

# Chronic wound biofilms: diagnosis and therapeutic strategies

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## Abstract

**Objective:** To review the diagnosis of chronic wound biofilms and discuss current treatment approaches.

**Data sources:** Articles included in this review were obtained from the following databases: Wanfang, China National Knowledge Infrastructure, PubMed, and the Web of Science. We focused on research published before August 2019 with keywords including chronic wound, biofilm, bacterial biofilms, and chronic wound infection.

**Study selection:** Relevant articles were selected by carefully reading the titles and abstracts. Further, different diagnosis and clinical treatment methods for chronic wound biofilm were compared and summarized from the selected published articles.

**Results:** Recent guidelines on medical biofilms stated that approaches such as the use of scanning electron microscopy and confocal laser scanning microscopy are the most reliable types of diagnostic techniques. Further, therapeutic strategies include debridement, negative pressure wound therapy, ultrasound, antibiotic, silver-containing dressing, hyperbaric oxygen therapy, and others.

**Conclusion:** This review provides the identification and management of biofilms, and it can be used as a tool by clinicians for a better understanding of biofilms and translating research to develop best clinical practices.

**Keywords:** Biofilm; Chronic wound; Diagnosis; Therapy

## Introduction

A wound is termed chronic when it cannot achieve anatomical and functional integrities through normal, orderly, and timely repair processes under the influence of various internal or external factors.<sup>[1]</sup> Wounds are injuries that have not healed and have no tendency to heal after more than one month of treatment.<sup>[2]</sup> A bacterial biofilm (BBF) in a chronic wound is a membranous tissue formed by bacteria attached to the wound bed and fused with extracellular matrix (ECM) secreted by the film.<sup>[3]</sup> It is composed of bacteria and their products, ECM, necrotic tissue, and so on.<sup>[2]</sup> Clinics are more common in pressure ulcer, diabetic foot ulcer, lower extremity arteriovenous ulcer, and other chronic wounds.<sup>[4,5]</sup> For example, the annual incidence of foot ulcers in diabetic patients is 1% to 4% in the United States, with a lifetime risk of occurrence between 15% and 25%.<sup>[3]</sup> In 2006, the cost of the treatment, amputation, rehabilitation, and long-term care of diabetic foot ulcers in the United States totaled \$10.9 billion<sup>[6]</sup>; approximately 85% of amputations are preceded by this types of ulcers. These figures will increase as the number of diabetes diagnoses is expected to rise.

Pressure/decubitus ulcers are a common problem in nursing homes, rehabilitation clinics, and home care patients. Further, venous leg ulcers affect 1% of the worldwide population.<sup>[3]</sup> Surgical site infections occur in 5% of procedures and are an increasingly common type of post-operative complication; an average of 0.5% of the total hospital budget in the United States is allocated to manage these infections in affected patients.<sup>[7]</sup> The science of how a wound heals is fascinating, and new discoveries clarifying the mechanisms of physiologic wound repair are constantly being reported. In recent years, the role of BBFs in the formation of chronic wounds has attracted increased attention. The BBF may be an important factor that impairs the healing of chronic wounds.<sup>[8]</sup> In this review, the basic concepts of BBF will be discussed, with a focus on current practices in the treatment of chronic wounds and future directions in wound care.

## Definition and Structural Characteristics of BBF

In 1978, the Canadian scholar Costerton first proposed the concept of “biofilm.” Thereafter, scientists used this

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concept to describe microbial colonies embedded in an extracellular polymeric matrix secreted by themselves. A BBF has a growth pattern corresponding to plankton cells formed on the surface of inert or active materials, and they adapt to the living environment during the growth of the bacteria. Its structure includes bacteria and extracellular polymeric substance (EPS) secreted by themselves.<sup>[9]</sup> This phenotype is the best condition for bacteria to inhabit, and is different from free bacteria that have been widely studied in the laboratory. The main components of a BBF include proteins, polysaccharides, extracellular DNA (eDNA), water, and so on. The formation of a biofilm is a dynamic process; it has been found that bacteria can form a mature biofilm on a wound within 24 h.<sup>[10]</sup> The formation of a BBF includes four stages.<sup>[5,11-13]</sup> (1) Adhesion: the wound bed contains organic or inorganic nutrients on which bacteria get attached, and most are implanted biomaterials and their own tissue lesions. (2) Reproduction: when bacteria are adhered to the wound surface, they initiate gene expression, secrete a large number of EPS, attract each other to form a microbial colony, and thereafter form a mushroom structure. (3) Maturation: bacteria are buried deep in the matrix and become mature biofilms. (4) Shedding: when the biofilm matures, a small cluster of bacterial cells separate from the biofilm, spread to other environments, and cause infections; thus, chronic wound infection occurs repeatedly in clinics. Further, recent studies have found biofilms resistant to anti-microbial agents; biofilms may be 1000 times more resistant to anti-microbial agents than ordinary-free organisms.<sup>[14]</sup> The eDNA in biofilm plays an important role in drug resistance. Chiang *et al* found that the protective effect of eDNA makes *Pseudomonas aeruginosa* resistant to aminoglycoside drugs.<sup>[15]</sup> Evidence showed that eDNA had anti-microbial activity; chelating cations could make the cells split, stabilize the lipopolysaccharide and outer membrane of bacteria.<sup>[16]</sup> By adding DNA lyase to the biofilm formation process, it was found that DNA lyase could not act on the mature biofilm or mucinous biofilm of *P. aeruginosa*.<sup>[17]</sup> eDNA is an important component of biofilm matrix in both Gram-negative or Gram-positive bacteria.<sup>[18]</sup> The production of eDNA is related to quorum sensing (QS) in the wild-type biofilm of *P. aeruginosa*, and the regulation of QS system can lead to cell cleavage and provide eDNA for the biofilm.<sup>[15]</sup>

### Clinical Diagnosis of BBF in Chronic Wound

It is unlikely that bacterial aggregates in biofilms in the wound can be visualized with the naked eye because they are often less than 100  $\mu\text{m}$  in size and lack macroscopically distinguishable features.<sup>[19]</sup> Clinical workers usually need to use a bacterial culture to detect bacteria in the wound; however, the diagnosis of chronic infection caused by BBF lacks accuracy. Currently, there is no specific clinical manifestation for the diagnosis of biofilm.<sup>[20]</sup> Previous studies have shown that the clinical symptoms of BBF that colonize wounds are similar to those of chronic infection wounds, such as pale wound bed, yellow exudate, necrotic tissue, and clear tissue fluid.<sup>[21]</sup> Some scholars have used granulation tissue morphology and color as the criteria for identifying BBF.<sup>[22]</sup> Bacterial species and distribution in the biofilm of the wound were summarized and used as one of

the diagnostic criteria by extracting the specimens of different kinds of chronic wounds.<sup>[23]</sup> In 2003, Parsek *et al*<sup>[24]</sup> put forward the diagnostic criteria of Parsek-Singh experiment: (1) the relationship between the bacterial infection and wound surface tissue; (2) the pathological examination of the wound tissue, which showed that bacteria were gathered and encapsulated by a matrix; (3) infection in local tissues, with or without systemic infection; and (4) the resistance of bacteria to conventional antibiotics. In 2012, a World Biofilm Seminar summarized the clinical diagnostic criteria of a biofilm infection: (1) pale and edema wound bed; (2) a fragile granulation tissue; (3) large amount of yellow exudate; (4) necrotic and rotting tissue; (5) wound pain; and (6) pungent smell.<sup>[25]</sup> This criteria was updated in 2017, which included: (1) recalcitrant to treatment with antibiotics or antiseptics; (2) treatment failure despite using appropriate antibiotics or antiseptics; (3) delayed healing; (4) cycles of recurrent infection/exacerbation; (5) excessive moisture and wound exudate; (6) low-level chronic inflammation; and (7) low-level erythema.<sup>[26]</sup> Recent guidelines on medical biofilms by the European Society of Clinical Microbiology and Infectious Diseases study group for biofilms stated that approaches such as the use of scanning electron microscopy (SEM) and confocal laser scanning microscopy are the most reliable types of diagnostic techniques. The SEM technique can identify biofilms in wounds that do not show any evidence of acute infection.<sup>[27]</sup> However, these imaging techniques are highly specialized and not practical in a typical clinical setting.<sup>[28]</sup> To improve the accuracy and scientific nature of the clinical diagnosis of BBF, some new methods have been developed, such as polymerase chain reactions, fluorescence *in situ* hybridization, and denaturing gradient gel electrophoresis.

### Therapeutic Strategies

Wound debridement is the first key step in the removal of BBF. Sharp debridement is commonly used in clinical practice to remove inactivated tissue, slough and necrotic tissue, foreign bodies, and poor healing tissues, which provide an attachment point for bacterial colonization and biofilm formation; thus, it is important to remove necrotic tissue and foreign bodies in time.<sup>[29]</sup> Late debridement and residues of necrotic tissues and foreign bodies can lead to bacterial colonization of *Staphylococcus aureus* and *P. aeruginosa*, which can cause secondary infections. To improve patient tolerance to debridement, painless debridement has gained considerable attention.<sup>[30]</sup> Hydro-surgical debridement is a painless debridement technique developed recently, and its basic principle is the application of precisely controlled ultrasonic fine water flow to remove carrion, tissue fragments, colonies, and so on from the wound bed based on liquid jet technology, while keeping the wound bed clean and moist. Caputo *et al*<sup>[31]</sup> used this technique to debride wounds in 22 patients with chronic leg ulcer; 19 patients with similar ulcers were treated with surgical debridement as the control group. Results showed that ultrasonic atomization of water flow debridement was quicker than that of surgical debridement. In addition, the use of gauze, physiological saline, and other materials was reduced, in addition to the decrease in the pain. Therefore, ultrasonic atomization technology can improve the

effectiveness and safety of debridement. In future applications, it is necessary to explore its characteristics, operating methods, and cost-effectiveness, to popularize this technique.

Negative pressure wound therapy (NPWT) has been a widely used method for wound treatment in the last 20 years.<sup>[32,33]</sup> This therapy can improve local blood flow, reduce tissue edema, promote the growth of granulation tissue, and effectively reduce the number of bacteria.<sup>[34]</sup> In 2012, Ngo *et al*<sup>[35]</sup> first reported that the number of bacteria in BBF significantly decreased after treatment using negative pressure combined with silver foam for 2 weeks, by establishing an *in vitro* model of *P. aeruginosa* biofilm. It is speculated that the micromorphology of the wound tissue caused by negative pressure may destroy the original thickness structure of BBF, control the spread of bacteria in the membrane, and thus effectively reduce the wound infection. Further, Phillips *et al* used an infected pig-skin biofilm as a research object.<sup>[36]</sup> It was found that NPWT combined with different flushing solutions can effectively remove bacteria in the biofilm in the wound. In 2016, Wang *et al*<sup>[37]</sup> used concanavalin A staining *in vitro* and found that negative pressure environment could reduce biofilm formation compared with normal pressure environment based on observations through a fluorescence microscope. In the model of a rabbit ear biofilm infection, the early treatment of *S. aureus* infection with NPWT could effectively inhibit the formation of the biofilm; however, it could not clear the mature biofilm.<sup>[13]</sup> In addition, in this *in vitro* experiment, the authors found that a negative pressure environment can reduce the total amount of eDNA in the *S. aureus* biofilm. Further, as eDNA plays an important role in bacterial drug resistance, it is suggested that negative pressure may play a role in reducing bacterial drug resistance. However, there is a lack of research and direct evidence in this area. Negative pressure wound therapy instillation is an improvement on NPWT and one of the treatment methods for biofilms.<sup>[13]</sup> Phillips *et al*<sup>[36]</sup> found that flushing NPWT-binding active anti-bacterial substances could enhance the bacterial clearance of the wound by NPWT and destroy the BBF effectively.

Ultrasound can destroy and remove biofilms via electron-hole pairs and foaming. Nursing staff certified to perform wound treatments can administer ultrasonic treatment independently after training. The effect of ultrasound combined with antibiotics on micro-organisms was studied by Teresa *et al*<sup>[38]</sup> It was found that ultrasound could significantly enhance the bactericidal efficacy of gentamicin against *P. aeruginosa* and *Escherichia coli*. Zhu *et al*<sup>[39]</sup> found that when the parameters of high intensity focused ultrasound were set to a focal length of 150 mm and an output frequency of 40 W, linear scanning radiation, scanning speed of 3 mm/s, scanning length of 10 mm, and scanning interval of 5 mm, it can kill *P. aeruginosa* and destroy its biofilm structure to a certain extent. Ultrasound, as a physical cleaning method, has been well developed in clinical wound nursing in recent years.

Antibiotic treatment of wounds has always been controversial; generally, only when the wound is accompanied by

inflammatory reactions such as redness, swelling, heat, pain, or symptoms of bacteremia, and whole-body anti-bacterial treatment is considered.<sup>[26]</sup> For chronic wounds with BBF but no symptoms of infection, the efficacy of systemic anti-bacterial therapy was reduced by 25% to 30%. The drug resistance of bacteria after biofilm formation can increase to 1000 to 1500 times of that in the free state,<sup>[40]</sup> and improper use of antibiotics can promote membrane formation.<sup>[41]</sup> Antibiotic resistance of micro-organisms within a biofilm can have a significant influence on wound healing in mammalian medicine. When wound isolates are grown in the biofilm phenotypic state, they exhibit enhanced tolerance to antibiotics. This tolerance of a biofilm occurs through phenotypic rather than genotypic changes. Many studies have reported the evidence of antibiotic-resistant isolates in biofilms, in particular methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus*, and multi-drug resistant *Acinetobacter baumannii*,<sup>[22,42]</sup> and; therefore, it is suggested that antibiotics should be used in combination with other antibiotics. Meanwhile, antibiotics should be used according to the structural characteristics of BBF.<sup>[43]</sup> For example, fluoroquinolones have the strongest scavenging effect on biofilms, but imipenem and ceftazidime have a weaker effect. Macrolides have the strongest penetrating effect on the bacterial extracellular polysaccharide matrix, while fluoroquinolones and  $\beta$ -lactams are the second, with aminoglycosides being the weakest.<sup>[44]</sup> At present, the combination of traditional antibiotics and specific anti-BBF agents against BBF is the research focus, and it includes using the combination of linazolamine and acetylcysteine. Acetylcysteine can degrade extracellular polysaccharides and destroy bacterial adhesion; therefore, it can inhibit the formation of BBF. Further, the combination of the two can play a synergistic effect, effectively reduce the formation of BBF in *Staphylococcus epidermidis*.<sup>[45]</sup> Although new anti-bacterial agents are being developed globally, there are many research studies on single active ingredients, lacking clinical trials and comprehensive pharmacokinetic analysis, and this is expected to become another research hotspot to conquer BBF in the future.

Nanoparticles are versatile and bioactive, and they are becoming increasingly popular for use as a biofilm-targeting approach. Nanoparticles with intrinsic antimicrobial activity, primarily inorganic materials such as silver, can act as biofilm-targeting agents or as nano-coatings. Owing to their flexible chemical structures, they can also function as drug delivery vehicles (nanocarriers) with organic nanoparticles, accounting for over two-thirds of the systems approved for use in humans. Further, both inorganic and organic nanoparticles can be combined or modified by adding molecules (hybrid nanoparticles) to enhance their biological properties or provide multifunctionality. Excellent in-depth reviews on the principles and current applications of nanoparticles, particularly silver, are available.<sup>[9]</sup> Silver-containing dressing is recognized as a broad-spectrum anti-bacterial dressing. Silver ion dressing is the first choice in the treatment of BBF wounds. When the concentration of silver ion is as high as 5 to 10 g/mL, 90% of the bacteria in the wound BBF could be cleared within 24 h and 100% within 48 h.<sup>[46]</sup> The silver

ions can prevent various micro-organisms including bacteria and fungi from competing with the host cell for oxygen and nutrients, inhibit the production of the metabolic toxin, reduce the expression of the growth factor and the local anti-inflammatory effect, and effectively control the growth of the micro-organisms in the wound environment<sup>[47]</sup> thereby significantly improving the healing of the wound.

Honey has a very high osmotic pressure and low pH value, and it contains hydrogen peroxide and acetone aldehyde and other bactericidal components; it can reduce bacterial adhesion, inhibit biofilm formation, interfere with QS, hinder the formation of early biofilm structure, and remove or destroy established biofilms.<sup>[48]</sup> According to the guidelines of the European Wound Management Association, clinical wound nurses can choose different types and concentrations of honey according to the type of bacteria and the stage in which they are located, to perform effective clinical nursing care of the wound.<sup>[49]</sup> Maddocks *et al*<sup>[50]</sup> found that honey inhibited the specific adhesion of *S. aureus*, *P. aeruginosa*, and *Streptococcus pyogenes* to fibronectin, fibrinogen, and collagen, respectively, and prevented it from attaching to human keratinocytes. Meluca honey at 8% concentration inhibited 95% of the biofilm formation of *S. aureus*, which could reach 97% if the concentration reached 10%, while only 50% of the biofilm formation was inhibited by artificial honey. The minimum inhibitory concentrations of Meluca honey on *S. aureus*, *P. aeruginosa*, and *S. pyogenes* biofilms were 16%, 50%, and 30%, respectively.

Traditional Chinese medicine (TCM) has a long history in the treatment of chronic wounds and has unique advantages in the prevention and treatment of BBF infection. Wound nurses should actively learn TCM anti-bacterial therapy in clinics, understand the advantages of TCM anti-bacterial therapy, and actively organize multi-disciplinary joint diagnosis and treatment in clinical nursing, for example, carry out joint wound treatment with the TCM department.<sup>[39]</sup> The advantages of TCM should be integrated into the clinical nursing of wound. Study by Gong *et al*<sup>[51]</sup> showed that the oral decoction of peony bark (125 mg/L) and ginger (250 mg/L) could inhibit *Candida albicans* biofilms to a certain extent. It was found that a wet compress of gallnut ethanol extract had a scavenging effect on *P. aeruginosa* biofilms. The minimum inhibitory concentration (MIC) was 19.5 µg/mL, and two times ethanol extract of gallnut had a complete scavenging effect on the *P. aeruginosa* biofilm.<sup>[52]</sup> Chen *et al*<sup>[53]</sup> found that andrographolide with the concentration of 30 µg/mL could interfere with the bacterial aggregation of *P. aeruginosa* wild strain, reduce the adhesion force of *P. aeruginosa* and destroy the biofilm structure by wet dressing or lavage within 72 h.

Maggot debridement therapy refers to the use of sterile medical maggots to nibble away necrotic tissue and bacteria that hinder wound healing, reduce inflammation, and promote tissue regeneration.<sup>[54]</sup> The advantage of this therapy is that the debridement using maggots does not affect the healthy tissue around the wound. Maggots can enter deep wounds and pathogens that are difficult to reach

by surgery, such as lurking pathogens and sinuses, which can easily form BBF because of anaerobic bacteria. The excrement or secretion of maggots after ingesting rotten meat contains unique collagenase, trypsin, chymotrypsin, and anti-bacterial phthalein, which decomposes necrotic tissue into semi-liquid foam, and then it is digested to degrade the bacteria.<sup>[55]</sup> An aseptic maggot is used to remove Gram-positive bacteria biofilms, but bacteria-pre-treated maggots fed with many kinds of bacteria can inhibit Gram-negative bacteria biofilms more effectively than the aseptic maggot, such as *P. aeruginosa* and others.<sup>[56]</sup> The debridement of maggots in the future may be a promising method for the removal of biofilms; however, the mechanism needs to be further studied to better grasp the application methods and timing in clinical practice.

Some metal ions have a certain bactericidal ability. In addition to the extensive use of silver ions in the management of infected wounds, transition metal gallium has recently been found to inhibit and kill *P. aeruginosa* in zooplankton and BBF.<sup>[57]</sup> In the model of pulmonary infection in rats, early gallium therapy can reduce the number of bacteria by about 1000 times, which indicates that gallium has good potential in treating both acute and chronic infections.<sup>[39,58,59]</sup>

### Phage therapy

Phages are found in abundance and can be isolated from a wide range of environments. They are usually specific to narrow host ranges, and due to their self-replication, a low dosage is sufficient. Their high mutation rate helps them to adapt as the host bacteria undergoes genetic alterations to survive in a given environment. Phages have been effective in eradicating biofilms of single or mixed bacterial species and can lyse a biofilm grown on a chronic wound.<sup>[60]</sup>

### Lactoferrin

It is an important non-heme iron-binding glycoprotein in milk, and its anti-bacterial activity is the most remarkable. Lactoferrin can inhibit and kill many micro-organisms, including Gram-positive, Gram-negative aerobes, anaerobes, and some fungi.<sup>[59]</sup> *In vitro* experiments showed that through adhesion and decomposing the extracellular polysaccharide of BBF, lactoferrin accelerates infiltration into the membrane to kill bacteria.<sup>[61]</sup>

### Extracellular polymeric substance

Treatment for the composition and structure of EPS has been a popular therapy in recent years. Extracellular polysaccharide degrading enzymes are typical examples, such as the glucose hydrolase (glucanase and insoluble glucanase), dispersin B, which can destroy the matrix of pathogenic biofilms in the oral cavity. Glycoside hydrolases are used to degrade biofilms on wounds infected with mixed bacteria (*S. aureus* and *P. aeruginosa*).<sup>[62-64]</sup> Lysozyme (bacteriophage-encoded peptidoglycan hydrolases) can destroy bacteria in biofilms by degrading peptidoglycan in the bacterial cell wall.<sup>[65]</sup> The engineered peptidoglycan hydrolases can bind to different anti-

bacterial substances, and then cleave bacteria by binding to different binding sites of peptidoglycan. This method has been applied specifically to *S. aureus*.<sup>[66]</sup> It has been proved to be effective in killing bacteria and removing biofilms.

### Cationic anti-microbial peptides

These peptides are a new family of anti-bacterial peptides found in recent years. It is a low molecular cationic peptide rich in arginine, which is widely distributed in animals, plants, and insects.<sup>[67]</sup> It has high efficiency and broad spectrum anti-bacterial activity, and does not cause drug resistance and adverse reactions like other antibiotics; thus, it has good application prospects. It has been reported that RNA III inhibitory peptide is very effective in the treatment of severe microbial infections, including highly resistant bacteria such as methicillin-resistant *S. aureus*.<sup>[68]</sup>

### QS system

Because a quorum-sensing signal system plays a central role in regulating bacterial pathogenic factors, researchers assume that it may be a new target for the control of infectious diseases.<sup>[59]</sup> It is hoped that it can inhibit the expression of pathogenic factors to achieve a therapeutic purpose.<sup>[69]</sup> Therefore, signal molecular inhibitors in the QS system have been paid increasing attention in biomembrane therapy. For example, an *in vitro* study found that QS system autoinducer inhibitor can effectively inhibit bacterial adhesion and dissemination.<sup>[70]</sup> Quorum-sensing inhibitor type I autoinducing peptide can dissolve methicillin-resistant *S. aureus*, which is clustered on the surface of titanium, making it more sensitive to rifampicin and levofloxacin.

### Phytochemicals

Plant-based chemicals called phytochemicals are being increasingly explored as possible anti-therapeutic agents as they can kill micro-organisms with diverse mechanisms of action with a minimal chance for bacteria to develop resistance. Phytochemicals such as 7-hydroxycoumarin (7-HC), indole-3-carbinol (I3C), salicylic acid, and saponin have shown inhibitory activity against the planktonic culture of *E. coli* and *S. aureus*, and they were also able to restrict the growth of the biofilm partially. The phytochemicals I3C and 7-HC had a more pronounced effect on QS inhibition and bacterial motility for both *E. coli* and *S. aureus*.<sup>[71]</sup>

Hyperbaric oxygen therapy (HBOT) uses 100% oxygen at pressures greater than atmospheric pressure. HBOT has been successfully used as adjunctive therapy for wound healing.<sup>[72]</sup> It can increase tissue metabolism, which can not only reduce the exudation and edema of damaged tissue, improve the local blood circulation, but also promote the formation of neovascularization, accelerate the establishment of collateral circulation, and accelerate the repair of epithelial tissue.<sup>[73]</sup> Zhang *et al*<sup>[74]</sup> studied the efficacy of hyperbaric oxygen in the treatment of submandibular cellulitis in children, and found that hyperbaric oxygen could promote the absorption of inflammation, accelerate wound healing, reduce the

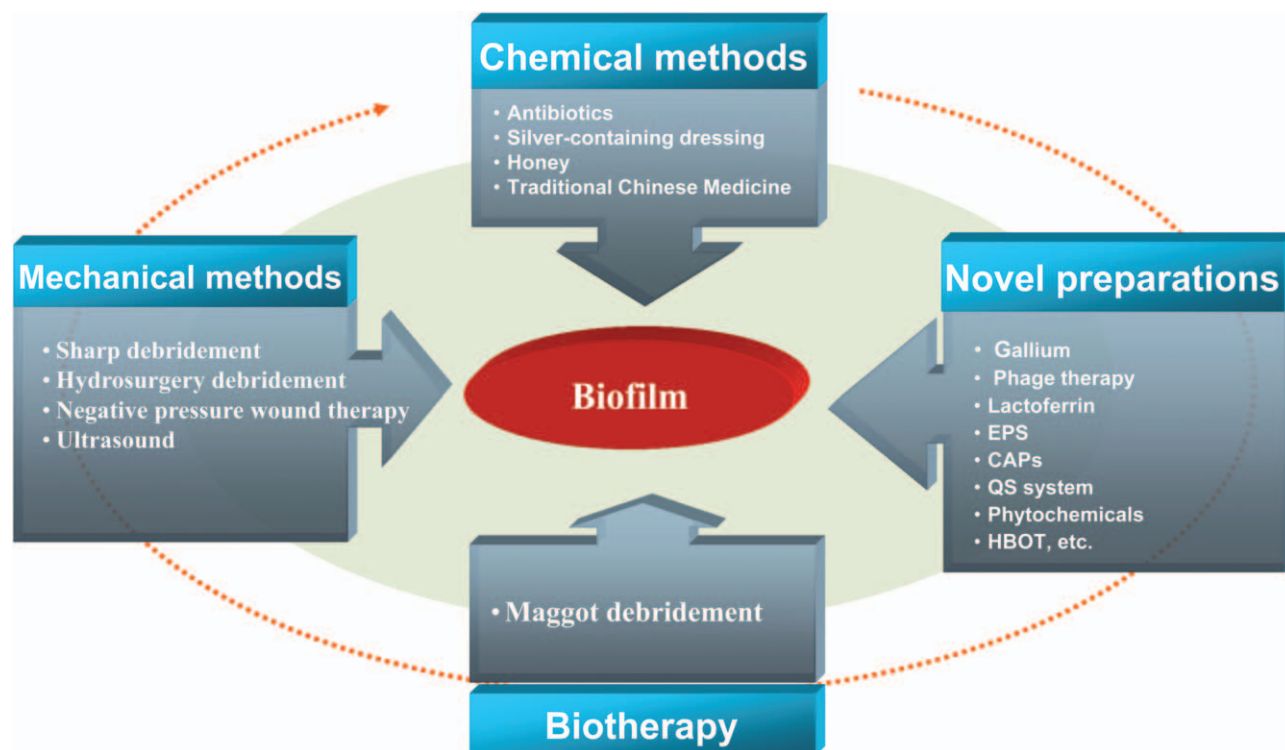
infection rate of post-operative incision, and shorten hospitalization time. Cimsit *et al*<sup>[75]</sup> also suggested that hyperbaric oxygen can effectively control wound infection and accelerate wound healing. However, if hyperbaric oxygen is overused or treated incorrectly, adverse reactions such as barotrauma, oxygen poisoning, and decompression sickness will occur. Therefore, for the application of hyperbaric oxygen in biofilm chronic wounds, it is necessary to follow normal operation, strictly control the oxygen pressure and speed, and so on, to avoid unnecessary injury.

Other methods include scavenging enzyme and anti-oxidant enzymes, including alginase lyase, deoxyribonuclease I, poly-phosphate kinase, and others.<sup>[75]</sup> Natural products such as proanthocyanidins in North American cranberry juice, ursolic acid in black sandalwood, and green tea polyphenols all have a good inhibitory effect on biofilms.<sup>[76]</sup> The type II DNA-binding proteins can destroy the integrity of the eDNA structure.<sup>[77]</sup> Integration host factor with high affinity can specifically bind to nucleic acid protein in biomembranes, and it has been widely used in animal models.<sup>[78,79]</sup> There are also genetic engineering drugs and stem cell therapy.<sup>[80]</sup> Current therapeutic approaches being devised and used in the clinic are shown in Figure 1.

### Future Prospects

This review provides clarity on the identification and management of biofilms, and it can be used as a tool by clinicians seeking to gain a better understanding of biofilms and a way for translating research to best clinical practice. Diagnostic guidelines are essential for evaluating the treatments of BBF; the efficacy of anti-biofilm treatment must indicate a significant reduction in bacteria as an outcome.<sup>[25]</sup> BBFs are difficult to diagnose because cultures are not necessarily an accurate indicator of BBF. Thus, to investigate biofilms *in vivo*, identify an infectious etiology, or evaluate treatments, clear clinical signs, and symptoms of BBF are required.

Currently, researches on biofilms are still in the exploratory stage. With the extensive and in-depth development of related research, people will have a deeper and comprehensive understanding of the chronic recurrent infection caused by biofilms and its drug-resistance mechanism. From as early as 2008, the concept of "biofilm-based wound care," which aims to successfully remove BBF by inhibiting BBF re-formation, led to the improvement of other therapeutic care schemes (such as skin grafting, skin flap, or negative pressure wound treatment), which could change the wound from a difficult state to a treatable state.<sup>[81]</sup> An increasing number of wound care experts believe many factors that delay wound healing, such as diabetes mellitus, endocarditis, periodontitis, osteomyelitis, and other systemic diseases; the use of graft and prosthesis like catheter indwelling, artificial heart valve, and joint replacement; patients with low autoimmune function, systemic malnutrition, and cell dysfunction,<sup>[25]</sup> also increase the risk of BBF formation. Therefore, constantly updating the knowledge of BBF can help identify clinically high-risk patients and treat wounds.



**Figure 1:** Current therapeutic approaches. CAPs: Cationic antimicrobial peptides; EPS: Extracellular polymeric substance; HBOT: Hyperbaric oxygen therapy; QS: Quorum-sensing.

Although successful cases of the clinical removal of BBF have been reported, specific methods and strategies are still in their initial stages. Thus, we predict that future studies will focus on the following: (1) increase in clinical studies on biofilm infections through the combination of clinical and laboratory identification tools to explore the effects of different intervention methods on the removal of biofilm; (2) analysis of the therapeutic target, considering how to exert the synergy and efficiency maximization of combined therapy according to the individual differences of wounds, and summarizing the nursing process of BBF wounds; (3) large-sample randomized controlled trials using active biofilm dressing in clinics and obtaining the best practice evidence; (4) joint diagnosis and treatment of multi-disciplinary medical nursing and emphasizing the importance of wound specialist nursing; and (5) consideration of a drug to penetrate existing biofilms as this feature affects both potential cytotoxicity and anti-bacterial efficacy, and the potential for *de novo* emergence of anti-microbial resistance.

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### Conflicts of interest

None.

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