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Review Article

Antisense RNA, the modified CRISPR-Cas9, and metal/metal oxide nanoparticles to inactivate pathogenic bacteria

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ABSTRACT

Finding efficient therapeutic strategies to fight antibiotic-resistant bacteria is a complicated affair specifically in the therapy of chronic bacterial infections related to hospital-acquired infections. Recently, three major antibacterial systems based on antisense RNA, CRISPR-Cas9, and metal/metal oxide nanoparticles particularly silver (Ag) nanoparticles have shown more effective antibacterial activity compared to conventional antibiotics. ROS generation, attachment to the cell membrane, disruption of bacterial envelop, inactivation of electron transport chain, decreasing the local pH, modulation of cell signaling, and denaturation of biological macromolecules such as proteins and nucleic acids have been found as the main antibacterial functions of Ag nanoparticles. Antisense RNA, a single-stranded RNA, can hybridize with complementary genes in messenger RNA (mRNA) followed by blockage translation of these genes into proteins. Moreover, CRISPR (clustered regularly interspaced short palindromic repeats) is a family of viral DNA sequences derived from bacteriophages, which can target and destroy foreign DNA by nuclease activity. There are 2 classes and 6 subtypes (I-VI) of CRISPR-Cas systems, which may be engineered as potential antibacterial agents to target specific sequences. Therefore, here, recent advances and challenges for the antibacterial application of these three therapeutic agents are presented.

1. Introduction

Infectious diseases are always a serious threat to the health of humans. According to the World Health Organization (WHO), there are a high proportion of antibiotic-resistant bacteria resulting from common infections such as urinary tract infections, pneumonia and bloodstream infections, and dire clinical requirements [1]. Ineffectiveness of the drug against infection means an increase in the rate of disability and death and imposes huge costs on the health sector [2]. Antibiotics are a valuable source of medicine employed to treat bacterial infections and their use in humans and animals is only allowed with the prescription of a physician and health experts and should be used in a full course of treatment. With the discovery of antibiotics, deaths from infectious diseases have been significantly reduced; however, with the misuse of antibiotics and resistance to them, these diseases are emerging [3-5].

Due to the increasing resistance to antibiotics, the world is in dire need of changing the pattern of consumption and prescribing this source of medications. If drug application remains the same, even the production and development of new drugs cannot prevent increased resistance to antibiotics. In addition to not using antibiotics arbitrarily, surveys are needed to reduce the

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spread of infection through regular vaccinations, regular hand washing, and attention to food hygiene [6].

In fact, antimicrobial resistance is the resistance of a microorganism (including bacteria, fungi, viruses, and parasites) to antimicrobial drugs. When these microorganisms become resistant to antibacterial, antifungal, and antiviral drugs, standard drugs become ineffective, the infection remains in the body such as chronic diabetic foot ulcer, and it is not easily treated [7, 8]. The evolution of resistant strains is a natural phenomenon and occurs when microorganisms are mistakenly propagated or resistant traits are exchanged between several microorganisms. Frequent and inappropriate use of antibiotics is one of the main reasons for the increase in drug-resistant bacteria [9].

There is a plethora of strategies to inhibit antibiotic-resistant bacteria. We have presented three main antibacterial therapies based on antisense RNA, CRISPR-Cas9, and metal/metal oxide nanoparticles (NPs). Antisense RNA, a single-stranded RNA, can hybridize with complementary genes in messenger RNA (mRNA) followed by blockage translation of these genes into proteins [10]. mRNA degradation and translation inhibition via hybridizing with sequences flanking the ribosome binding site as well as the start codon of the mRNA are main routes for silencing target genes [11]. CRISPR (clustered regularly interspaced short palindromic repeats) is a family of viral DNA sequences derived from bacteriophages, which can target and destroy foreign DNA by nuclease activity (Figures 1a and b). There are 2 classes and 6 subtypes (I-VI) of CRISPR-Cas systems, which may be engineered as potential antibacterial agents to target specific sequences [12]. These palindromic repeats can be found as ~50% and ~90% in bacterial and archaeal genomes, respectively [13]. In the case of CRISPR-Cas9, the DNA sequences may be modified to silence bacterial virulence genes and bacterial resistance genes, which can be transferred to pathogenic bacteria by bacteriophages or plasmids [14]. It should be noted that this technology is in the preclinical phase and several companies such as Eligo Bioscience, Locus Biosciences, and Intellia Therapeutics are working on this type of therapy. Although phage capsids such as the temperate phage phiNM1 and the M13 phage were employed to deliver CRISPR antimicrobials, nevertheless, one main problem for using this technology is lacking efficient delivery systems with the ability to discriminate between pathogenic bacteria and other cells. [12, 15, 16].

Recently, nanomaterials having specific properties (large surface area to volume ratio and aspect ratio) compared to bulk materials have shown excellent inactivation of microorganisms [17-19]. There are various methods for the fabrication of organic and inorganic nanomaterials, which can be functionalized in a smart way to target microorganisms [20-22]. For instance, natural sources such as plants, fungi, alga, lichens, bacteria, and bacteriophages having particular secondary metabolites can be used to modify organic and inorganic nanomaterials [23, 24]. Metal and metal oxide NPs encompassing silver, zinc oxide (ZnO), copper (Cu), and titanium dioxide (TiO_2) have attained more attention to inactivate microorganisms specifically bacteria compared to other nanomaterials [25, 26]. Metal ion release from these NPs and production of reactive oxygen species (ROS) are considered as main antibacterial mechanisms [27, 28]. According to the above discussion, here, these three new strategies are presented to get a deep insight into future researches.

2. Antisense RNA

For inhibitor screening, essential proteins that are conserved among pathogens are considered suitable targets. The bacterial protein YidC (60-kDa) is critical for membrane protein translocation and insertion by its operation with the Sec machinery in eukaryotic organelles, archaea, and bacteria, which can be targeted for antibacterial therapy (Figure 1b) [29]. Downregulation of *yidC* in *Escherichia coli* was caused using the RNA silencing approach and eugenol/carvacrol essential oils treatment. By downregulation of this gene in *E. coli*, functions of cytochrome o oxidase, F_1F_o ATPase, and proton-motive force are reduced [11]. In addition, hypersensitivity to specific

antibiotics may be caused by the incorporation of antisense RNA targeting the particular gene in bacteria species. For example, anthrax is a serious bacterial infection eventuated from rod-shaped, Gram-positive bacteria of *Bacillus anthracis*, which can affect wild animals, domestic and humans. Hypersensitivity to beta-lactam antibiotics and MetS-particular antibacterial agents were found for *B. anthracis* under induced transcription of RNA from *murB2* and *metS1* antisense-oriented genes related to enzymes of UDP-N-acetylenolpyruvoylglucosamine reductase and methionyl-tRNA synthetase, respectively [30].

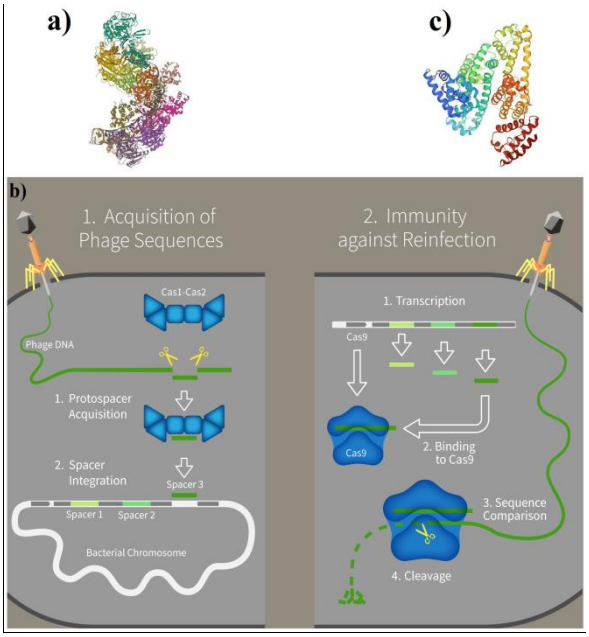

Fig. 1 (a) The crystal structure for CRISPR cascade bound to a single-stranded DNA target (PDB accession no.: 4QYZ), (b) The CRISPR can protect bacteria by a nuclease defense mechanism from repeated phage infections at two steps of 1) acquisition of phage sequences and 2) obtaining immunity against reinfection. (Licensed under the Creative Commons Attribution-Share Alike 4.0 International license), and (c) YidC gene of *E. coli* (PDB accession no.: 3WFV).

3. The modified CRISPR-Cas9

Gram-positive round-shaped bacterium of *Staphylococcus aureus* can induce infections in soft and skin tissue (furuncles, cellulitis, and abscesses) and bone (osteomyelitis). As illustrated in Figure 2a, modified CRISPR-Cas9 bacteriophage (delivering of ϕSaBov) may be prescribed for significant mitigation of the growth of *S. aureus* relative to untreated strains [31]. Formulation of this modified CRISPR-Cas9 by alginate hydrogel (at a concentration of 3% (w/v)) showed a biofilm reduction as separate fragments on orthopedic screws and bone after 8 days of treatment (Figures 2b and c) [32]. *Enterococcus faecalis* is a Gram-positive bacterium related to hospital-acquired infections, which can have antibiotic resistance genes. Pheromone-responsive plasmids as CRISPR-Cas-encoding delivery plasmids were engineered to target antibiotic resistance genes of *ermB* through conjugative delivery to combat erythromycin-resistant *E. faecalis* [33]. Moreover, targeted-antibacterial-plasmids may be employed to deliver CRISPR-Cas systems with a strain-specific antibacterial activity through bacterial conjugation [34].

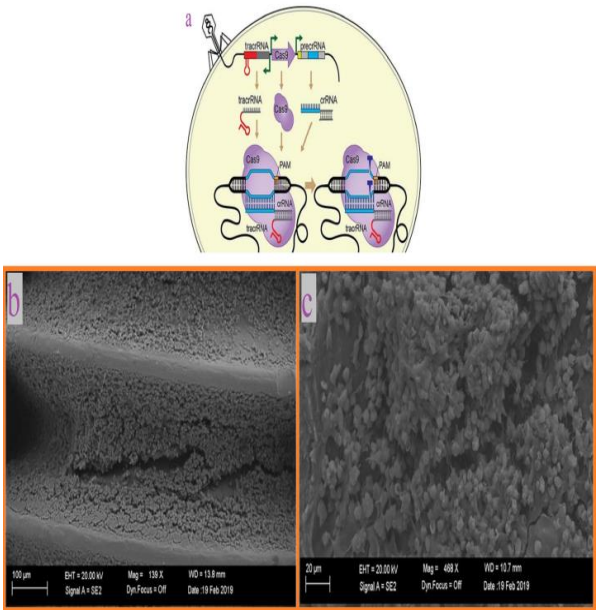

Fig. 2 (a) The CRISPR-Cas9 system (Cas9, CRISPR RNA (crRNA), and trans-activating crRNA (tracrRNA)) is expressed and scanned the PAM (protospacer adjacent motif) sequence followed by recognition of the target gene in the chromosomal bacterial DNA, leading to DNA cleavage [31]. (Licensed under a Creative Commons Attribution 4.0 International License). (b) SEM micrographs of biofilm formation on the distal portion of orthopedic screws (c) and dispersed biofilm

of *S. aureus* under treatment by CRISPR-Cas9 modified bacteriophage at eighth day [32] (Copyright: © 2019 under Creative Commons Attribution License).

4. Metal and metal oxide NPs

ROS generation, attachment to the cell membrane, disruption of bacterial envelop, inactivation of the electron transport chain, decreasing the local pH, modulation of cell signaling, and denaturation of biological macromolecules such as proteins and nucleic acids via oxidative stree (production of superoxide radical, hydroxyl radical, and hydrogen peroxide) have been found as major antibacterial functions of metal/metal oxide NPs especially AgNPs (Figure 3) [35]. The antibacterial mode for AgNPs was mainly dependent on the size and shape of NPs. For instance, smaller sizes (the size range of 5-10 nm) of AgNPs have illustrated cell membrane damage and degradation of the bacterial chromosome. Attachment to the cellular envelope, penetration inside the bacteria and inactivation of respiration were indicated for sizes at the range of 20-25 nm, while NPs with 90-100 nm have shown hindrance of metabolic pathways [35].

In the green synthesis (Figure 4), Surface modification of AgNPs with organic materials such as primary and secondary metabolites of living organisms including plants, fungi, alga, lichens and bacteria can synergize antibacterial and biocompatibility properties in vitro and in vivo. For example, there are various therapeutic activities such as antimicrobial, anticancer, anti-inflammatory and reduction of drug side effects for curcumin ($C_{21}H_{20}O_6$; isolated from turmeric, *Curcuma longa* plant species) as herbal secondary metabolite [7, 36, 37]. This herbal drug or other phytochemicals such as polyphenols can neutralize the antibiotic-resistance mechanisms and increase the antibacterial capacity of drugs [38]. As an example, a combination of curcumin with AgNPs showed lower cytotoxicity toward human normal fibroblast and a striking reduction of multidrug-resistant *Pseudomonas aeruginosa* under photodynamic therapy as a non-invasive antibacterial approach [39].

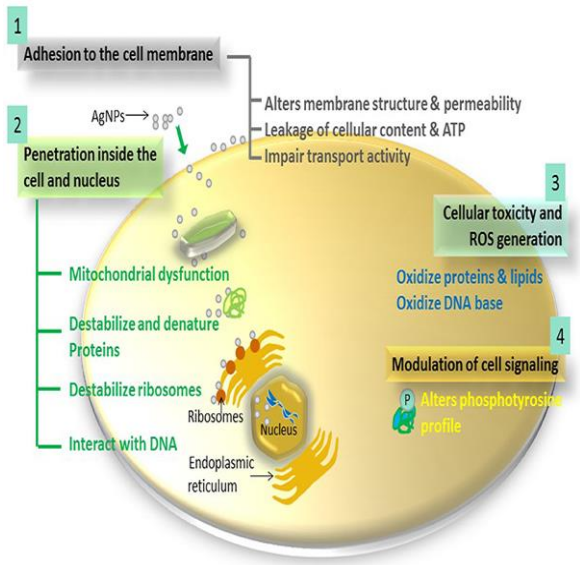

Fig. 3 The main antibacterial mechanisms of AgNPs [35] (Copyright © 2016 under the terms of the Creative Commons Attribution License).

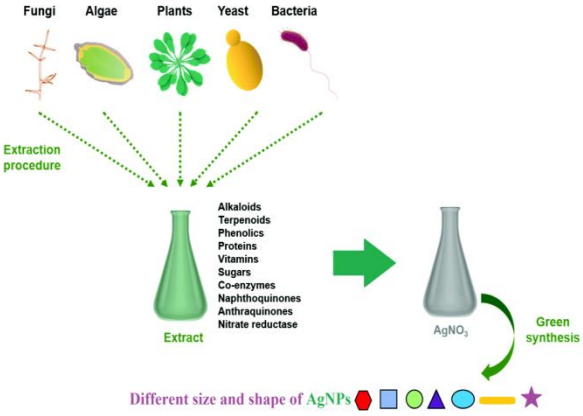

Fig. 4 Biosynthesis methods for fabrication of various sizes and shapes of AgNPs in a one-pot way [40] (Attribution-NonCommercial 3.0 Unported (CC BY-NC 3.0)).

5. Conclusion

Finding new therapeutic approaches to fight antibiotic-resistant bacteria is a complicated affair in the therapy of bacterial infections specifically hospital-acquired infections. There is a plethora of approaches to inactivate pathogenic bacteria, which we have presented three main antibacterial therapies based on antisense RNA, CRISPR-Cas9, and metal/metal oxide NPs. In the case of antisense RNA, essential proteins that are conserved among pathogens are considered suitable targets. In this regard,

hypersensitivity to specific antibiotics or other antibacterial agents such as herbal or bacterial metabolites may result from the incorporation of antisense RNAs targeting particular genes in pathogenic bacteria. For the CRISPR-Cas9 system, a significant reduction of soft tissue infection upon treatment of the modified CRISPR-Cas9 bacteriophage was equal to fosfomycin antibiotic. It can be concluded that further study is required to determine the therapeutic efficacy of CRISPR systems as antibacterial agents. Lacking robust delivery systems is a major challenge in the application of CRISPR antimicrobials, which should be considered comprehensively in future investigations.

Abbreviations

CRISPR: clustered regularly interspaced short palindromic repeats

CRISPR-Cas9: CRISPR-associated protein 9

crRNA: CRISPR RNA

mRNA: messenger RNA

NPs: nanoparticles

ROS: reactive oxygen species

tracrRNA: trans-activating crRNA

WHO: World Health Organization

Conflict of interest

None of the authors have any conflict of interest to declare.

Consent for publications

The authors read and proved the final manuscript for publication.

Availability of data and material

Not applicable.

Authors' Contribution

M.A has written the first draft of this manuscript and **M.R.** revised it.

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Ethics approval and consent to participate

No human or animals were used in the present research.

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