies employing repeated removal of the biofilm help wound healing to progress. However, despite this approach, it remains difficult to remove a

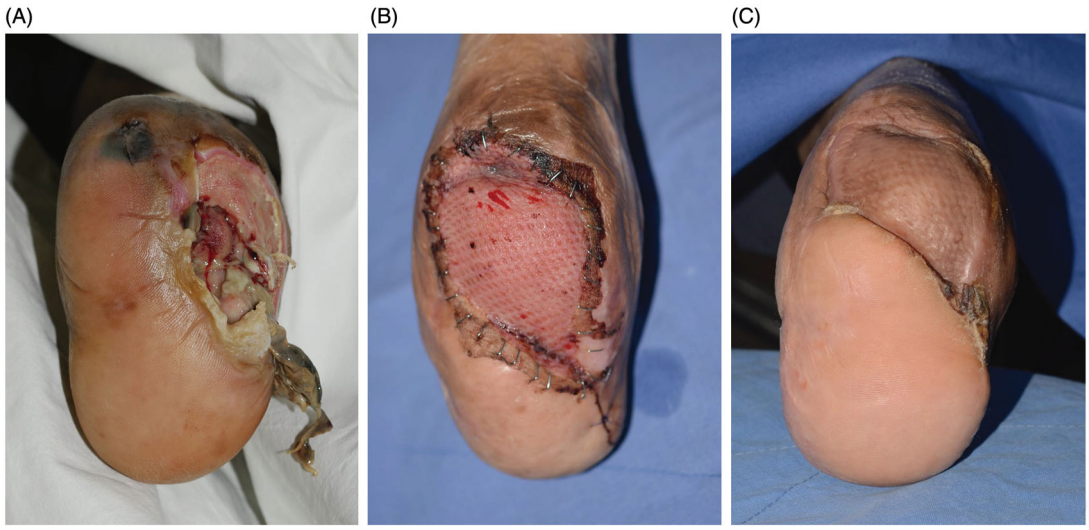

Figure 4. A 60-year-old women with a 20-week-old biofilm associated diabetic foot infection on the plantar area (A) Preoperative view; (B) Ten days after the mesh split-thickness skin graft applied immediately after radical surgical debridement; (C) 12 months after the graft.

Table 2. Microorganisms isolated from wounds.

Name of isolates	Number (%)
Gram positive cocci	129
Staphylococcus aureus (MRSA)	39 (52.0)
Enterococcus faecalis	27 (36.0)
Staphylococcus epidermidis (MRSE)	14 (18.7)
Staphylococcus aureus (MSSA)	11 (15.7)
Streptococcus agalactiae	10 (13.3)
Enterococcus faecium	8 (10.7)
Enterococcus faecium (VRE)	3 (4.0)
Others	17 (22.7)
Gram negative bacilli	112
Acinetobacter baumannii	32 (42.7)
Pseudomonas aeruginosa	18 (24.0)
Enterobacter cloacae	14 (18.7)
Escherichia coli	12 (16.0)
Klebsiella pneumoniae	8 (10.7)
Stenotrophomonas maltophilia	8 (10.7)
Proteus mirabilis (anaerobic)	3 (4.0)
Bacteroides fragilis (anaerobic)	1 (1.3)
Others	16 (21.3)
Fungal	7
Candida albicans	5 (7.8)
Others	2 (2.7)
Total	248

biofilm completely. As mentioned earlier, successful wound healing rates for a BAI are 16.7–77%.

Our treatment method for BAI is based on the hypothesis that bacteria residing in the depths of a biofilm are slow-growing or quiescent, giving rise to metabolic quiescence, whereas bacteria near the biofilm surface are more rapidly growing and have more robust metabolic activity. Therefore, metabolically active bacteria are thought to be removed effectively by surgical debridement, and the remaining bacteria are relatively dormant. However, these dormant bacteria, which are located deep in the biofilm before debridement, become more active as they are moved to the wound surface and readily nourished by oxygen and nutrients in exudates after debridement. A biofilm reaches its pre-debridement resistance level within 72 h after debridement; thus, strategies to impede the formation of a biofilm should be implemented soon after surgical debridement. Considering this 72-h window after debridement, we hypothesized that application of a mesh split-thickness skin graft immediately after surgical debridement, rather than waiting until clean healthy granulation tissue develops by repeated debridement, would reduce the chance for bacterial regrowth and biofilm formation. Applying NPWT also improves graft take, reduces seroma formation, and removes exudates and bacteria [17]. Localized seroma or exudates between the graft and wound bed are a common cause of biofilm reformation and graft failure. In the present study, we focused on biofilm-associated infections and employed a meshed skin graft immediately after surgical debridement of chronic wounds with biofilm-associated infections. Thus, we maintained a shorter period than usual with NPWT as a bolster after skin grafting to reduce the risks of skin graft failure due to infection that may develop in a sealed space. This allowed the graft to survive by reducing seroma formation and removing exudates particularly for the first 72 h after debridement, which is the most effective therapeutic window.

Wounds can also be covered with various flaps (if available) instead of a skin graft immediately after surgical debridement, as a flap can diminish secondary wound contraction when compared to skin grafts, and is less likely to desiccate. However, a kind of 'dead space' forms between the flap and the wound bed during the critical window period, and exudates, fibrinoid materials, and seroma in the 'dead space' can cause bacterial growth and reformation of the biofilm. Thus, a meshed skin graft with NPWT is likely more suitable, as it is not associated with these issues; however, further studies may be necessary to confirm this.

Although a large number of studies on biofilms have been published, only a few clinical studies exist in the literature. Wolcott et al. performed a retrospective study using a biofilm-based wound care algorithm in patients with critical limb ischemia. The main components, in addition to standard care and debridement, were anti-biofilm agents, including quorum sensing inhibitors, antibiotics, silver, and chemicals. They reported that 77% of wounds healed completely, while 23% failed to heal [4]. Lenselink and Andriessen reported that a polyhexanide dressing reduces biofilm formation in stagnating wounds [9]. Beele et al. also performed a randomized study to evaluate the antimicrobial performance of an ionic silver alginate/carboxymethyl cellulose dressing on chronic biofilm wounds, compared with a non-silver calcium alginate fiber dressing [8]. They reported that the silver alginate/carboxymethyl cellulose dressing significantly improved healing as indicated by a reduced wound surface area. Of the 18 wounds, complete wound healing occurred in three (16.7%) in

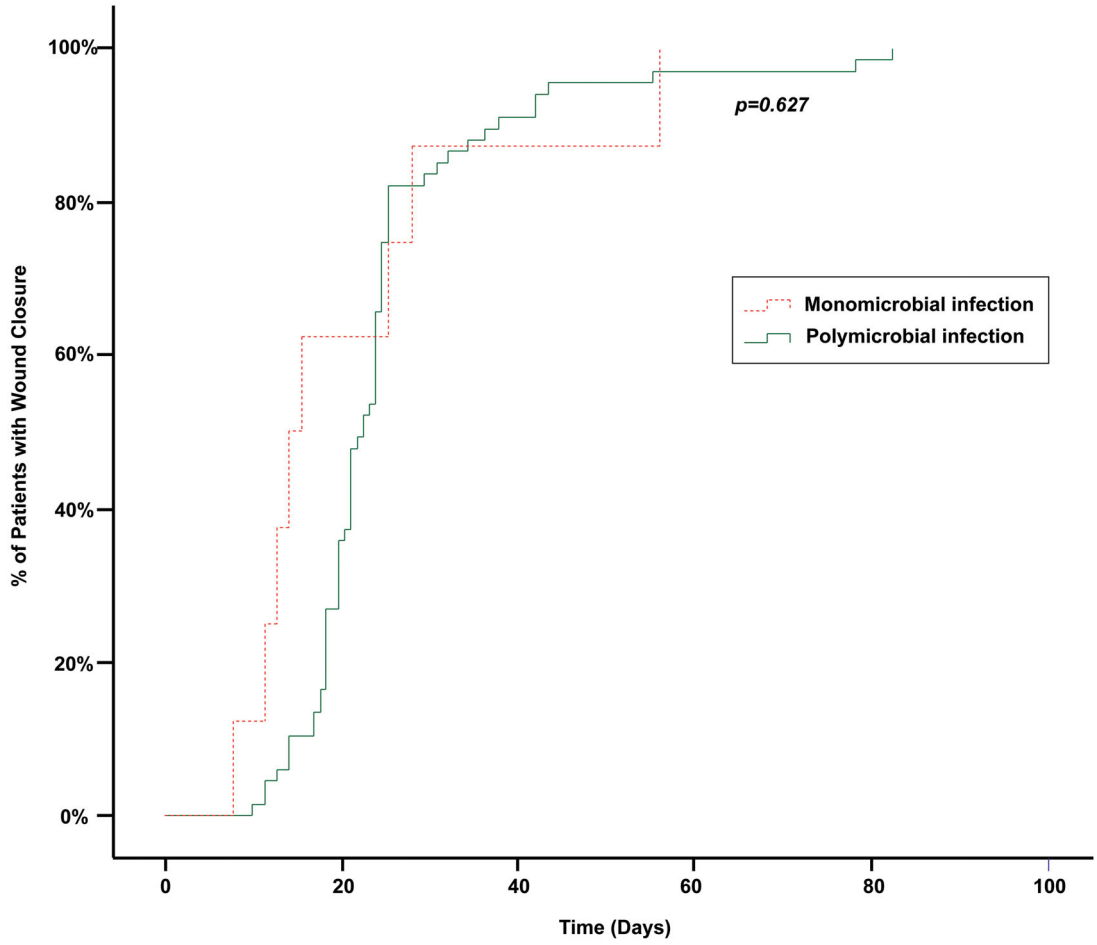

Figure 5. Kaplan-Meier analysis of the time to complete healing by study group ($p = 0.627$, Log Rank test).

Table 3. Comparisons of the polymicrobial and monomicrobial groups.

Variable	Patients with monomicrobial infection ($n = 8$)	Patients with polymicrobial infection ($n = 67$)	p-Value
Age, years	59.0 ± 19.4	59.3 ± 10.4	0.69
Sex, *n* (%)			0.05
Male	4 (50.0)	55 (82.1)	
Female	4 (50.0)	12 (17.9)	
HbA1C, %	7.3 ± 2.5	8.8 ± 2.3	0.08
Dialysis, *n* (%)	0 (0)	13 (19.4)	0.32
Baseline $TcPO_2$, mmHg	32.1 ± 20.8	33.8 ± 22.4	0.48
Wound duration, weeks	10.5 ± 5.8	12.6 ± 6.9	0.84
Wound area, cm^2	48.8 ± 61.3	32.3 ± 55.4	0.38
Location, *n* (%)			0.50
Dorsum	3 (37.5)	31 (46.3)	
Plantar	2 (25.0)	21 (33.3)	
Border	3 (37.5)	15 (22.4)	
Presence of osteomyelitis, *n* (%)	2 (25.0)	26 (38.8)	0.17
Follow-up duration, weeks	45.8 ± 11.5	58.8 ± 47.9	0.66
Time to wound healing, weeks	3.0 ± 2.2	3.6 ± 1.8	0.06
Wound healing outcome			0.48
No graft loss	7 (87.5)	50 (74.6)	
Partial graft loss	0 (0)	15 (22.4)	
Healing by additional graft	1 (12.5)	2 (3.0)	

HbA1C: hemoglobin A1c; $TcPO_2$: transcutaneous partial oxygen tension. Values were reported as means ± standard deviations (SDs) for continuous variables and proportions or percentages for categorical variables.

the treatment group during the 4-week treatment period (Table 4).

The clinical assessment of a wound biofilm is vital for diagnosis. The presence of a biofilm can be pronounced, or be imperceptible to the naked eye depending on the bacterial numbers as well as individual patient factors. To date, no clear diagnostic criteria have been used by clinicians to indicate a biofilm infection. Microscopic examination using specialized techniques, such as confocal or scanning electron microscopy, may be useful to identify the EPS covering the attached bacteria in samples taken from chronic wounds; however, there are limitations to these methods in the clinical setting. Evidence of 'persistent slough' in a chronic wound and identification of bacterial microcolonies on microscopic examination have been proposed as clinical markers of a

Table 4. Previous biofilm management studies.

Authors and reference numbers	*n*	Methods	Biofilm species	Complete healing rate (observation time)
Clinical studies				
Namgoong *et al.* (Our study)	75	Surgical debridement, immediate meshed skin graft, and NPWT	Polymicrobial	77.3% (4 weeks), 100.0% (12 weeks)
Wolcott et al.[4]	190	Biofilm-based wound care algorithm	Not mentioned	77% (not mentioned)
Lenselink and Andriessen[9]	12	Polyhexanide dressing	Not mentioned	75% (24 weeks)
Beele et al.[8]	18	Silver alginate/carboxymethyl cellulose	Not mentioned	16.7% (4 weeks)
Animal studies				
Seth et al.[24] (rabbit ear model)		Initial debridement, daily lavage, and silvaden	*P. aeruginosa*	Not mentioned (show improved healing rate and decreased bacterial count)
Watters et al.[25] (diabetic mice model)		Gentamicin treated gauze	*P. aeruginosa*	80% (2.3 weeks)
Davis et al.[19] (Porcine model)		Mupriocin cream or triple antibiotic ointment	*S. aureus*	Not mentioned (show decreased bacterial count)

biofilm [2]. Many studies have reported the clinical features of chronic wounds considered to contain a bacterial biofilm, including indicators such as a pale wound bed, yellow discharge, necrotic tissue, friable granulation tissue, and unresponsiveness to antimicrobial interventions [18]. However, clinical diagnosis remains highly subjective; at present, tissue biopsies combined with microscopic identification techniques are required to confirm the presence of a wound biofilm [19]. Microscopic techniques can be used to visualize and identify bacterial clusters/microcolonies, indicative of biofilm bacteria within wound tissues. In our center, clinical diagnostic criteria for BAI are presence of a wound for >6 weeks, highly persistent slough, positive microbiological result of a tissue biopsy culture, and presence of bacterial microcolonies within wound tissue on microscopic examination.

Use of antibiotics and the duration of antibiotic administration for the treatment of diabetic foot infections have been an issue of debate for clinicians because overuse of broad-spectrum systemic antibiotics may contribute to the development of antibiotic-resistant bacteria. In 2014, Lipsky et al. [20] emphasized that there is no reason to prescribe antibiotic therapy for an uninfected wound, either as prophylaxis against infection or to hasten wound healing, and that the rationale for prescribing topical, oral, or parenteral antibiotics for patients with a diabetic foot wound should be based on treatment of a clinically evident infection. Our study focused on patients who were diagnosed as having biofilm-associated foot infections, and those who were included in the study had infections confirmed by microbiological tissue biopsy results. According to the Cochrane Database Systematic Reviews in 2015, most diabetic foot infections require systemic antibiotic therapy [21]. The Infectious Disease Society of America Diabetic Foot Infection Guidelines also suggest that diabetic foot infections often require surgical debridement or resection and/or prolonged antibiotic therapy [22]. The duration of the antibiotic therapy should be 2–5 days when no residual infected tissue remains after surgery, 4–6 weeks when residual infected bone exists, and >3 months when no surgery is performed or when residual dead bone is left postoperatively, particularly for bone or joint involvement. In addition, Johani et al. [23] studied the efficacy of topical antimicrobial/antiseptic wound solutions, including povidone iodine and chlorhexidine, against microbial biofilms by using *in vitro*, *ex vivo*, and *in vivo* model systems at clinically relevant exposure times. They concluded that wound solutions should not be used as a monotherapy and that clinicians should consider multifaceted strategies that include sharp debridement and other treatment modalities to eradicate biofilm-associated infections. As we previously mentioned, the polymicrobial nature of biofilm-associated infections in chronic diabetic ulcers is well known; 89% of the patients in our study also had a polymicrobial infection. A total of 248 bacterial colonies were isolated, with a mean of 3.3 colonies per wound. These polymicrobial biofilms may be responsible for the chronicity of diabetic foot ulcers. Wound culture can confirm the presence or absence of infection, and tissue biopsy is the gold standard for wound cultures. To reduce the possibility of developing antibiotic resistance, we obtained tissue biopsy samples from deep tissue, as the microbiologic characteristics of superficial and deep tissues are different in chronic wounds. These discrepancies are well known as the origin of the development of antibiotic resistance. Furthermore, we treated all the enrolled patients in accordance with the proper antibiotic regimens after consultation with the department of infectious diseases.

We included patients who had chronic wounds for >6 weeks that had highly persistent slough, positive microbiological tissue biopsy culture, and bacterial microcolonies within the wound tissue on microscopic examination. Therefore, proper and appropriate antibiotic treatment was essential for the treatment of biofilm-associated infection in our study. Comparison of the results of a treatment group given only an antiseptic treatment without antibiotics may yield interesting findings. However, this could not be conducted in the present exploratory study, which was initially designed to find the clues to treat biofilm-associated infection in patients with chronic diabetic ulcers, which remain challenging to completely eliminate and have a poor successful treatment rate of 16.7–77%.

This was an observational pilot study without a control arm; consequently, the true effect size of the investigated intervention remains unknown. However, the purpose of this explorative study was to report the clinical results of the treatment strategy based on the hypothesis that an immediate skin graft could obliterate the space for the formation of a biofilm owing to the tight contact between the skin graft and the recipient wound bed. In spite of the limitations of the statistical analysis that are inherent to observational studies, our study was conducted in a large population with diabetic foot ulcers between January 2010 and December 2014. We also narrowed down the cohort reflecting the effectiveness of our treatment strategy for biofilm-associated infections based on certain criteria, which were elaborately designed to overcome the limitations of an explorative retrospective study. Thus, the results of the present study could be less biased or misconstrued in terms of the interpretation of the clinical results of our treatment strategy. We believe that clinicians who have struggled to treat biofilm-associated infections will be interested in these results. Further well-designed studies may be needed to fully evaluate the efficacy of surgical debridement and simultaneous meshed skin graft combined with NPWT for BAI.

Conclusion

This exploratory study now suggests that surgical debridement and simultaneous meshed skin graft combined with NPWT may be successfully used to combat BAI in patients with chronic diabetic ulcers. We look forward to larger pivotal studies to confirm or refute these initially promising findings.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Costerton JW, Geesey GG, Cheng KJ. How bacteria stick. Sci Am. 1978;238(1):86–95.

[2] Percival SL, Hill KE, Williams DW, et al. A review of the scientific evidence for biofilms in wounds. Wound Repair Regen. 2012;20(5):647–657.

[3] Hoiby N, Bjarnsholt T, Givskov M, et al. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents. 2010;35(4):322–332.

[4] Wolcott RD, Rhoads DD. A study of biofilm-based wound management in subjects with critical limb ischaemia. J Wound Care. 2008;17(4):145–155.

[5] Cardinal M, Eisenbud DE, Armstrong DG, et al. Serial surgical debridement: a retrospective study on clinical outcomes in chronic lower extremity wounds. Wound Repair Regen. 2009;17(3):306–311.

[6] Harrison CA, MacNeil S. The mechanism of skin graft contraction: an update on current research and potential future therapies. Burns. 2008;34(2):153–163.

[7] Wolcott RD, Rumbaugh KP, James G, et al. Biofilm maturity studies indicate sharp debridement opens a time- dependent therapeutic window. J Wound Care. 2010;19(8):320–328.

[8] Beele H, Meuleneire F, Nahuys M, et al. A prospective randomised open label study to evaluate the potential of a new silver alginate/carboxymethylcellulose antimicrobial wound dressing to promote wound healing. Int Wound J. 2010;7(4):262–270.

[9] Lenselink E, Andriessen A. A cohort study on the efficacy of a polyhexanide-containing biocellulose dressing in the treatment of biofilms in wounds. J Wound Care. 2011;20(11):534–536-9.

[10] Ogawa R, Hyakusoku H, Ono S. Useful tips for successful skin grafting. J Nippon Med Sch. 2007;74(6):386–392.

[11] Scherer-Pietramaggiori SS, Pietramaggiori G, Orgill DP. Skin graft. In: Neligan PC, Gurtner GC, editors. Plastic surgery. 3rd ed. London (UK): Elsevier Saunders; 2012. p. 319–338.

[12] Davies D. Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov. 2003;2(2):114–122.

[13] Rumbaugh KP, Armstrong A. The role of quorum sensing in biofilm development. In: Rumbaugh KP, Ahmad I, editors. Antibiofilm agents. Berlin, Heidelberg: Springer; 2014. p. 97–113.

[14] Brandner JM, Zacheja S, Houdek P, et al. Expression of matrix metalloproteinases, cytokines, and connexins in diabetic and nondiabetic human keratinocytes before and after transplantation into an *ex vivo* wound-healing model. Diabetes Care. 2008;31(1):114–120.

[15] Mottola C, Mendes JJ, Cristino JM, et al. Polymicrobial biofilms by diabetic foot clinical isolates. Folia Microbiol. 2016;61(1):35–43.

[16] Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004;2(2):95–108.

[17] Ngo QD, Vickery K, Deva AK. The effect of topical negative pressure on wound biofilms using an *in vitro* wound model. Wound Repair Regen. 2012;20(1):83–90.

[18] Percival SL, Vuotto C, Donelli G, et al. Biofilms and wounds: an identification algorithm and potential treatment options. Adv Wound Care (New Rochelle). 2015;4(7):389–397.

[19] Davis SC, Ricotti C, Cazzaniga A, et al. Microscopic and physiologic evidence for biofilm-associated wound colonization *in vivo*. Wound Repair Regen. 2008;16(1):23–29.

[20] Lipsky BA. Treating diabetic foot osteomyelitis primarily with surgery or antibiotics: have we answered the question? Dia Care. 2014;37(3):593–595.

[21] Selva Olid A, Sola I, Barajas-Nava LA, et al. Systemic antibiotics for treating diabetic foot infections. Cochrane Database Syst Rev. 2015;(9):Cd009061.

[22] Lipsky BA, Berendt AR, Cornia PB, et al. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis. 2012;54(12):e132–e73.

[23] Johani K, Malone M, Jensen SO, et al. Evaluation of short exposure times of antimicrobial wound solutions against microbial biofilms: from *in vitro* to *in vivo*. J Antimicrob Chemother. 2018;73(2):494–502.

[24] Seth AK, Geringer MR, Gurjala AN, et al. Treatment of Pseudomonas aeruginosa biofilm-infected wounds with clinical wound care strategies: a quantitative study using an *in vivo* rabbit ear model. Plast Reconstr Surg. 2012;129(2):262e–274e.

[25] Watters C, DeLeon K, Trivedi U, et al. Pseudomonas aeruginosa biofilms perturb wound resolution and antibiotic tolerance in diabetic mice. Med Microbiol Immunol. 2013;202(2):131–141.