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Wound Biofilm and Therapeutic Strategies

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Abstract

Since the turn of the millennium, an evolving body of scientific and clinical evidence indicates that biofilm is implicitly linked to delayed wound healing and infection. Currently, wound anti-biofilm strategies rely on non-specific wound bed preparation techniques involving physical debridement and cleansing, and innovative technologies designed to specifically manage biofilm have only just begun to emerge. The first output of anti-biofilm research and product development in wound care show great promise for patients, clinicians and healthcare institutions. The aim of this chapter is to address the current clinical biofilm problem, describe existing and emerging strategies to combat wound biofilm and review the available evidence.

Keywords: wound, anti-biofilm, dressing, healing, infection

1. Introduction: the clinical problem

Fossil evidence of microorganisms existing as surface-attached microcolonies dates back 3.4 billion years [1], establishing biofilm as one of the oldest life-forms on earth. The scientific study of surface-attached microorganisms dates back to the seventeenth century [2], but it is only in recent decades that their relevance has been appreciated in both natural and pathogenic ecosystems [2, 3]. Although the term 'biofilm' has been used to describe surface-attached, matrix-encased microbial communities in industrial and environmental applications since the 1930s, it was not until 1985 that Bill Costerton introduced the term into medical microbiology [2]. The importance of biofilm in chronic infections is now widely accepted and there has been an exponential rise in related medical publications since 1975, reaching a number of 3251 in 2013 alone [2].

The refractory nature of many infections has been largely attributed, in recent decades, to the continuing rise in antibiotic resistance, but the involvement of biofilm in microbial tolerance to antimicrobial agents and immune cells is increasingly recognised. The combined effect of biofilm tolerance and antibiotic resistance are the two most important microbial defence strategies, and in combination present a significant risk to public health. In 1999, Costerton *et al.* [4] reported bacterial biofilm as being a common cause of persistent infections that include conditions such as periodontal disease, otitis media, cystic fibrosis, pneumonia and device related infections. Although the authors also considered necrotising fasciitis and osteomyelitis as biofilm infections, wound infections in more general terms were not considered.

Any wound that is not healing and that has not followed a normal wound healing trajectory is likely to involve biofilm. Healthy skin is an effective microbial barrier; therefore dermal tissues are intrinsically sterile. However, the surface of the skin is heavily colonised. Damage to or removal of the epidermal barrier layer will inevitably lead to wound contamination and opportunistic microbial colonisation. The innate human immune system can usually counter this invasion but, if the initial contamination event is overwhelming (such as a severe traumatic wound), or contamination is repetitive (for example for a faecally incontinent subject suffering from a sacral pressure ulcer), or if the casualty has a weakened immune system (as a result of age, disease, malnutrition, obesity, smoking, etc.) then biofilm may become established.

The earliest indirect indication of wound bacteria existing in biofilm form involved the detection of extracellular polysaccharide capsules surrounding the cells of both aerobic and anaerobic wound pathogens, using light and scanning electron microscopy [5]. Capsule production is a key component of a biofilm mode of life that can protect bacterial communities from the host immune system [6]. The earliest scientific research into wound biofilm was reported by Serralta *et al.* in 2001 [7]. In this *in vivo* study, both planktonic and biofilm bacterial lifestyles were observed, with biofilm bacteria (*Pseudomonas aeruginosa*) exceeding planktonic bacteria by approximately 100-fold. Whereas the planktonic *P. aeruginosa* could be removed by vigorous flushing, adherent biofilm *P. aeruginosa* could only be removed by forceful scrubbing with a detergent agent.

The development and evolution of wound biofilm from contamination to a pathogenic state was proposed in 2004, and the point at which an evolving biofilm begins to interfere with wound healing and increase the risk of infection has largely replaced the previously-used term 'critical colonisation' [8]. In 2008, a hypothesis (that was considered novel at the time) relating to why chronic wounds fail to heal was reported [9]. Based on their previous experiences with chronic *P. aeruginosa* infections in patients suffering from cystic fibrosis, Bjarnsholt *et al.* [9] proposed that the inability of polymorphonuclear leukocytes and antibiotic treatment to eliminate *P. aeruginosa* biofilm was the cause of recalcitrance in chronic wounds. Subsequent clinical studies using microscopy and molecular analytical techniques demonstrated that biofilm existed in a majority of non-healing chronic wounds [10, 11]. Since 2008, an increasing number of studies have demonstrated the presence of biofilm in wounds of varied aetiology [10–21] as indicated in **Table 1**.

Since the turn of the millennium, wound biofilm has been proposed, investigated and confirmed, as a factor in chronic wound pathogenesis. From initial evidence of their

existence [7], role in wound healing [8], and the simultaneous and pioneering confirmation of their clinical existence in 2008 [10, 11], a large body of scientific and clinical evidence now suggests that biofilm is inextricably linked to wound infection and delayed healing [7, 22–37] (Table 2).

Wound types	No. Methods	Observations	Ref.
Chronic (mixed)	50 Light microscopy, scanning electron microscopy (SEM)	30 chronic wounds observed to contain biofilm (60%)	10
Chronic (mixed)	22 Confocal laser scanning microscopy (CLSM)	13 chronic wounds observed to contain biofilm (59%)	11
Chronic (mixed)	9 Fluorescence microscopy, CLSM	<i>P. aeruginosa</i> observed deeper in wound than <i>S. aureus</i>	12
Chronic (mixed)	10 Fluorescence microscopy, CLSM	<i>P. aeruginosa</i> elicited greater inflammation than <i>S. aureus</i>	13
Chronic	1 Fluorescence microscopy	Both samples contained biofilm	9
Mixed aetiologies	15 Fluorescence microscopy	7 wounds observed to contain biofilm (47%)	14
Full-thickness burns	11 Light & transmission electron microscopy, SEM	Only ulcerated and escharotomy sites contained biofilm	15
Diabetic foot ulcers (DFU)	2 CLSM	Both samples contained biofilm	16
Acute	16 Light microscopy, SEM	1 acute wound contained biofilm (6%)	10
DFU	4 Light & fluorescence microscopy, environmental SEM	Microcolonies associated with biofilm observed in all wounds	17
Surgical sternal	6 Light & fluorescence microscopy, CLSM, SEM	3D biofilm aggregates observed in all 6 infected wounds	18
Venous leg ulcers (VLU)	45 Transmission electron microscopy	Biofilm matrices of polysaccharides, proteins & DNA observed	19
Malignant	32 Fluorescence microscopy	Biofilm observed in 35% of wounds	20
Mixed & non-wound	113 Biofilm-forming capacity of isolates by culture & SEM	Significantly more biofilm formed by wound isolates than others	21

Table 1. Key scientific evidence for the presence of biofilm in human wounds.

Model	Biofilm species	Observations	Ref.
Porcine acute	<i>S. aureus</i> (<i>S.a</i>)	Challenge with antimicrobial agents confirmed the recalcitrance of biofilm bacteria	7
Porcine acute	<i>S.a</i>	Polymorphonucleocytes observed on the surface of, but not within, biofilm	22
Porcine partial thickness	MRSA, <i>P. aeruginosa</i> (<i>P.a</i>)	Interactions between MRSA and <i>P.a</i> were observed, delaying healing due to suppression of epithelialisation and expression of	23

Model	Biofilm species	Observations	Ref.
		virulence factors	
Murine burn	<i>P.a</i>	Microscopic biofilm observed that was not readily removed by rinsing with saline	24
Murine diabetic chronic	<i>P.a</i>	Biofilm-colonised wounds highly inflamed; 8 weeks for biofilm-colonised wounds to heal, 4 weeks for controls	25
Murine diabetic chronic	<i>P.a</i>	Biofilm significantly delayed wound healing, even in diabetic mice treated with insulin	26
Murine infected surgical	<i>P.a</i>	Biofilm highly-tolerant to antibiotics & sodium hypochlorite once established over several days	27
Murine chronically-infected surgical	<i>S.a, P.a, E. faecalis, F. magna</i>	Polymicrobial biofilm maintained for 12 days, & delayed healing more than <i>P.a</i> biofilm, by wound closure	28
Murine splinted	<i>S.a/S. epidermidis</i>	Biofilms significantly delayed epithelialisation; inhibition of biofilm restored normal healing	29
Rabbit ear	<i>S.a</i>	Biofilm and active infection significantly delayed healing; biofilm-colonised wounds expressed significantly lower levels of inflammatory cytokines than infected wounds	30
Rabbit ear	<i>P.a</i>	Biofilm significantly delayed healing; debridement, lavage and silver sulphadiazine in combination were more effective at restoring healing than individual treatments	31
Rabbit ischaemic ear	<i>K. pneumonia (K.p), S.a, P.a</i>	<i>K.p</i> biofilm was least virulent, <i>P.a</i> biofilm most virulent, measured by healing inhibition and inflammation; extracellular polymeric substances (EPS)-deficient <i>P.a</i> did not delay healing	32
Rabbit ear	<i>S.a, P.a</i>	2-species biofilm elicited significantly elevated inflammatory response & impaired epithelialisation & granulation tissue formation compared to single-species	33
Rabbit ear	<i>P.a</i>	Dressing designed specifically to manage biofilm gave significant reductions in biofilm count & significantly improved wound healing (granulation & epithelialisation)	34
Murine	Natural skin microflora	Biofilm developed over time in chronic wounds (similar to humans); reducing oxidative stress increased their susceptibility to antibiotics & dismantled biofilm	35
Rabbit ear	<i>K.p, P.a</i>	Wounds showed increased inflammation and delayed healing with <i>P.a</i> biofilm infection as determined by of wound healing cells transcriptome analysis	36
Porcine burn	<i>P.a, A. baumannii</i>	Biofilm-infected wounds, tolerant to silver dressings, eventually closed, but skin barrier function compromised	37
Diabetic mouse	<i>S.a</i>	Diabetic wounds had significantly more biofilm & less neutrophil activity, thus poorer healing than wild type	38

Table 2. Evidence that biofilm delays wound healing from porcine, murine and rabbit ear models.

In our laboratory, we have used microscopy techniques to better understand the structure and development of wound biofilm. Planktonic cultures of *P. aeruginosa* were grown on track-

etched membrane cell culture inserts in culture wells for up to 48 hours, and biofilm growth was examined at various stages of development by light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Biofilm was shown to form within 6 hours, and becoming established after 24–48 hours (**Figure 1**). This favourably compares with previous work reported [39] in which mature *P. aeruginosa* biofilm formed within 5 hours of initial inoculation. Stages of biofilm observed *in vitro* may correlate with our understanding of wound biofilm development and its link to chronicity and infection (**Figure 1**).

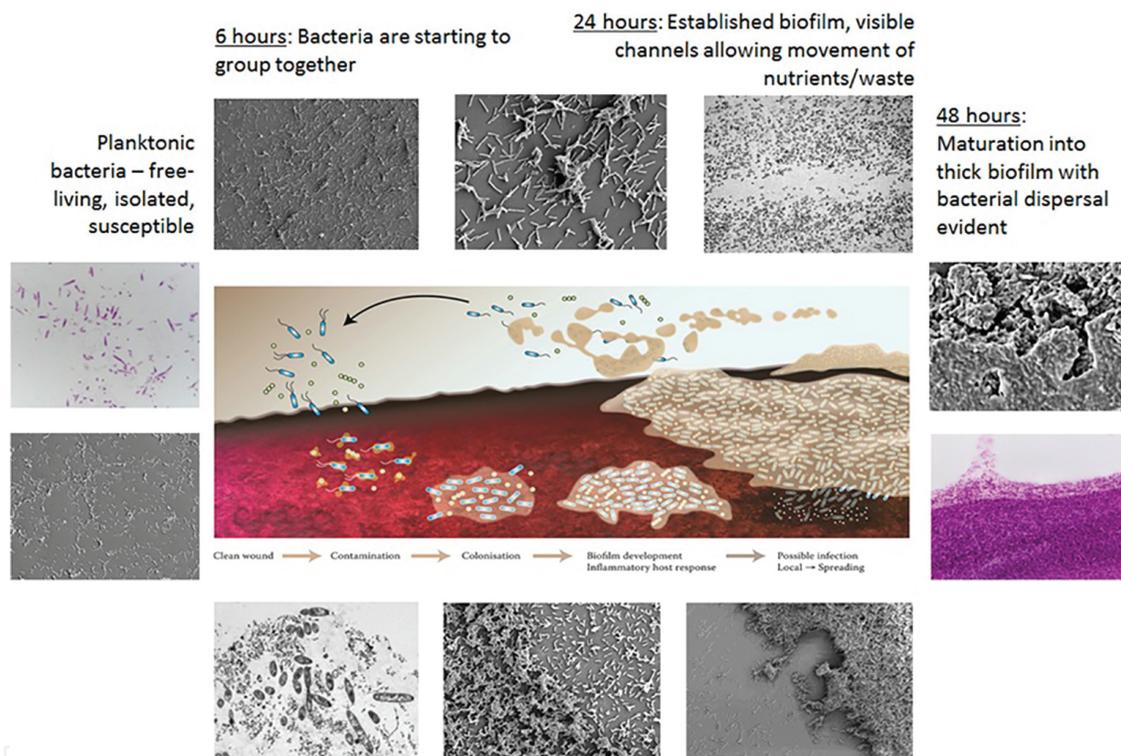


Figure 1. Evolution of *P. aeruginosa* biofilm over 48 hours, with evidence of microcolony formation, maturation and dispersal.

Numerous *in vivo* models have been reported in recent years, and these have been critically reviewed and compared [40]. The rabbit ear model developed by Tom Mustoe's team in Illinois, USA is perhaps the most developed and validated of the models [30], has demonstrated clear links between wound biofilm and healing, and has also been used to compare the effectiveness of anti-biofilm strategies [31, 34, 41–43].

Scientific, clinical and animal evidence strongly suggests that biofilm delays wound healing [44], and efforts are underway to understand and develop ways to visualise and control biofilm to aid clinical practice [44, 46]. It has been shown that by targeting and suppressing biofilm

healing can be improved, so the onus is now on wound care product developers and manufacturers to offer technologies with anti-biofilm effectiveness.

2. Therapeutic anti-biofilm strategies

With only relatively recent recognition of the existence of biofilm in wounds and consequent role it plays in delayed healing and chronicity [8, 10, 11], the development of effective therapeutic treatments and strategies to date has been very limited. However, this late recognition does mean that wound care researchers can benefit from the knowledge gained in other industries and in related healthcare areas such as dentistry and indwelling medical devices. Here treatment strategy options are well developed and broadly similar. Although the intention to prevent, remove and kill bacterial biofilm is the same, there is a significant challenge in selecting wound treatments that have an appropriate balance of safety versus efficacy. There are also challenges in simultaneously addressing the other clinical needs of the wound as, unlike inert medical devices or tooth enamel, the surface of a wound, particularly a chronic wound, can be acutely sensitive and fragile.

Wound biofilm is generally initially attached to the wound bed which is a dynamic mixture of viable and non-viable (slough) tissues. Exudate permeates through this underlying tissue

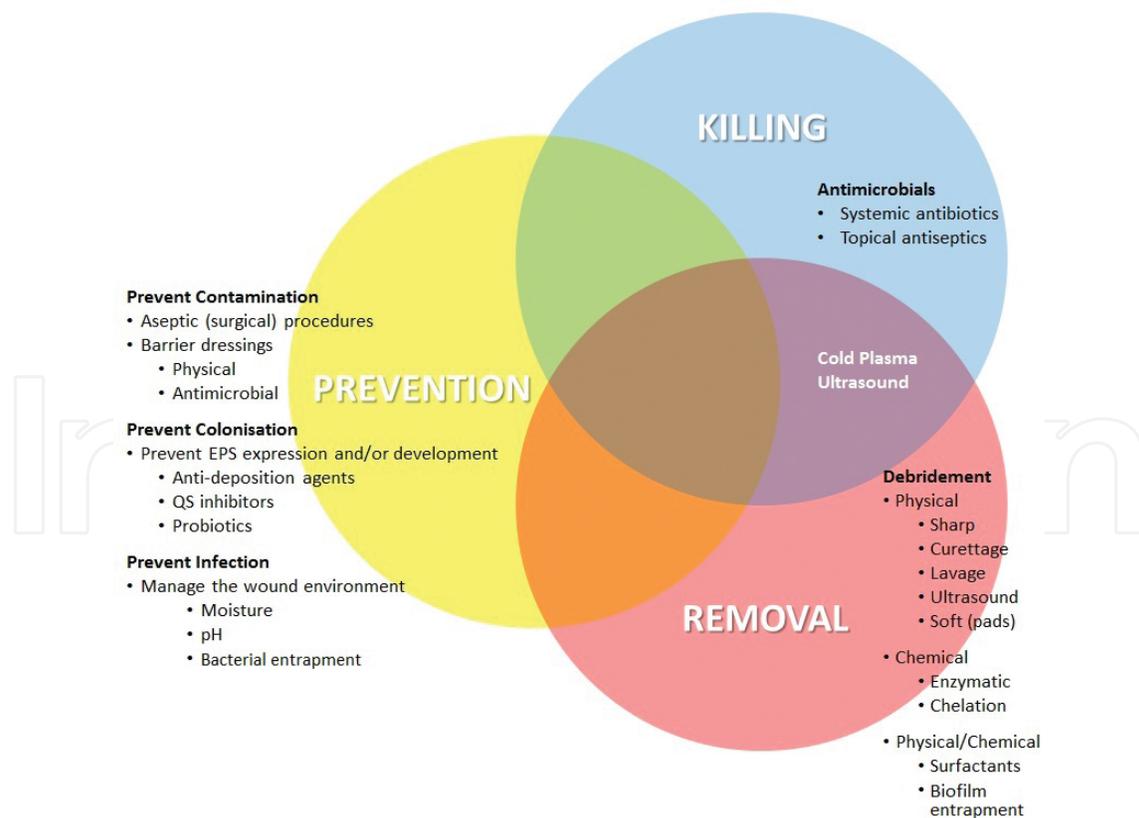


Figure 2. Anti-biofilm strategies for wound care—prevention, removal, killing.

providing moisture and nutrients to developing biofilm. Treatment and prevention of wound infection usually consists of systemic antibiotics and/or topical antiseptics. The polymicrobial nature of wound bioburden [5] and difficulty in identification of species present means that antibiotic selection and coverage is imperfect. Chronic wounds are often poorly perfused with blood (a causative factor) therefore delivery of systemic antibiotics, at a sustained therapeutic concentration, for the period necessary to diffuse into a biofilm and take effect, is an additional challenge [47]. Topical antiseptics are preferred because of their broad spectrum of activity, but they suffer from a lack of selectivity towards bacteria and therefore can be toxic to host tissues. General reaction with organic matter and continual dilution and removal with wound exudate mean that an antimicrobial product needs to be continuously instilled [48] or formulated for slow release and physical retention within the wound or dosed at high concentration. Clinical evidence suggests that none of the above existing systemic or topical treatments are particularly effective against wound biofilm. Therefore, different strategies are required.

It is convenient to consider these strategies in broad groups aligned with the clinical intent as mentioned above—to prevent, to remove, to kill biofilm-associated organisms (**Figure 2**)—and many treatments will involve combinations of these with the best being a combination of all three. We will also discuss the anti-biofilm effectiveness of existing methods together with associated devices, and how some of these are providing therapeutic advances in the emerging 'biofilm era' of wound care.

2.1. Prevention of biofilm

Microbial contamination of breaches in skin integrity is inevitable, unless the wound is created under aseptic surgical conditions, and wounds that are not successfully managed can become chronic and at risk of infection. The ideal situation is to prevent bacteria entering the wound in the first instance. The risk of wound contamination post-surgery can be addressed by applying effective microbial barrier dressings. In the 1990s *DuoDERM*[®] hydrocolloid adhesive dressings were shown to reduce the rate of infection (compared to traditional gauze dressings) [49] and these dressings were later shown to provide a physical barrier to both bacteria and viruses [50]. More recently, combined physical and antimicrobial barrier dressings have been developed to minimise the risk of post-operative infection [51].

Although barrier dressings have an important role to play in minimising infection, it is most likely that any open wound will become contaminated to some extent (chronic wounds will become significantly more contaminated than most surgical wounds). Consequently, an important infection control strategy at this point is to prevent microbial attachment to the wound tissue. This might be achieved by chemical or biological treatment of the wound surface. Examples of the former are lactoferrin and xylitol. Lactoferrin [52, 53] is a protein that is believed to inhibit the effectiveness of bacterial adhesins by its ability to sequester iron from acidic environments, particularly for Gram-negative bacteria [54]. It has proven useful in the preservation of meat but in a living system with a functioning circulating system supplying an excess of iron and buffering to neutral pH there will be challenges in maintaining efficacy. If derived from non-human sources there is also the possibility that lactoferrin may be

identified by the immune system as a threat and illicit an inflammatory response. Xylitol is a naturally occurring sugar that binds to the surface of Gram-positive bacteria preventing adhesion [53], inhibiting glycocalyxes (exopolymeric substances) and disrupting cell wall growth [54]. But xylitol faces the same challenge as lactoferrin in that it is a freely soluble substance and will be difficult to maintain at an effective concentration in an exuding wound. Gallium is also mooted in this space as Ga^{3+} is similar to Fe^{3+} in size but does not undergo the same redox reactions ($\text{Fe}^{3+} \leftrightarrow \text{Fe}^{2+}$) and therefore interferes with bacterial attachment and proliferation [52].

If microorganisms gain the opportunity to attach to wound tissue and acclimatise to the environment, then subsequent colonisation will lead to the development and maturation of a predominantly biofilm population that is protected from the immediate hostilities within the wound environment (**Figure 1**). Two potential biological approaches for controlling biofilm development are quorum sensing (QS) inhibitors and probiotics. Quorum sensing is an active field of research with over 100 bacterial species identified as having the ability to communicate by release of small signalling molecules [55]. At a critical concentration, microbial communication between cells triggers a change in gene expression which results in a change in behaviour. QS is involved at all stages in the biofilm life cycle (initial attachment, EPS expression, proliferation, maturation and dispersal) and is implicated in biofilm virulence. In practical terms, this minimum concentration dependence translates into a minimum threshold bacterial population density. However, it must be borne in mind that wound biofilms are polymicrobial [56–58] and although approximately 50% of all known QS bacterial species have the ‘universal’ autoinducer 2 (AI-2) [55], QS signal molecules vary between species and strains. Therefore, a universal inhibitor for wound biofilm formation seems unlikely in the near future. Probiotics [59, 60] offer the interesting possibility of prophylactically colonising the wound with non-pathogenic bacteria. *Lactobacillus* species have been shown to successfully out-compete and inhibit the pathogenic activity of *P. aeruginosa* and *S. aureus* possibly through QS inhibiting effects [59]. However, there are significant challenges, particularly for chronic wounds, in how to selectively provide conditions for the survival and growth of probiotic microorganisms in an already contaminated and inflammatory wound.

Finally, often overlooked is the management of the wound environment itself—establishing the best conditions in which the body’s immune system can function and/or creating conditions which reduce bacterial proliferation and biofilm development. The optimal moisture balance in the wound bed is reported to be 100% humidity with no free liquid [61], and it has recently been suggested that poor exudate control is likely to encourage the development of biofilm [62]. Chronic, non-healing wounds are often characterised by a high pH (7.15–8.9) and healing wounds tend to have a lower pH [63]. The increased production of *S. aureus* EPS with increasing pH has been reported [64], but other examples describe the opposite, so the relationship between biofilm and pH appears to be complex [65, 66].

2.2. Removal of biofilm

By the time wounds are presented to a wound care specialist, the majority of non-healing traumatic and chronic wounds are likely to be biofilm impeded. There is a long history of

removing non-viable and necrotic host tissue from wounds (debridement) in order to encourage the inflammatory, granulation and epithelialisation processes of wound closure. Surgical debridement techniques can range from aggressive surgical or sharp removal of tissue to less invasive techniques such as curettage and lavage. This practice is likely to have coincidentally been removing biofilm with beneficial effect. With increasing familiarity with the appearance of biofilm or more likely, the symptomatic signs of its presence, clinicians are seeking methods to physically remove biofilm from wounds. In recent years, debridement devices utilising a number of very different technologies have emerged.

Sharp debridement is the most radical approach and requires expertise [67]. Excision of devitalised host tissue (i.e. necrosis) or infected/biofilm tissue via scalpel or other surgical instrument until the exposed tissue is bleeding would certainly be expected to remove a majority of any biofilm residing in the wound, but the deleterious effects on healing tissues need to be balanced with the need to remove unhealthy tissue. However, sharp debridement has proven successful and advocates such as Wolcott have developed protocols where regular sharp debridement has provided a 'healing window' during which improved effectiveness of concurrent antimicrobial treatment has been observed [68]. Hurlow has also reported the atraumatic removal of biofilm above a non-healing surgical wound with exposed tendon using curettage and antimicrobial cleansers [69, 70] (**Table 3**).

A number of other devices that can be used for wound debridement are now commercially available. Examples include devices that emit energy in the form of water jets (lavage), ultrasound and cold plasma (**Table 3**). High pressure lavage using hand-held devices [67] has been assessed in several laboratory and clinical investigations, and there is evidence that removal of unwanted tissue (which may include biofilm) using this method encourages wound progression [71]. Ultrasonic wound debridement has proven effective in clinical cases [72], and scientific studies support the ability of ultrasound to disrupt biofilm and encourage healing *in vivo* [42], as well as potentiate the effects of antiseptics via its anti-biofilm action *in vitro* [73]. Finally, encouraging *in vitro* anti-biofilm effectiveness of cold atmospheric pressure plasma technology suggests that the reactive oxygen species produced in precise beams by these devices may disrupt and kill biofilm while sparing host tissues [74].

Unfortunately, in many clinical institutions the skills, training and equipment for the use of advanced debridement techniques or devices may not be available. Under these circumstances simple cleansing, enhanced with 'soft debridement' using engineered textiles, may be helpful. Recently, debridement pads or wipes have emerged which aim to gently brush and lift away wound debris. A polyester filament pad has generated encouraging clinical effectiveness data [75, 76] and cost-saving estimates [77]. In addition to disrupting and lifting surface-associated wound debris (which is likely to include biofilm), these soft debridement devices are simple and safe to use, gentle on patients and relatively low-cost, compared to most other debridement techniques and devices discussed in **Table 3**.

More thorough biofilm removal may be achieved by degrading the structure of the EPS such that it flows away from the wound or can be more readily irrigated or absorbed by absorbent dressings. General proteolytic enzymes have been used for many decades to remove slough and necrotic tissue, but, as EPS is not primarily comprised of extracellular proteins for its

structural integrity, these are ineffective. This fact has been utilised by an aid to detect the presence of wound biofilm [45]. Alternative enzymatic candidates that are effective against polysaccharides have been identified and reviewed [78, 79], and include: α -amylase (mammalian), polysaccharide depolymerase (bacteriophage), alginate lyase (bacterial) and glycoside hydrolase (DspB) (bacterial). Generally, the kinetics of enzyme reactions are known to be sensitive to pH, for example Dispersin B, despite demonstrating some activity *in vitro* [80], has optimal activity at pH 5, and as proteins, enzymes will be vulnerable to the high concentrations of proteolytic enzymes often associated with chronic wounds. Hence, careful formulation of any enzyme based anti-biofilm treatment would be required. Sun *et al.* also point out that the current high cost of industrial enzyme production makes the application of these enzymes relatively expensive [79].

Debridement method	Evidence for effectiveness against biofilm	Ref.
Curettage	(Clinical) Gentle scraping of suspected biofilm (in combination with other antimicrobials) improved healing in case studies	69,70
Lavage/water jets	(Clinical) Indirect anti-biofilm evidence; debridement with <i>Versajet II</i> (Smith & Nephew) removed unwanted tissue & encourages healing	71
Ultrasound	(<i>In vitro</i>) Anti-biofilm action demonstrated in agar biofilm model using <i>Ultrasonic-Assisted Wound (UAW)</i> device (Soring)	73
	(<i>In vivo</i>) Leporine ear model showed <i>MIST</i> (Celleration) reduced <i>P. aeruginosa</i> biofilm & inflammation, & improved healing parameters	42
	(Clinical) Indirect anti-biofilm evidence; case study evidence that <i>UAW</i> can effectively debride unwanted tissue	72
Cold plasma	(<i>In vitro</i>) Biofilm significantly reduced using <i>Coblation</i> (Smith & Nephew) compared to lavage or curettage in porcine explant model	74
Soft debridement pads	(Clinical) Indirect anti-biofilm evidence in case studies where sloughy wounds were well managed using <i>Debrisoft</i> (Lohmann & Rauscher)	75
	(Clinical) Debridement was classed as effective in 94% of patients, removing debris and slough, in a 57-patient study using <i>Debrisoft</i>	76

Table 3. Biofilm removal using debridement methods and devices.

Structural degradation of biofilm EPS can also be achieved chemically. Divalent cations such as calcium and magnesium are known to be involved in cross-linking polysaccharides within EPS and manganese and iron are involved in bacterial metabolism and cell wall structure [81]. Competition for these ions or their removal (chelation) will therefore affect biofilm formation and strength. Metal chelating agents are a diverse set of compounds but biocompatibility and safety considerations restrict those that can be considered for wound care to ethylenediamine tetra acetic acid (EDTA) and its homologues and polyanionic compounds such as phosphates and citrate. The most widely discussed of these as an anti-biofilm agent and the one with the greatest affinity for calcium and magnesium cations is EDTA. The literature primarily focuses on the tetra-sodium salt form but only at high pH (>pH 10) [81], which is incompatible with wound management practices. Lower pH forms of EDTA, such as

the di-sodium salt, are effective but all anionic chelating agents are pH-sensitive. A water-soluble gel formulation that contains 0.1% EDTA, acetic acid, citric acid and carbopol has demonstrated anti-biofilm effectiveness against *P. aeruginosa* and *S. epidermidis in vitro* [82].

Empirical experience in other industries such as food, laundry, personal washing and dental products [83] has shown the utility of surfactants as anti-biofilm agents to facilitate penetration of combination agents through biofilm EPS [84], leading to detachment from surfaces and prevention from re-deposition by micelle formation. An anti-biofilm gel comprising a surfactant and calcium chelator has shown *in vitro* and *in vivo* anti-biofilm activity. Although not yet commercially available, use of this gel has shown clinical superiority to standard wound care, as well as apparent synergy with standard care [85].

2.3. Killing of biofilm microorganisms

The efficacy of existing antimicrobial therapies in wound care has almost exclusively been based on their activity against susceptible planktonic bacteria. Whilst associated devices may be useful in controlling this bacterial phenotype by reducing the risk of contamination and dispersal, their effectiveness against biofilm is unproven. Indeed, the prevalence and recurrence of chronic wounds suggests that most antimicrobial therapies are ineffective.

Considering the selective and specific action of antibiotics, the polymicrobial nature of wound bioburden and the increasing threat of multi-drug resistant organisms (MDROs), the effectiveness of antibiotics in chronic wound care is questionable. However, utilising state of the art molecular microbiological techniques, personalised cocktails of topically-applied antibiotics yielded better results than patients receiving systemic antibiotics prescribed using the same diagnostic techniques who, in turn, yielded better outcomes than a standard-of-care group treated upon data from standard culture techniques [86]. Unfortunately, this level of diagnostic sophistication is not within the reach of most health care systems, and therefore we must await further technological advancements so that it becomes generally affordable.

The majority of wound treatments do not have the benefit of sophisticated microbiological analysis; therefore any antimicrobial therapy administered must have broad-spectrum activity. Choice then becomes restricted to antiseptics which can only be applied topically. Antiseptics are chemically reactive species that are largely non-selective in their action, therefore potential cytotoxicity (local toxicity to skin cells) and systemic toxicity must be taken into account. Toxicity is generally managed by limiting the concentration and time of exposure to the antimicrobial agent. Therefore, antimicrobial cleansing at dressing change may involve slightly higher concentrations of antiseptic than an antimicrobial dressing which may stay *in situ* for several days. However, given that the minimum biofilm eradication concentration (MBEC) for antiseptics in wound relevant bacteria is often between 10–1000 times greater than its minimum inhibitory concentration (MIC) for planktonic bacteria (the value upon which most products are formulated) [87], the balance of safety versus efficacy of antiseptic agents is challenging. Topical wound antiseptic treatments typically involve 0.5 to 12% for silver in dressings (depending on the form of silver), approximately 1% for molecular iodine in dressings (or 10% as povidone iodine), 2 to 4% for chlorhexidine gluconate in cleansers and

0.1% for polyhexamethylene biguanide (PHMB) in cleansers. Increasing the concentration of the antiseptic component to be effective against biofilm may not be possible, practical or safe.

Clinical experience and safety reviews have limited the number of usable antiseptic substances. Currently, silver is the most widely-used topical antiseptic agent, primarily due to its good safety versus efficacy balance [88]. Silver is the most studied topical antiseptic [52, 53], and ionic silver—the antimicrobial active form—has a particularly high affinity for sulphur atoms, binding irreversibly to thiol groups. Ionic silver also binds to nitrogen atoms in amines and oxygen atoms in carboxylates, although less strongly. These three interactions lead to very efficient denaturing of peptides, proteins and enzymes—all of which are essential to bacterial structure and metabolism. However, carboxylate functional groups are also found within the polysaccharide in EPS. Therefore, although ionic silver may be inactivated by EPS and other organic matter within the biofilm, there is a theoretical basis for it to have some biofilm disruptive effects. Evidence for this effect was a reduction in EPS mechanical strength of an *S. epidermidis* biofilm after the application of dilute silver ion solution [89] (**Table 4**). Similar observations have been made for a silver-containing carboxymethylcellulose dressing [90, 91], and it was reported wound dressings with hydrophobic base material impregnated with silver had sustained anti-biofilm activity [92] (**Table 4**).

Molecular iodine has proven too toxic for direct application but, by complexation with a carrier molecule and careful formulation, acceptable slow release products have been developed. Although the mode of action of molecular iodine is not fully understood [52], studies suggest that in common with silver, sulphur atoms are a reaction target resulting in protein denaturing and subsequent changes to cell wall structure [93]. Iodine will react with unsaturated fats and lipids and organic matter within the wound, and is known to be trapped by polysaccharides. There is limited evidence that molecular iodine has anti-biofilm properties, aside from in simple *in vitro* models [94], but *ex vivo* studies of a formulated cadexomer iodine product suggest that sustained release may result in biofilm penetration [95] (**Table 4**).

Evidence for the anti-biofilm effects of the cationic, nitrogen containing, surfactant-like antibacterials—chlorhexidine (CHG), PHMB and octenidine—in wound care is limited. CHG has been shown to have limited effect against some biofilms *in vitro* but to be ineffective against others, and the theoretical electrostatic effect on bacterial cell walls as the antimicrobial mechanism is believed to be negated by biofilm, so the observed effects are unexplained [96]. PHMB is similar in structure to CHG and is proposed to accumulate within biofilm by electrostatic interactions [97], i.e. cationic PHMB binds to the anionic polysaccharide of EPS [98]—initially, at least, this will have an inactivating effect on the antimicrobial action. Available anti-biofilm data focus on formulated products [99–104], so laboratory and clinical results cannot be attributed solely to PHMB (**Table 4**). Octenidine has been tested *in vitro* against *S. aureus* biofilm, and above a critical concentration bioburden reduction rate was seen to increase, but biofilm was possibly removed to surfactancy rather than specific anti-biofilm activity [105].

The next most popular traditional antiseptic substances are the molecular halogens and related oxidising compounds. Chlorine itself is too toxic to be used and hypochlorite-based bleaches are considered too cytotoxic for general wound care. Hypochlorous acid (HOCl) and chlor-

ine dioxide (ClO₂) are under consideration [52] as potent, fast-acting cleansing solutions, and some early anti-biofilm effectiveness has been observed *in vitro* or inferred clinically for various HOCl formulations [104, 106–108] (**Table 4**).

Antimicrobial agent	Evidence for effectiveness of formulated product against biofilm	Ref.
Ionic silver	(<i>In vitro</i>) Reduction in <i>S. epidermidis</i> biofilm EPS mechanical strength after application of dilute (50 ppb [ng/ml]) silver solution	89
	(<i>In vitro</i>) Anti-biofilm activity of silver Hydrofiber (AQUACEL Ag; ConvaTec) shown over 48 hours using range of biofilm models	90,91
	(<i>In vitro</i>) Sustained anti-biofilm activity evident for at least 7 days, independent of the microbial strain	92
Molecular iodine	(<i>In vitro</i>) Povidone iodine dressing (<i>Inadine</i> ; Systagenix) demonstrated greater anti-biofilm activity than silver dressings	94
	(<i>In vitro</i>) Cadexomer iodine dressing (<i>Iodoflex</i> ; Smith & Nephew) demonstrated anti-biofilm activity in <i>P. aeruginosa ex vivo</i> model	95
PHMB with alkylamidopropyl betaine solution /gel	(<i>In vitro</i>) Biofilm matrix on human dermal cell line was disrupted, releasing bacteria for killing, by <i>Prontosan</i> (B. Braun)	104
	(<i>In vivo</i>) Anti-biofilm effectiveness of <i>Prontosan</i> was significantly more effective than inactive controls in porcine dermal wounds	100
	(Clinical) Signs of biofilm & infection reduced, healing progression observed in 124-patient study using <i>Prontosan</i> gel	101
Hypochlorous acid	(<i>In vitro</i>) 0.01% HOCl killing of CDC reactor-grown <i>P. aeruginosa</i> biofilm by live-dead staining with confocal microscopy	106
	(<i>In vitro</i>) Biofilm matrix on human dermal cell line was disrupted, releasing bacteria for killing, using a concentrated HOCl solution	104
	(Clinical) Signs of infection reduced & progress toward healing in a 31-patient study using <i>Vashe</i> (PuriCore)	107
	(Clinical) Infection controlled & wounds healed in 14 osteomyelitis patients using <i>Dermacyn</i> (Oculus)	108

Table 4. Examples of existing topical antimicrobial products with some anti-biofilm activity.

2.4. State of the art today

2.4.1. Multi-modal strategies

Perhaps the most straightforward way for wound care clinicians to implement more effective biofilm management strategies today is to consider how dental care has embraced multiple strategies to manage dental plaque biofilm. By using combinations of debridement (brushing, flossing), surfactants with antimicrobials (toothpaste), and antimicrobial rinses (mouthwash), most consumers manage biofilm effectively on a daily basis to maintain oral hygiene, and prevent conditions such as dental caries and periodontitis.

Biofilm-based wound care (BBWC) is an emerging and evidently effective way of combining multiple modes of wound treatment to improve the health of chronic and infected wounds [68, 85, 109]. Practised initially by the pioneering wound care physician, Randall Wolcott in Texas, a first assumption of BBWC is made that most (if not all) chronic or infected wounds contain biofilm. A further assumption is that one mode of treatment may not suffice, therefore the use of combinations of vigorous debridement, cleansers or gels, topical antimicrobial or anti-biofilm agents, and wound dressings, is required. Wolcott *et al.* [85, 109–111] have shown how BBWC can result in significantly improved outcomes compared to standard wound care in several large patient cohorts. A number of case studies reported by Hurlow and Bowler [69, 70] have also described how protocols of care designed to target biofilm result in improved wound outcomes. Combining lactoferrin and xylitol (see Section 2.1) in a hydrogel in conjunction with a silver wound dressing demonstrated good efficacy against biofilms [53].

We also firmly believe that the multi-modal approach is the most effective way of rapidly improving wound health in chronic wounds that are likely compromised by biofilm or infection. A key component in such protocols of care is undoubtedly efficacious wound dressings which can provide effective, sustained and safe antimicrobial and anti-biofilm action. Although the focus here is on therapeutic approaches towards wound biofilm, biofilm cannot be considered in isolation. Other challenging wound conditions must be considered alongside biofilm—exudate must be managed, infection must be controlled, the wound must be protected, and pain must be considered—to provide outcomes that can improve quality of life.

Most established antimicrobial dressings are very efficient at managing planktonic bacteria, thereby limiting initial contamination and spread of infection. However, they all suffer the same challenge in the treatment of biofilm in that the antimicrobial agent must penetrate the EPS in order to reach the target bacteria and, when they do so, they largely rely on metabolism to draw them into the bacterial cell for them to act. EPS can restrict the movement of antimicrobial agents by binding them and increasing the likelihood of reaction with other organic matter. If the agent is able to reach the target bacterial cell it must do so in a concentration sufficient to be cidal for the sessile (biofilm) phenotype. Therefore, it is clear that universally successful antimicrobial therapy using a topical antiseptic agent can only be achieved by a sustained application or release in combination with some form of EPS (biofilm) disruption.

2.4.2. An anti-biofilm wound dressing

In 2009, the authors of this chapter undertook a substantial research project to design a wound dressing specifically to manage biofilm. The starting point was taken as an existing antimicrobial dressing, *AQUACEL*[®] Ag. This dressing has a well-documented clinical history for patient acceptance, safety, management of exudate and reducing the risk of infection [112–115]. *In vitro* studies have demonstrated this product to be effective against a broad spectrum of pathogenic bacteria in their planktonic form, including pathogenic multi-drug-resistant species and clinical wound isolates that have shown high levels of antiseptic tolerance

in vivo [116–121]. In addition, this dressing has shown some *in vitro* anti-biofilm activity in simple models [90, 91]. Using the MBEC method [122] the ability of combinations of anti-biofilm agents, surfactants and ionic silver to eliminate mature *P. aeruginosa* biofilm after 30 minutes contact was investigated. Component concentrations were varied, as was pH, and in all over 60000 tests were performed. Very few combinations proved to be beneficial, but a number of strong synergistic effects were identified, in particular between ionic silver, quaternary ammonium surfactants and metal-chelating agents (especially EDTA), all at a slightly acidic pH [123]. These synergistic components (termed *Ag+ Technology*) were incorporated into the dressing and subjected to extensive safety testing. We believe that the resultant dressing, *AQUACEL® Ag+ Extra™*, is the first commercially available wound dressing specifically designed to manage biofilm.

In the laboratory *AQUACEL Ag+ Extra* dressings have demonstrated effectiveness against biofilm microorganisms in sophisticated *in vitro* wound models. Here, thick biofilms of multi-drug-resistant *S. aureus* or *P. aeruginosa* were grown on cotton gauze substrates, and placed on to a simulated wound bed of nutrient agar, within model peri-wound skin [124] (**Table 5**). Further studies using isothermal microcalorimetry demonstrated how neither the standard silver dressing alone (*AQUACEL Ag*) nor silver nitrate solution showed a marked anti-biofilm activity, while the *AQUACEL Ag+ Extra* dressing eradicated the *S. aureus* biofilm *in vitro* [125]. Interestingly, in this study the anti-biofilm agents alone, without silver, were also shown to be ineffective unless combined with silver, demonstrating the synergistic nature of this anti-biofilm formulation (**Table 5**).

The efficacy of this combination of ionic silver, metal chelator and surfactant has also been demonstrated in an FDA-recognised *in vivo* model of wound healing [30]. Here, the controlled formation of *P. aeruginosa* biofilm and polymicrobial biofilm (*P. aeruginosa* and *S. aureus*) in an acute wound of defined size, and its subsequent treatment, was assessed over time by measuring parameters such as viable biofilm counts, granulation tissue formation and epithelialisation. The anti-biofilm dressing was found to be significantly superior to a PHMB-containing dressing in improving these wound parameters [34] (**Table 5**).

Most encouraging is the early clinical performance data emerging for this new anti-biofilm technology. Harding *et al.* [126] demonstrated the safety and effectiveness of this dressing containing *Ag+ Technology* in a 42-patient study in VLU patients. In particular, the authors highlighted a subset of 10 clinically-infected wounds (where biofilm was assumed to be a problem) that responded in a more dramatic fashion (**Table 5**). European and Canadian clinical evaluations summarised 113 cases which were selected on the basis of being difficult-to-heal wounds, with suspected involvement of infection or biofilm. Following an average of 4.1 weeks of use of the new dressing in otherwise standard wound care protocols, an average wound closure of 73% was achieved, with 17% of wounds healing completely [127] (**Table 5**). More detailed individual case studies from these evaluations have also been presented [128]. In more recent UK-based evaluations of *AQUACEL Ag+ EXTRA*, a 29-case evaluation reported reductions in all described signs of clinical infection, including a reduction in suspected biofilm from 76 to 45%. This was accompanied by an average wound closure of 62%, with 34% of

wounds fully healing after an average of 5.4 weeks of dressing use [129] (**Table 5**). Finally, a 112-patient post-market surveillance study further demonstrated the safety and effectiveness of this dressing, shifting stagnant or deteriorating wounds, that had previously been managed with a large variety of standard antimicrobial products, onto healing trajectories [130] (**Table 5**).

Study type	Summary of results	Ref.
<i>In vitro</i>	Assessed quantitatively by viable counts & confocal scanning laser microscopy, <i>AQUACEL Ag+ Extra</i> gave 6 log ₁₀ kill of <i>P. aeruginosa</i> and CA-MRSA biofilm after 4 & 5 days; standard <i>AQUACEL Ag</i> dressing did not fully eradicate either biofilm	124
	Dressing & silver nitrate+EDTA+BC eradicated <i>S. aureus</i> biofilm; silver CMC dressing, CMC dressing, EDTA+BC, & silver nitrate alone did not eradicate biofilm; demonstrating synergy of silver with metal chelator & surfactant	125
<i>In vivo</i>	The new dressing technology gave 2 log ₁₀ reductions in <i>P. aeruginosa</i> or polymicrobial biofilm after 4 & 6 days compared to PHMB gauze & CMC dressings; granulation tissue formation & epithelialisation significantly better after new dressing	34
Clinical study	An acceptable safety profile was demonstrated; after 4 weeks of the new dressing then 4 weeks CMC 12% of wounds healed, 76% showed improvement; mean ulcer size reduction 55%; subset of 10 infected wounds reduced in area by 70%	126
Clinical evaluation	The new dressing resulted in an average wound closure of 73% after average of 4.1 weeks of use in 127 cases; 17% of wounds healed completely	127
	62% average wound closure after 5.4 weeks of <i>AQUACEL Ag+ Extra</i> dressing use; 34% of wounds healed completely; exudate & signs of suspected biofilm & infection reduced in 29-case evaluation	129
	Safety & effectiveness demonstrated in 112-case evaluation; suspected biofilm coverage of wound reduced; 13 of wounds healed completely, 65% improved after 3.9 weeks of <i>AQUACEL Ag+ Extra</i> dressing use	130

Table 5. Evidence for a dressing designed specifically to manage exudate, infection and biofilm.

3. Conclusions and future perspectives

Biofilm is increasingly accepted as an integral component of wound recalcitrance and infection, and is likely a key reason for the frequent failure of antibiotics and antiseptics in wound healing. Strategies for combating wound biofilm are currently limited and non-specific physical debridement techniques—from physical removal with absorbent dressings, pads and wipes, to sharp and surgical tissue removal—remain the most effective approach. Despite the limited available anti-biofilm wound strategies, efforts are in progress to develop durable medical devices and wound dressings that combine anti-biofilm and antimicrobial activity. To-date and to our knowledge, only one dressing has been designed to combat biofilm (**Figure 3**), and there is a growing body of evidence demonstrating the exceptional clinical

effectiveness of this dressing (*AQUACEL Ag+ Extra*) [126–130]. It is likely that future efforts will continue to investigate combination technologies that will disrupt biofilm to enhance the antimicrobial efficacy of antiseptics and antibiotics. Certainly, eradication of wound biofilm is critical to promotion of healing and hence improving the lives of patients debilitated by wound recalcitrance.

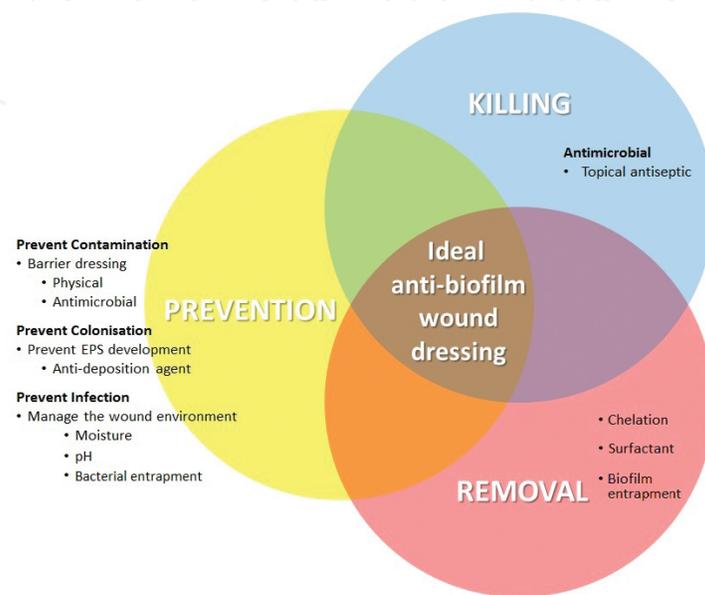


Figure 3. The ideal anti-biofilm wound dressing—prevention, removal, killing.

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