Photo-Stimuli-Responsive Antibacterial Applications of CuS and Its Nanocomposites

Subjects: Infectious Diseases

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Photo-stimuli-responsive therapeutic nanomaterials have gained widespread attention as frontline materials for biomedical applications. The photoactivation strategies are classified as single-modality (based on either reactive oxygen species (ROS)-based photodynamic therapy (PDT), hyperthermia-based photothermal therapy (PTT)), or dual-modality (which combines PDT and PTT). CuS has excellent photoactivated antibacterial properties and shows promising antibacterial activity when irradiated in the so-called biological window (i.e., NIR region).

Keywords: photo-stimuli-responsive nanomaterial ; CuS ; photodynamic therapy ; photothermal therapy

1. CuS Nanomaterials

CuS nanomaterials exhibit inherent photoactivated antibacterial potential, especially in the NIR region. For example, Qiao et al. [1], developed ultrasmall copper sulfide (covellite) nanodots (CuS NDs), which showed effectiveness in the treatment of multidrug-resistant bacteria-infected chronic non-healing wounds. The dual functional mode of the CuS nanosystem enabled eradication of extended-spectrum β-lactamase (ESBL) *E. coli* and methicillin-resistant *S. aureus* (MRSA) in both in vitro and in vivo studies, while simultaneously expediting wound healing. The NIR laser (808 nm, 2.5 $W{\cdot}cm^{-2}$)-mediated photothermal effect and remote control of copper-ion (Cu^{2+}) release were the main origins of the dual-mode activity. Ren et al. [2] utilized CuS nanoparticles (NPs) to prepare a photothermal antibacterial silk fabric. A chitosan quaternary ammonium salt (QCS) was used as a template. The antibacterial efficiency of CuS NP-deposited silk fabric with QCS as a template (SF/QCS/CuS) resulted in 99.99% bacterial death of *S. aureus* and *E. coli* within 5 min of irradiation (400 $mW{\cdot}cm^{-2}$). The nanocomposite also showed good UV-resistance and superb light-to-heat conversion efficiency. Even after 10 washes, the antibacterial activity of the nanocomposite remained at a high level, demonstrating excellent bonding between the photothermal nanomaterials. In a similar experiment, CuS NPs were coated onto glass slides, and the antibacterial properties were investigated [3]. The CuS NP–glass samples showed good stability and an admirable photothermal effect when irradiated with NIR light. The samples were also effective for the controlled release of copper in water. Hence, the antibacterial activity of the CuS NP-glass samples against *E. coli* and *S. aureus* was attributed to the dual mechanism of hyperthermia-induced NIR laser irradiation and the slow and sustained copper release. As discussed earlier, CuS has shown morphology-dependent photodynamic and photothermal antibacterial activities [4].

2. CuS with 2D Nanomaterials

In recent times, 2D nanomaterials have revolutionized research due to their unique physiochemical properties, non-toxicity, and excellent utility in various biomedical applications, including antibacterial treatments [5][6][7]. After the immense success of graphene [8][9], researchers have been exploring different 2D nanomaterials as antibacterial agents, namely graphitic carbon nitride ($g\text{-}C_3N_4$) nanosheets [10][11], titanium carbide (Mxene) [12], boron nitride (BN) [13], transition metal dichalcogenides [e.g., molybdenum disulfide (MoS_2)] [14], BP [15][16][17][18], and others. The basic advantage of using 2D nanomaterials with CuS is the synergetic improvement in the antibacterial activity, with less chance of toxicity, due to the biocompatibility of 2D materials.

In this respect, Lv et al. [19] demonstrated the importance of CuS nanocomposites with graphene oxide (GO) for photothermal antibacterial applications by utilizing visible light. A simple one-pot hydrothermal method was utilized to synthesize flower-like CuS anchored on GO sheets. Due to the morphology-dependent high surface area of CuS and defects in GO, the nanocomposite showed excellent photocatalytic performance. The nanocomposite showed excellent antibacterial activity against *E. coli* and *S. aureus*. This antibacterial activity was attributed to the excellent photocatalytic activity of the nanocomposite, coupled with the visible-light-induced (0.2 $W{\cdot}cm^{-2}$) photothermal response and release of Cu ions. The nanomaterial was also biocompatible with L929, a murine fibroblast cell line. Another 2D nanomaterial,

graphitic carbon nitride (g-C_3N_4) nanosheets [20], were utilized with CuS nanomaterials for the photothermal eradication of bacteria. In that study, a simple hydrothermal process was again utilized to fabricate CuS nanomaterial-decorated graphitic carbon nitride (g-C_3N_4) nanosheets. Thereafter, polyethylene glycol (PEG) was capped with the nanocomposite to make it more biocompatible. The PEG-CuS@g-C_3N_4 nanocomposite was effective as an antibacterial agent, killing up to 99% of both *E. coli*. and *S. aureus* in a 200 μg·mL^{-1} suspension within 20 min of NIR irradiation (808 nm, 2.5 W·cm^{-2}). The biocompatibility of the nanocomposite was also verified using mouse skin fibroblast NIH-3T3 cell lines. $Ti_3C_2T_x$ Mxene was also used with CuS to achieve PDT- and PTT-mediated multimodal activity against *E. coli* and *S. aureus*, with bactericidal rates of 99.6 and 99.1%, respectively [21]. $Ti_3C_2T_x$@CuS composites were synthesized by a simple hydrothermal method, where the CuS NPs were distributed on the surface of $Ti_3C_2T_x$, affording synergistic effects. ROS generation, which eventually facilitated destruction of the bacterial cells, was attributed to the formation of the $Ti_3C_2T_x$@CuS heterojunction, which promoted the separation of electrons and holes, leading to improved electron transport efficiency. Furthermore, the nanocomposite had a stronger photothermal effect than the individual components, confirming its synergistic action. Recently, the researchers reported a BP-based CuS nanoplatform (CB) with photothermal activity, demonstrating antibacterial activity against environmental bacterial pathogens [16]. In the work, a low-temperature solution synthesis method was utilized to prepare CuS nanomaterials, which were then immobilized on BP nanosheets. The synthesized CB nanocomposites showed excellent antibacterial activity against *P. aeruginosa* and *S. aureus*, and also showed good activity against the MDR strains of these bacteria after irradiation with NIR light (808 nm, 2.5 W·cm^{-2}). The CB nanocomposite also showed exceptional ROS generation ability under NIR irradiation relative to the non-NIR-irradiated sample.

3. CuS with Polymers

CuS nanomaterials were also utilized with different polymers to achieve synergistic photoactivated antibacterial activity. Dai et al. [22] synthesized a collection of poly(5-(2-ethyl acrylate)-4-methylthiazole-g-butyl)/copper sulfide nanoclusters (PATAC4@CuS), which were applied to levofloxacin-resistant Gram-negative and Gram-positive bacteria for efficient capture and effective ablation by NIR laser irradiation. The nanoclusters not only showed excellent photothermal activity under NIR irradiation, but also demonstrated admirable photodynamic properties. PATAC4@CuS nanoclusters showed excellent antibacterial activity against *B. amyloliquefaciens*, *E. coli, P. aeruginosa*, and levofloxacin-resistant *S. aureus* at 5.5 μg·mL^{-1} under NIR laser irradiation (980 nm, 1.5 W·cm^{-2}, 5 min). The heat and ROS generated from the NIR-irradiated PATAC4@CuS nanoclusters were stated as the reasons for the effective elimination and prevention of regrowth of the bacteria. The nanocluster also showed wound healing potential in bacteria-infected rat wounds without nonspecific harm to normal tissue. In another similar experiment, Wang et al. [23] introduced a thiol-terminated, alkyl-containing short-chain poly(ethylene glycol) (HS-$(CH_2)_{11}$-$(OCH_2CH_2)_6$-OH, abbreviated as MUH, onto CuS nanoclusters (NCs) to achieve photothermal antibacterial activity under NIR irradiation. The MUH-coated CuS NCs exhibited excellent stability in solutions with various pH values and stayed stable in pure water for at least 10 months. The MUH-coated CuS NCs showed strong antibacterial activity toward *E. coli* bacteria at 800 μM (76.8 μg·mL^{-1}) concentration.

4. CuS with Protein

Despite numerous advantages of CuS nanomaterials for biomedical applications, toxicity remains an issue that needs to be resolved. To address this issue, researchers have tried to coat CuS with proteins, especially bovine serum albumin (BSA). BSA is selected as a coating for CuS due its various advantageous properties, such as versatility, non-toxicity, stability, and biodegradability. Additionally, its functional groups allow easy binding and capping of nanomaterials, such as CuS.

Zhao et al. [24] applied BSA-CuS NPs to the treatment of diabetic wound infection in vivo as a method of photothermal therapy. A facile biomineralization method was used to synthesize BSA-CuS nanomaterials, which were successfully used to destroy *A. baumannii*, *S. aureus*, and *S. haemolyticus* under NIR irradiation (808 nm, 8.0 W·cm^{-2}) for 10 min at 200 mg·L^{-1} concentration. Both in vitro and in vivo experiments were performed in this case. The BSA-CuS NPs showed excellent NIR-mediated photothermal antibacterial activity, with low cytotoxicity, in vivo toxicity, and excellent water solubility. Dual-mode PTT and PDT was applied using Ce6-labeled BSA-CuS NPs, enabling a synergistic effect against *E. coli and S. aureus* both in vitro and in vivo [25]. Most importantly, the same NIR light source was utilized to generate the ROS required for PDT activity and for PTT. The CuS NPs were prepared in the presence of BSA, which prevents aggregation of the CuS NPs. BSA also acts as a linker between the CuS and Ce6 molecules through amide bonds. The Ce6-labeled BSA-CuS NPs showed an overall bacterial killing efficacy of 97% in vitro due to the synergistic effects of combined PTT and PDT. The nanocomposite also successfully eliminated bacterial infection in vivo. BSA was also utilized as a template to synthesize uniform CuS NPs by a biomineralization method, and its NIR-mediated photothermal

antibacterial activity against *E. coli* and *S. aureus* was evaluated. The as-prepared BSA-CuS nanocomposites showed good biocompatibility with skin fibroblast cells. Moreover, the nanocomposites showed excellent antibacterial activity under NIR light (980 nm, 1.59 W·cm^{-2}). The biocompatible CuS-BSA/lysozyme nanocomposite was also effective against *B. subtilis* and *E. coli* under NIR irradiation (980 nm, 0.7 W·cm^{-2}) [26].

5. CuS with Metals

The use of intrinsic antibacterial agents, such as Ag [9][14], Au [15], and ZnO [14][27], in combination with CuS to achieve photothermal antibacterial activity, is still in the infancy stage. Addae et al. [28] demonstrated the strong photothermal antibacterial potential of Au/CuS NPs against *Bacillus anthracis*. The antibacterial effect was dependent on both the NP concentration and treatment time. Similarly, Park et al. [29] showed the photothermal antibacterial potential of Au@CuS against *E. coli*. The Au@CuS yolk-shell nanomaterials on a PDMS film induced excellent photothermal sterilization against *E. coli*, where the photothermal efficiency of the nanocomposite reached 79.8%. Further research in this area is still necessary to completely understand the antibacterial mechanism of the nanocomposites.

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