Nanoparticle-based Therapies for Wound Biofilm Infection: Opportunities and Challenges

Min-Ho Kim

Department of Biological Sciences, Kent State University, Kent, OH 44242, USA

Min-Ho Kim: mkim15@kent.edu

Abstract

Clinical data from human chronic wounds implicates biofilm formation with the onset of wound chronicity. Despite the development of novel antimicrobial agents, the cost and complexity of treating chronic wound infections associated with biofilms remain a serious challenge, which necessitates the development of new and alternative approaches for effective anti-biofilm treatment. Recent advancement in nanotechnology for developing a new class of nanoparticles that exhibit unique chemical and physical properties holds promise for the treatment of biofilm infections. Over the last decade, nanoparticle-based approaches against wound biofilm infection have been directed toward developing nanoparticles with intrinsic antimicrobial properties, utilizing nanoparticles for controlled antimicrobials delivery, and applying nanoparticles for antibacterial hyperthermia therapy. In addition, a strategy to functionalize nanoparticles towards enhanced penetration through the biofilm matrix has been receiving considerable interest recently by means of achieving an efficient targeting to the bacterial cells within biofilm matrix. This review summarizes and highlights the recent development of these nanoparticle-based approaches as potential therapeutics for controlling wound biofilm infection, along with current challenges that need to be overcome for their successful clinical translation.

Keywords

biofilm infection; nanoparticles; extracellular polymeric substances; antimicrobial elivery; hyperthermia; wound healing

1. Introduction

The U.S. Centers for Disease Control and Prevention (CDC) reports that up to 70% of infections in the western world are associated with biofilms. Biofilms are consortia of microbial cells adhered to a surface and embedded in a self-produced extracellular matrix that is primarily composed of extracellular polymeric substances (EPS). The EPS is composed of exopolysaccharides, proteins, dead bacteria, bacterial DNA, and enzymes and acts as a protective barrier against antibiotic penetration and cellular attack by host innate immune cells [1–4]. A critical challenge in treatment of biofilm infection is the alarming rate by which they frequently develop a resistance to traditional antimicrobial therapies as well as to host immune responses [5–7]. Biofilm formation in the cutaneous wounds especially raises challenging problems for the management of wound infection. Indeed, many clinical

data from human chronic wounds implicates the formation of biofilm in the wound as a major mechanism that contributes to the wound chronicity [3, 8–12].

A number of biochemical and biophysical approaches have been studied as potential therapeutic strategies to control wound biofilm formation. Biochemical approaches include the use of quorum sensing inhibitors [13], new classes of antimicrobial peptides [14], and enzymes that dissolve biofilms [15]. Biophysical approaches include the application of infrared and light pulsing [16, 17], direct-current electrical stimulation [18, 19], ultrasound [20, 21], and alternating electric fields [22]. However, many of these approaches have demonstrated a modest antimicrobial efficacy and still have limitations in successfully controlling biofilm infection.

The use of materials at the nanometer or submicron scale has shown to be promising for biomedical applications including diagnosis and therapy. This is attributed to the increased reactivity of nanomaterial due to large surface area to volume ratio, as well as the flexibility in controlling its chemical and physical properties [23]. In view of this, nanoparticle-based approaches have received considerable attention over the last decade as a new therapy for the treatment of wound biofilm infection. Approaches have been directed toward developing a new class of nanoparticles that can confer antimicrobial effects. For example, nanoparticles made of metal or metal oxide can be synthesized to exhibit intrinsic antimicrobial properties. These nanoparticles exhibit antimicrobial mechanisms against bacterial pathogens by disrupting the cell membrane directly or producing free radicals [24]. Properties of certain nanoparticles that enable controlled and sustained delivery of drugs, such as liposomes or polymeric nanoparticles, can be used for controlled delivery of therapeutic dose of antimicrobial drugs to the target site of biofilm infection [25]. The strategy can overcome the current limitations of conventional antibiotic treatments associated with toxicity, inefficient delivery or enzymatic inactivation of drugs in vivo. In addition, new approaches of physically disrupting bacterial cells within biofilms, by applying external energy sources, have been introduced recently. For example, nanoparticles such as gold nanoparticles or magnetic nanoparticles (MNPs, such as γFe_2O_3 maghemite or Fe_3O_4 magnetite nanoparticles) can be excited to generate heat on their surfaces when they receive external energy such as near-infrared (NIR) light [26] or alternate magnetic field (AMF) [27], which can impose an irreversible thermal damage to the target cell when they are properly targeted. These approaches can provide a promising opportunity for treating wound biofilm infections in minimally invasive and on-demand manners. Lastly, a strategy to engineer the size and surface functionality of nanoparticles towards a facilitated penetration through the biofilm matrix can be effective in targeting bacterial cells [28]. This review article highlights recent studies on the application of nanoparticle-based strategies as potential therapeutics for controlling wound biofilm infections.

2. Why biofilm infections are difficult to treat in the cutaneous wound

The deleterious influence of microbial infection on wound healing has been recognized for centuries and has motivated a plethora of approaches to alleviate bacterial burden within a wound site and promote a normal healing. The loss of skin integrity and barrier to cutaneous wounding elicits the exposure of open wounds to bacterial colonization and proliferation.

The pathogens including *Staphylococcus aureus (S. aureus)*, *Pseudomonas aeruginosa (P. aeruginosa)*, *Staphylococcus epidermidis* (*S. epidermidis*) and *Enterococcus spp.* have been identified as predominant ones in skin wound infections in hospitalized patients [29]. Skin wounding and infection triggers a cascade of inflammatory events that lead to rapid recruitment of phagocytes (neutrophils and macrophages) from the blood circulation to the local site of injury [30]. In normal physiological conditions, microbes are successfully cleared by the host's immune system, which leads to a normal wound healing process. However, under conditions associated with immune deficiency or dysregulation, microbes frequently attach to the wound surface and a biofilm then begin to develop with the secretion of EPS [31]. Accumulating evidence supports that the chronicity of non-healing wounds, including venous leg ulcers, pressure ulcers, and diabetic foot ulcers, are associated with increased incidences of biofilm formation [32–35].

A critical challenge in treatment of infections associated with biofilms is that they are resistant to antibiotics and readily evade innate immune attacks by phagocytes. It has been shown that bacteria within biofilms are 10–1000 times less sensitive to treatments with antimicrobial agents compared to planktonic cells [36]. Current topical and systemic antibiotics are minimally effective in the treatment of chronic biofilm infections in wounds [8]. This has been associated with slow or incomplete penetration of antimicrobials to bacterium since the presence of EPS forms protective barrier for the diffusion of antimicrobials [37]. In addition, antimicrobial agents may react chemically with the extracellular components of the biofilm or attach to the anionic polysaccharides without reaching to the target bacterial cells [38] (Figure 1A).

Importantly, the advent of a bacterially secreted EPS may also act as a protective barrier against cellular attacks by phagocytic macrophages and neutrophils [1–4]. It has been well characterized that neutrophils and macrophages play a crucial role in innate immune protection against infectious pathogens [39, 40]; their defense mechanisms are highly effective against planktonic bacteria. However, the formation of biofilm structures provides a shielding mechanism that results in protection from the phagocytic activity of neutrophils and macrophages [10]. In addition, biofilms have the capacity to promote the production of leukocyte-inactivating substances, which results in a phenomenon termed "frustrated phagocytosis" [41] (Figure 1B). The quorum sensing compounds derived from EPS, including alginate and rhamnolipids, have been attributed to the inhibitory capacity as the potential to resist the leukocyte attack [42, 43]. Although the phenomenon of frustrated phagocytosis has been well studied in a model of P. aeruginosa biofilm associated with pulmonary infection, S. aureus biofilm has also shown to utilize a similar mechanism by interfering with macrophage polarization towards enhancing their own survival. The classically activated profile of macrophage (M1) is necessary to confer an appropriate host defense against invading bacterial pathogens by producing pro-inflammatory cytokines. A recent study using a cutaneous biofilm infection with S. aureus revealed that macrophages exhibited limited phagocytosis and killing of bacteria in the presence of biofilm matrix, which was associated with skewing of gene expression patterns from M1 to an alternatively activated phenotype (M2) [44].

Since treatment options for clinicians fighting biofilm infections are limited, there is, therefore, an urgent need for an alternative antimicrobial treatment that efficiently and rapidly disrupts biofilms and is non-toxic to host tissue in infection clearance and wound resolution.

3. Nanoparticle-based strategies for controlling biofilm infections

Recent advances in nanoparticle-based technology provide new and promising opportunities for effectively defending wound infection associated with biofilms. Over the last decade, nanoparticle-based approaches against biofilm infection have been directed towards designing nanoparticles to exhibit specific chemical and physical properties towards anti-biofilm activities. This section is focused on highlighting recent studies on the application of various nanoparticle-based approaches as potential antimicrobial therapeutics for controlling biofilm infection in the cutaneous wounds (Table 1). The schematic on the mechanism of actions for various nanoparticle-based approaches for anti-biofilm activities is depicted in Figure 2. For more detailed understanding on the design and synthesis of these nanoparticles for anti-microbial effects, readers may refer to recently published review articles [24–26, 45–51]

3.1. Nanoparticles as an intrinsic antimicrobial agent

Conventional antimicrobial therapy relies on antibiotics to either kill or interfere with the growth of infectious pathogens. However, the use of antibiotics has been limited by toxicity or allergic reactions to host cells [52]. In addition, the frequent administration of sub-lethal dose of antibiotics has been shown to lead to a multi-drug resistance [53]. The antimicrobial activity of metals or metal oxides has been well reported [54]. As such, nanoparticles made of metals and metal oxides have been employed as topical antimicrobial agents.

Metal nanoparticles such as silver, copper, gold, titanium, and zinc have shown to exhibit an antimicrobial activity against wound biofilm infection. Among them, silver-based nanoparticles have received considerable attentions. The mechanism of the antimicrobial action of silver ions results from their interaction with sulfhydryl groups [55, 56], which interferes with bacterial cell membrane integrity, respiratory chains, enzyme activities, and cell proliferation [57]. Moreover, silver has shown to destabilize the biofilm matrix by compromising intermolecular forces [58, 59]. However, the use of free silver ions in the wound bed resulted in rapid sequestration by proteins and other cellular components, which reduced bioavailability of silver ions and substantially diminished their antibacterial effects [60]. The advantage of using silver-based nanoparticles for the wound biofilm management lies in achieving the prolonged release of silver ions in the target cells, which in turn triggers an antibacterial activity by generation of reactive oxygen species (ROS) [61]. Silver nanoparticles can be synthesized by either chemical method involving the reduction of silver nitrate using a reducing agent or physical method utilizing thermal or electric power energy [62]. The silver nanoparticles were effective in reducing formation of biofilm from both gram-positive and gram-negative bacteria *in vitro* [63]. The study showed that the 24 hours treatment of silver nanoparticles could result in more than 95% inhibition in biofilm formed by *P. aeruginosa* and *S. epidermidis*. The coating of silver nanoparticles on the material

surface could successfully restrict biofilm formation by methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE) isolated from human wounds [64]. However, despite the considerable interest in the use of silver nanoparticles as an antimicrobial agent, their use has been challenged with diminished therapeutic effects for prolonged treatment [65]. This is in line with the reported silver resistance for prolonged treatment in clinical isolates of methicillin-resistant *S. aureus* (MRSA) [66]. Another limitation is that high dose of silver nanoparticles may delay wound repair by triggering a toxic effect on keratinocytes and fibroblasts [67]. The cytotoxic effect of silver nanoparticles has been shown to be dependent on the size, concentration, and the rate of intracellular silver ion release [68]. However, it is interesting to note that biofilm bacteria, which survived the prolonged treatment of silver nanoparticles, were susceptible to antibiotics [65]. This implicates that combined treatment with antibiotics or antimicrobials can be synergistic for enhancing the anti-biofilm efficacy of silver nanoparticles while reducing the cytotoxic effect [69, 70].

Along with well-reported antibacterial activities of metal oxides, there have been considerable interests in developing nanoparticles made of metal oxides including zinc oxide (ZnO), magnesium oxide (MgO), iron oxide (Fe_2O_3), aluminum oxide (Al_2O_3), and copper oxide (CuO). Among them, ZnO nanoparticle has been widely used for the treatment of wound infection [24]. In a rat model of skin wound infection, the delivery of ZnO nanoparticle in combination with β-chitin dressing exhibited an improved wound healing and caused a reduction in the growth of bacteria [71]. Importantly, in a comparative study examining the antimicrobial activity of different types of metal oxide nanoparticles made of ZnO, CuO, and Fe_2O_3, ZnO nanoparticles were shown to exhibit the most antibacterial effect against multiple species of bacteria including gram positive strains of *S. aureus* and *Bacillus subtilis* (*B. subtilis*) and gram negative strains of *E. coli* and *P. aeruginosa* bacteria [72, 73]. It was further revealed that ZnO nanoparticles can confer antimicrobial activities against biofilms formed by *P. aeruginosa* [74, 75] and *S. aureus* [76] as well. Although the exact mechanism of antibacterial activity of ZnO nanoparticle has not been well understood, the generation of hydrogen peroxide [77] and cell membrane damage [78] have been suggested as possible mechanisms of antibacterial activity. However, despite its potent anti-biofilm effect, the use of ZnO nanoparticle for clinical translation may be limited by their cytotoxic effects to human cells. For example, concentrations of ZnO nanoparticle at 10 μg/ml exhibited a substantial decrease in viability of human epithelial cells [79].

3.2. Nanoparticles for controlled delivery of antimicrobial agents

The major limitation of conventional antibiotic or antimicrobial treatment against biofilm infections in non-healing chronic wounds relates to the insufficient delivery of the desired concentration of antibiotics to the target bacterial cells, which is largely associated with the presence of a complex matrix network of biofilms, along with the avascular nature in the chronic wounds [80]. This could not only substantially diminish the therapeutic effect of systemically or topically administered drugs, but also lead to the induction of drug resistance. The presence of EPS forming biofilm can not only limit the diffusion of antimicrobial agents to the individual cells of bacteria, but also result in the binding of soluble antimicrobials to matrix components, which prevents them from reaching target cells

[81]. Over the last few decades, considerable efforts have been directed to achieve controlled and sustained release of drugs by utilizing nanoparticles in pharmaceutical science. The strategy of delivering antimicrobials using nanoparticles offers several advantages that can substantially increase antimicrobial activity compared to the strategy of delivering free drugs only, which include the release of drugs at a sustained and controlled manner, protection of drugs from enzymatic inactivation, and targeted delivery of drugs to the target tissues [82].

3.2.1. Controlled release of antibiotics—Antibiotics that are encapsulated within nanoparticles can efficiently penetrate EPS to reach target cells compared to free drugs, which can facilitate the delivery of a therapeutic dose of antibiotics to the bacterial cells. Many biocompatible and biodegradable nanoparticles have been used as carriers for antibiotics to promote sustained antimicrobial effects. Among them, liposomes and polymeric nanoparticles have been widely used as carriers for antibiotics delivery against biofilms [45]. Liposome nanoparticle is composed of phospholipid bilayer and has been widely used as a drug delivery carrier in many biomedical applications due to its lipid bilayer structure that mimics cell membranes [83]. Liposome nanoparticle can readily fuse with bacterial cell wall and the encapsulated drug can be released to the cell membranes or the inside of the bacteria [84]. Recent studies have demonstrated the enhanced therapeutic efficacy of delivering antibiotics with liposomes to the biofilms compared to the delivery of free antibiotics. For example, the encapsulation of the β-lactam antibiotic, piperacillin, within liposomes could protect the drug from hydrolysis by staphylococcal β-lactamase [85], which was correlated with a higher activity for piperacillin encapsulated-liposomes against *S. aureus* compared to the free piperacillin. In another study, Mugabe et al. [86] demonstrated that the strategy of encapsulating gentamycin within liposomes could result in a significantly higher antimicrobial activity against *P. aeruginosa* biofilms. Importantly, in a mouse model of subcutaneous infection, the delivery of daptomycin encapsulated-liposomes was effective in inhibiting *S. aureus* biofilm growth at the site of infection, which was comparable to the treatment of intravenous injection of daptomycin [87]. However, despite its therapeutic potential as a topical anti-biofilm agent, the use of antibiotics-encapsulated liposome has been challenged by their chemical and physical instability that can lead to drug leakage during the storage [25].

Among polymeric nanoparticles, nanoparticles made of poly(lactic-co-glycolic acid) (PLGA) have been widely studied. The main advantage of PLGA nanoparticle lies in their biocompatibility and biodegradability, as well as flexibility for controlling the release kinetics of loaded drugs by tuning the degradation profile of PLGA [88]. The use of PLGA nanoparticles for the delivery of antibiotics has shown to be effective in treating multiple species strains of bacteria including *P. aeruginosa* [89], *S. aureus* [89], and *E. coli* [90]. In addition, lipid-polymer hybrid nanoparticles that combine the advantages of both liposomes and polymeric nanoparticles have recently emerged as a new class of drug delivery platform [91]. The hybrid nanoparticle is composed of polymeric nanoparticles core surrounded by lipid layers, which combine the advantages of the highly biocompatible characteristic of lipids with the structural stability and controllable biodegradability imparted by polymeric nanoparticles [92]. Although there have been very few studies of using lipid-polymeric hybrid nanoparticles for controllable release of antibiotics [92], the use of hybrid

nanoparticles have been demonstrated to exhibit an enhanced cellular delivery efficacy compared to that obtained from liposomes or polymeric nanoparticles for targeting cancer cells [93]. As such, the hybrid nanoparticles can be an alternative to liposomes or polymeric nanoparticles as an antibiotics delivery platform to treat wound biofilm infections.

Taken together, these studies support that a strategy of loading and delivering antibiotics using nanoparticles has a great therapeutic potential in that desirable doses of antimicrobial agents can be delivered directly into the bacteria, which will overcome current limitations in conventional antibiotic treatment involving low water-solubility, cytotoxicity to healthy tissues, and rapid degradation in the tissue [25, 45]. However, despite its potential for anti-biofilm activity, only few nanoparticle-based antimicrobial agents have been approved for clinical use, which appears to be associated with high cost and inefficient drug loading [94]. Achieving the higher encapsulation efficiency of antibiotics in the nanoparticles may reduce potential toxicity issues that might result from the use of high concentrations of nanoparticles. In addition, premature drug release from the antibiotic-loaded nanoparticles remains another challenge and a novel method to achieve a site specific release of antibiotics, for example by developing infectious environment-sensitive nanoparticles, will be beneficial [45].

3.2.2. Controlled release of nitric oxide—Nitric oxide (NO) has been well reported to exhibit an broad-spectrum of antimicrobial activity by interfering the process of DNA replication and respiration in bacteria [95]. More importantly, NO was shown to be effective in dispersing the formation of biofilm [96]. However, the use of NO as a therapeutic agent has been mainly challenged due to its short half-life and instability in the tissue *in vivo*. In view of this, a strategy for utilizing nanoparticles as a vehicle for sustained and controlled release of NO has been proposed for the antimicrobial treatment of wound infections. The treatment of NO-releasing silica nanoparticles could demonstrate an enhanced bactericidal efficacy against planktonic *P. aeruginosa* cells compared to the treatment of small molecule NO donors [97]. In another study, it was further demonstrated that NO-releasing silica nanoparticles are effective in killing bacterial cells within established biofilms [98]. The therapeutic efficacy of NO-releasing nanoparticles was further demonstrated from *in vivo* studies using murine models of wound infection, in which NO-releasing nanoparticles made of silane hydrogels were effective in reducing bacterial burden in wounds infected with MRSA [99], *Acinetobacter baumannii* [100], and *Candida albicans* (*C. albicans*) [101]. Another advantage for NO-releasing nanoparticles is that it can potentially accelerate wound healing not only by conferring bactericidal activity, but also by promoting angiogenesis and tissue remodeling in the wound bed [102]. Taken together, these studies support that NO-releasing nanoparticles can be a potent antimicrobial therapeutic for topical treatment in cutaneous wound infection. However, a critical challenge for NO-based therapy for wound healing has been associated with maintaining an optimal concentration of NO in the wound bed since either too high or too low levels of NO may hinder the normal process of wound healing [103, 104]. Thus, important design criteria for NO-releasing nanoparticles should ensure the release kinetics of physiologically desirable concentration of NO is precisely controlled in a sustained fashion [102].

3.2.3. Controlled delivery of photosensitizer—Photodynamic therapy (PDT) is a therapeutic approach to kill pathogens using a combination of light and photosensitizer (PS) (e.g., phenothiazine dyes, porphyrin, methylene blue, or rose bengals). Recently, there has been increasing interests in applying PDT as a treatment for various types of localized infections [105]. The application of PDT against wound infection is based on the mechanism that activation of PS by the exposure of light of the appropriate wavelength can trigger the transfer of energy to molecular oxygen, which generates cytotoxic ROS that triggers a necrotic cell death [106]. Although planktonic bacteria were susceptible to PDT, the extent of antimicrobial activity by PDT was shown to be substantially reduced in bacteria in biofilm [107]. One mechanism responsible for the reduced susceptibility of biofilms to PDT was attributed to the failure of PS drug penetration. The strategy for encapsulating PS agents within nanoparticles confers advantages by preventing potential inactivation of the drugs by EPS matrix, over treatment of free photosensitizing molecules. In addition, the characteristic of large surface to volume ratio of nanoparticles can increase the amount of PS that can be delivered to the target cells [108]. Nanoscale carriers including liposomes and biodegradable polymeric nanoparticles have been widely proposed as PS delivery vehicles for PDT [108]. The controlled delivery of PS, porphyrin, by liposome could result in significantly enhanced inactivation of MRSA compared with free dye treatment [109]. Nanoparticles functionalized with methylene blue [110], or rose bengals [111] have demonstrated an efficacy for reducing biofilm formation. However, although the use of PS carrying nanoparticles has a potential for the treatment and management of wound infections, it still has several limitations that need to be overcome for its successful clinical translation. It is necessary to ensure the selectivity of PS carrying-nanoparticles to the target bacterial cells to avoid any potential non-specific PDT damage to the host cells at the site of wound infection [105]. In addition, to be used in the clinical setting, several factors, including the physiochemical properties of the PS, dose of PS to be delivered, and rate of release, and right dosimetry of light, should be carefully understood [108].

3.3. Responsive nanoparticles for anti-bacterial hyperthermia treatment

Approaches of triggering bacterial damage using a combination of externally triggered energy sources and energy absorbing nanoparticles have been developed as new therapeutic options for antimicrobial treatments. The basic principle is to induce irreversible thermal damages to the bacterial cells by activating nanoparticles with externally applied energy source such as NIR light or high frequency AMF. The absorbed energy on nanoparticles can be quickly converted into heat energy, which triggers temperature increase on the surface of nanoparticles. As far as an appropriate targeting strategy is achieved, the nanoparticle-based hyperthermia may hold promise for future clinical application in that the method is non-invasive, tissue-specific, and capable of generating precisely localized heating in the target pathogens for eradication [27].

3.3.1. NIR light-triggered hyperthermia—Nanoparticles made of gold, iron oxide, and graphene have been used as photothermal agents that are responsive to NIR light illumination. Among them, gold nanoparticles have been widely studied for this purpose due to their excellent responsivity to NIR light [26]. Previously, Zharov *et al.* [112] first demonstrated that NIR light-trigged activation of gold nanoparticles was sufficient to

thermally inactivate *S. aureus*, by achieving a high affinity targeting of gold nanoparticles to *S. aureus* by conjugating nanoparticles with anti-protein A antibodies. An alumina-coated iron oxide magnetic nanoparticle was also used as a photothermal agent, which demonstrated to be effective in bacterial killing [113]. The temperature of the nanoparticles suspension under illumination with NIR light increased by 20°C for 5 minutes, which resulted in inhibition of the growth of antibiotic resistant strain of both gram-positive and gram-negative bacteria by 95%. Recently, Wu et al. [114] have used a graphene nanoparticles for NIR light-triggered hyperthermia against *S. aureus* and *E.coli*. They utilized a photothermal property of reduced graphene oxide upon NIR laser irradiation, in which 80 ppm solution of graphene nanoparticles were sufficient in inducing a rapid killing of both gram-positive and gram-negative bacteria by 99%, within 10 minutes upon NIR laser irradiation.

However, since the mechanism of antibacterial activity for NIR light responsive nanoparticles relies on hyperthermia, their use can be limited due to their potential to impose off-target thermal effects to host cells, unless a suitable targeting strategy is achieved. In view of this, a strategy to combine with other antimicrobial therapeutic modality will be advantageous to achieve a successful eradication of pathogens, which could reduce potential for non-specific thermal damage to host cells by enabling the use of reduced level of NIR light intensity. Recently, Chiang et al. [115] successfully applied a dual modalities of therapeutic platform for treating wound infection by combining a platform for NIR light-triggered hyperthermia with one for controlled release of antibiotics. For this, they have developed a hybrid microsphere made of a shell of PLGA and aqueous cores composed of polypyrrole nanoparticles and vancomycin, in which polypyrrole nanoparticles were used as a photothermal agent. The combination of photothermally-induced hyperthermia and antibiotic delivery could result in synergistic effect of eradicating bacteria in wound abscesses of mice, to an extent that is greater than the sum of the two treatments alone.

3.3.2. AMF-triggered hyperthermia—Recent advancements in developing MNPs with high heating efficiency have improved the efficacy of hyperthermia treatment on pathogens [49]. The MNP hyperthermia utilizes MNPs in conjunction with high frequency AMF (>100kHz), in which MNPs absorb electromagnetic radiation and efficiently transmit energy in the form of highly localized heat (i.e. nanometer range of distance) on the surface of MNPs [48]. This technology has recently proven successful in the treatment of various cancers, with therapies in clinical trials to treat glioblastoma, prostate carcinoma, and breast carcinoma [116–118].

Despite extensive studies on the use of MNPs for cancer hyperthermia treatment, relatively little attention has been devoted to the use of MNP hyperthermia as a potential for antimicrobial therapy. Thomas et al. [119] recently demonstrated that magnetic fluid hyperthermia could be successfully used for bacterial destruction from *in vitro* culture model of *S. aureus*. The effect of magnetic fluid hyperthermia on the eradication of bacterial biofilms was further demonstrated *in vitro*, in which MNPs were added to *P. aeruginosa* biofilm, and heat was generated by placing the nanoparticle-containing biofilm in an AMF [120]. More than 4 log inactivation of the *P. aeruginosa* biofilm was observed within 8 minutes when relatively high concentrations of MNPs (60 mg/mL) were used. Recently,

Kim et al. [121] successfully validated the ability for MNP heating to effectively disrupt *S. aureus* biofilms using both *in vitro* culture assay and *in vivo* study of a mouse model of *S. aureus* cutaneous wound infection. The schematic on the strategy of applying MNP hyperthermia for the treatment of mouse model of wound *S. aureus* infection is depicted in Figure 3A. In an *in vitro* study, the brief AMF exposure (~3 min) disrupted biofilms in proportion to the amount of MNPs and the applied magnetic field amplitude. A 2-log reduction in *S. aureus* bioluminescence (about 99% killing) was achieved at a low dose of 1 mg/mL and magnetic field magnitude of 31 kA/m (Figure 3B). The study implicates that the extent of bacterial inactivation in response to AMF excitation of MNPs is highly dependent on MNP dosing and AMF field parameters. In a subsequent *in vivo* study using a mouse model of *S. aureus* cutaneous wound infection, the subcutaneous injection of anti-*S. aureus* antibody conjugated MNPs followed by the application of short duration AMF (3 minutes) could result in a significantly enhanced *S. aureus* inactivation (80%) compared with non-specific IgG (50%) or compared with MNPs with no linked antibody (Figure 3C).

However, it should be noted that although magnetic nanoparticle hyperthermia can generate a rapid temperature increase at the surface of a single MNP [122], a theoretical study also showed that overdosing presents the potential for disseminated tissue heating due to simultaneous heat dissipation from a large number of MNPs dispersed in a macroscopic region of tissue [123]. In addition, the application of high intensity AMF on any conductive biological medium can induce Eddy-currents that result in non-specific inductive heating in the body. Therefore, critical design criteria for the safe application are necessary, which has been a major drawback in related therapies based on MNP hyperthermia for cancer treatment. In particular, the ability to safely heat bacterial cells and prevent tissue damage depends upon high affinity targeting of pathogen cells, facilitated by proper customization of treatment parameters of nanoparticles (size and surface coating) as well as AMF (field frequency, amplitude, and duration of exposure).

3.4. Nanoparticles for efficient penetration to the biofilm matrix

Since the presence of EPS matrix in the biofilm structure is a major limiting factor that hinders the free diffusion of antibiotics or therapeutic drugs into the biofilm, strategies to achieve an enhanced penetration of nanoparticles into the biofilm matrix, while ensuring an effective entrapment of antibiotics, are critical for successful anti-biofilm activity. Although nanoparticles are considered to be transported into the biofilm via diffusional movement, both the size and surface chemistry of nanoparticles might alter the nature of interaction between nanoparticles and biofilm matrix [25], which will influence the penetration efficiency of nanoparticles into the biofilm matrix.

Many earlier studies for developing nanoparticles for enhanced penetration through the biofilm matrix have been performed using an experimental model of pulmonary biofilm infections, in which the EPS produced by *P. aeruginosa* is essential for the formation of thick and mature biofilms [124]. It has been shown that the presence of functional groups on the EPS (e.g., carboxylate or sulfate group) can cause the biofilms to generally exhibit an overall negative charge [125]. For example, the EPS synthesized by *P. aeruginosa* has shown to contain an anionic polysaccharide of alginate and negatively charged biomolecules including

extracellular DNA, which implicates that either neutral or negatively charged nanoparticles can be efficient in diffusing through the biofilm matrix. Indeed, in a recent study for delivering antibiotic encapsulated liposome against *Burkholderia cepacia* complex (Bcc) biofilms, negatively charged particles were observed to be directed towards bacterial cell clusters, while positively charged particles became immobilized by interactions with extracellular DNA-like structures in the biofilm matrix [126]. The surface coating of antibiotics-loaded PLGA nanoparticles with DNase I could result in significantly enhanced anti-biofilm activity against *P. aeruginosa* biofilms than that of free free-soluble antibiotics. This appears due to the increased degradation of the extracellular DNA that stabilize the biofilm matrix [127]. Although relatively little attention has been devoted to the use of EPS penetrating nanoparticles for wound biofilm infection, similar strategies used for pulmonary biofilm models can be applied for enhancing the penetration of nanoparticles through the biofilm matrix in the wound in that the EPS comprises integral part of the biofilm structures in skin wounds as well [31].

For nanoparticles with same surface chemistry, smaller size of particles appear to be efficient in achieving the penetration through the biofilm structure. Slomberg et al. [128] evaluated the efficacy of NO-releasing nanoparticles made of silica against *P. aeruginosa* and *S. aureus* biofilms as a function of particle size. The study revealed that the extent of NO delivery and eradication of *P. aeruginosa* and *S. aureus* biofilms were significantly higher with decreasing size of particles (~14 nm vs 50 nm and 150 nm), which was correlated with increased penetration depth of nanoparticles.

Taken together, the rational design for the control of surface functionality by means of targeting the EPS matrix can be synergistic with various nanoparticle-based anti-biofilm approaches described above, by increasing the efficiency of nanoparticle delivery to target cells. For example, the combined treatment of silver nanoparticles with biofilm dispersing enzymes could result in a synergistic inhibitory effect on biofilm-embedded MRSA in the study using a mouse model of MRSA infection in a cutaneous wound [129].

4. Future perspectives

Despite the development of novel antimicrobial agents, the cost and complexity of treating chronic wound infections remain a serious challenge, which necessitates the development of new and alternative approaches for an effective treatment of wound infection associated with biofilms. As reviewed here, recent advancement in nanotechnology for developing and applying a new class of nanoparticles that exhibit unique chemical and physical properties holds promise as an alternative to conventional antibiotic treatment for controlling biofilm infections.

However, there are several key issues that remain to be resolved for the successful clinical translation of nanoparticle-based approaches for treating biofilm infections. First, most of reported studies that demonstrate anti-biofilm activities of nanoparticles were based on single species strain of bacteria and mostly relying on in vitro cell culture studies. However, recent clinical data implicate that human chronic infectious wounds are colonized with polymicrobial biofilms composed of multiple species of both gram positive and gram

negative pathogens, which is now considered to be a primary impediment to the healing of chronic wounds [3, 8, 9]. The polymicrobial biofilms can exhibit a higher level of antimicrobial tolerance than monospecies [130]. Thus, it is critical to develop a novel strategy that can target multiple gram positive and gram negative bacterial species simultaneously. In addition, the therapeutic efficacy of nanoparticles should be carefully evaluated using clinically relevant polymicrobial biofilm models in vivo.

Second, despite extensive studies on the evaluation of various nanoparticles in anti-biofilm efficacy, limited studies have been performed on how these nanoparticles interact with biofilm structures. The ability of nanoparticles to effectively penetrate into the biofilm is critical for achieving successful biofilm eradication [128]. More detailed understanding on the important parameters that influence the penetration efficiency of nanoparticles into the biofilms will lead to an improved design of nanoparticles that increase anti-biofilm effects [131].

Third, there have been concerns over the potential health impacts of engineered nanoparticles, along with our limited knowledge of how nanoparticles interact with host cells and the subsequent biological pathways impacted [132]. Although nanoparticle-based approaches offer new opportunities for antimicrobial treatments, the treatment by engineered nanoparticles must not inhibit the tightly regulated functions of phagocytes (neutrophils, macrophages, etc.) that comprise a critical element of innate immune protection against invading pathogens. In addition, nanoparticles can be cleared from tissue in large by mononuclear phagocytic system, however, local host immune status in the site of infection alters the clearance process of nanoparticles [133]. It is necessary to have a complete understanding of the ultimate fate of nanoparticles aggregates that remain in the wound as well as an understanding of the physical interactions between the nanoparticles and host immune cells that they encounter.

In conclusion, although many challenges remain to be addressed for the successful translation into clinics, the extensive ongoing efforts in development and evaluation of nanoparticle-based therapeutics may lead to a needed tool for the treatment of patients who cannot be successfully treated with standard-of-care antibiotic treatment and establish a novel platform for smart treatments of antibiotic-resistant chronic wound infections.

Acknowledgments

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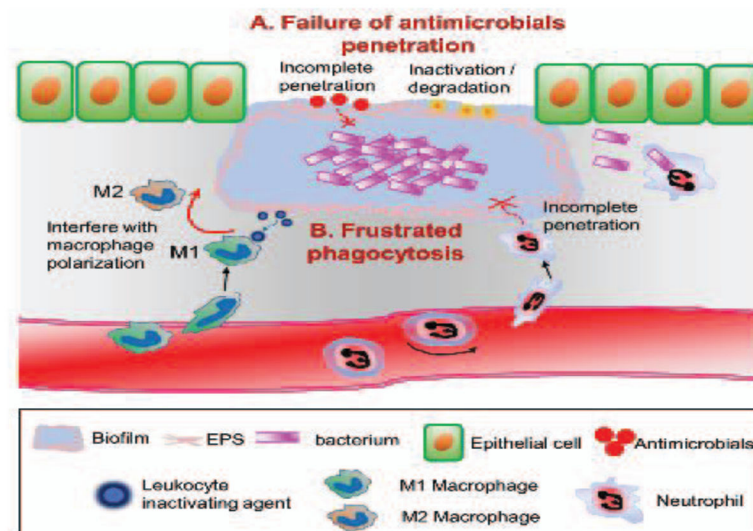
Figure 1.
Schematic on the potential mechanisms by which biofilm formation in the wound lead to the infection persistency and wound chronicity. The presence of EPS in the biofilm creates a protective environment for the residing microorganisms against antibiotics treatments and host innate immune response. A. The presence of EPS forms protective barrier for the diffusion of antimicrobials or results in inactivation of antimicrobials at the biofilm matrix. B. In addition, the formation of biofilm structures substantially diminishes the phagocytic activities of innate immune cells (neutrophils and macrophages), by not only providing a shielding mechanism from the penetration of neutrophils, but also promoting the production of leukocyte-inactivating substances, which impairs bactericidal activity of macrophages by skewing macrophage polarization towards M2-like phenotype.
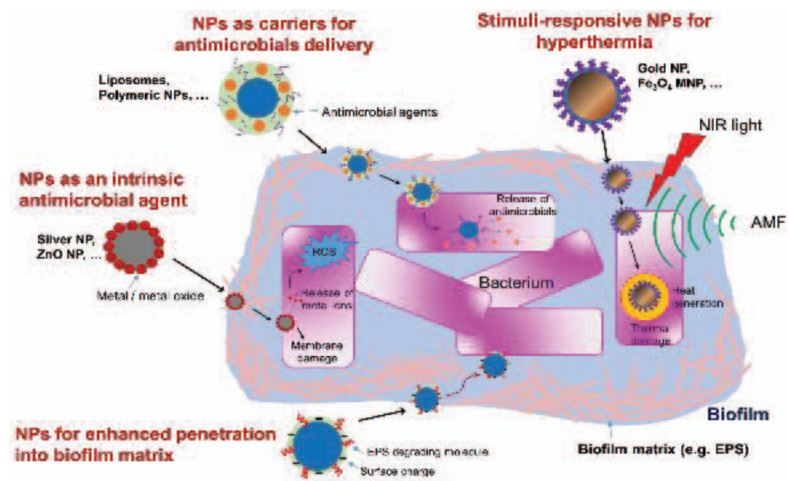
Figure 2.
Schematic on the mechanism of actions for various nanoparticle (NP)-based approaches for treating biofilm infections.

Figure 3.
In vitro and in vivo studies on the use of magnetic nanoparticle (MNP) targeted hyperthermia against *S. aureus biofilm*. A. Schematic on the strategy of MNP hyperthermia treatment in a mouse model of wound *S. aureus* infection. B. Representative bioluminescent image of *S. aureus* biofilm on 96 well plate before and after alternating magnetic field (AMF) application (for 3 min at 31 kA/m field amplitude) *in vitro*. The extent of bacterial killing was quantified based on the level of bioluminescence quenching that directly correlates with number of live bacteria (CFUs). C. Representative bioluminescent images of *S. aureus* in a mouse skin wound *in vivo*. Mice were infected with *S. aureus* (SA, 1×10^7 CFU) at wound sites and MNP-anti-*S. aureus* antibody (MNP-anti-SA mAb) conjugates were locally injected into wound at day 2 post-infection. Then, an AMF was applied for 3 min (31 kA/m field amplitude) for four experimental groups: (1) mice injected with MNP-anti-SA mAb conjugate but without AMF treatment, (2) mice injected with MNP only and with AMF treatment, (3) mice injected with MNP-IgG conjugate and with AMF treatment, (4) mice injected with MNP-anti-SA mAb conjugate and with AMF treatment. Reprinted with permission from reference [121].

Table 1

The mechanism of actions and limitations for various nanoparticle (NP)-based approaches for anti-biofilm activities.

		Types of NPs used	Mechanism of actions / advantages		Limitations
NPs as intrinsic antimicrobial agents		Silver NP [63, 64], Zinc oxide NP [71–76]	•	Generation of hydrogen peroxide and cell membrane damage.	Toxicity at high concentration
			•	Antibacterial efficacy for broad spectrum of bacteria.	
NPs for controlled delivery of antimicrobial agents	Antibiotics delivery	Liposomes [84, 85, 87], Polymeric NP [89, 90], Lipid-polymer hybrid NP [92]	•	Delivery of antibiotics to the site of infection at a sustained and controlled manner.	Insufficient drug loading
			•	Protection of drugs from enzymatic inactivation.	
	NO delivery	Silica NP [97], Silane hydrogel- based NP [99–101]	•	Interfere the process of DNA replication and respiration in bacteria.	Difficulty of controlling the release kinetics of physiologically optimal concentrations of NO in the wound bed
			•	Antibacterial efficacy for broad spectrum of bacteria.	
			•	Promote wound healing by angiogenesis and tissue remodeling.	
	Photosensitizer delivery	Porphyrin [109], Methylene blue [110], Rose bengals [111]	•	Triggers necrotic bacterial death by generation of cytotoxic reactive oxygen species to light exposure.	Non-specific cytotoxic reactive oxygen species damage to host cells
			•	Antibacterial efficacy for broad spectrum of bacteria.	
Responsive NPs for anti-bacterial hyperthermia treatment	NIR light- triggered hyperthermia	Gold NP [112], Fe_3O_4 MNP [113], Graphene NP [114]	•	Triggers irreversible thermal damage to bacteria in response to NIR light illumination.	Non-specific thermal damage to host cells
	AMF-triggered hyperthermia	Fe_3O_4 MNP [119–121]	•	Triggers irreversible	Non-specific thermal damage to host cells

		Types of NPs used	Mechanism of actions / advantages	Limitations
				thermal damage to bacteria in response to high frequency AMF application.
NPs for enhanced penetration to the biofilm matrix	Surface charge functionalization Surface coating with EPS degrading molecules	[127] [129]	•	Enhance the efficiency of nanoparticle transport to target bacterial cells by means of tuning surface charge and surface coating with EPS degrading molecules.