Novel Antimicrobial Strategies to Combat Biomaterial Infections

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1 Introduction

Bacteria are present in nature everywhere and the combat with them has the major priority especially in various industrial settings (i.e. food industry) or medical devices [1]. It was established earlier that most of bacteria found in nature exist in the form of biofilms (attached to surface of different objects and not as free floating organisms). Therefore, biofilm formation can be defined as a multistage process. It starts with bacteria adhesion to surface and continues with the formation of extracellular matrix. This matrix is composed of one or more polymeric substances (proteins, polysaccharides, humic substances, extracellular DNA) [1, 2]. Bacteria adhesion to surfaces depends on different surface parameters: wettability, roughness, chemistry, and charge of materials as well as of the nature of bacterial surface, environmental factors and the associated flow conditions etc. [3, 4].

There are several possible strategies to reduce or prevent bacterial infections among different populations: patients and medical staff [5]. Traditional hospital sterilization strategies are based on usage of high level disinfectants: hydrogen peroxide, peracetic acid, glutaraldehyde and low level disinfectants: alcohols, hypochlorites, iodine and iodophor. Advanced sterilization technology focuses on chemical-free technology such as UV rays or gas plasma components. However, there are several disadvantages of both chemical and chemical-free approaches. Firstly, they are toxic to some extent so medical personnel and patients have to evacuate the premises. Secondly, the quality of sterilization is proportional to human labor invested by cleaning personnel [6]. One of the alternative strategies independent of human labor, is to produce antibacterial coatings to reduce or eliminate bacteria colonization on surfaces by leaching of biocides, antibacterial surfaces

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with deposited metals such as copper, silver or gold, formation of superhydrophobic surfaces and surfaces encapsulated by photoactive nanoparticles [7–12].

The major drawback of biocides and metal deposited surfaces is their leaching from the surface in the environment. In this way those surfaces lose their antibacterial properties after some time. Besides, these surfaces develop bacterial resistance which causes more than 33,000 deaths and costs 1.5 billion euros per year in Europe [13]. The increase of patients infected in hospitals (in the developing countries the infection rate is 75%) was noticed [14]. The cost and cytotoxicity of the agents mentioned above might be a problem as well. As the price of the best antimicrobial additives (silver, titanium, gold, chitosan) is too high, companies are looking for cheaper and safer additives with strong antimicrobial potential. Permanent cytotoxicity of certain antimicrobial agents in concentrations larger than needed for antimicrobial action may cause many problems. A further limited factor of these materials usage is that silver and copper nanoparticles are prone to oxidation. After a certain time they don't show antibacterial effects.

In recent years new types of antibacterial surfaces have been designed by encapsulation of different photoactive nanoparticles in polymer matrices (polyurethane or dimethylsiloxane) [5, 15, 16].

2 State of the Art

Photodynamic therapy (PDT) is a treatment which includes the usage of light sensitive drugs in the healing of various diseases (for example skin or eye cancers). Antibacterial PDT (APDT) is used to eliminate multidrug-resistance pathogenic bacteria [17]. Based on principles stated above it is possible to design antibacterial surfaces from photoactive nanoparticles (in the form of hybrids or thin films/coatings) or by encapsulation of photoactive nanoparticles into various polymer matrices. One of the properties of these nanoparticles is their ability to produce reactive oxygen species-ROS (singlet oxygen, superoxide, hydroxyl radicals, hydrogen peroxide) or heat [18, 19]. ROS eradicates multidrug resistant bacteria, quickly disappears and does not represent a danger to the environment. Heat causes denaturation of bacteria but requires additional means for its control.

Photoactive nanoparticles called photosensitizers (PSs) produce ROS by the following mechanism: PSs have been excited to a singlet excited state by ultraviolet or visible light. From this state electrons are moving to a triple state or return to a ground state. Singlet oxygen can be generated if they transfer their electrons or energy to molecular oxygen as shown in Fig. 1. Molecular oxygen causes oxidative damage of bacteria cells. Since molecular oxygen simultaneously attacks several sites in bacteria, the bacteria are unable to mutate and develop resistance [20–23].

Different nanoparticles can be used as PSs: pristine and doped carbon quantum dots (CQDs) and graphene quantum dots (GQDs), chitosan nanodots (ChiNDs), ultra short single wall carbon nanotubes (US SWCNTs), gold nanoparticles (AuNPs)—Fig. 2. It was earlier reported that polymers (polyurethane,

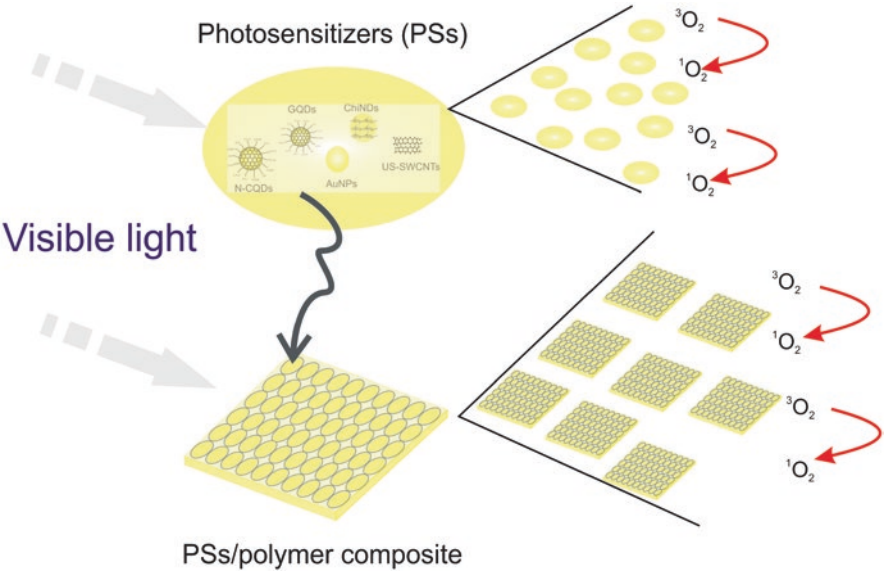

Fig. 1 Mechanism of singlet oxygen production by PSs

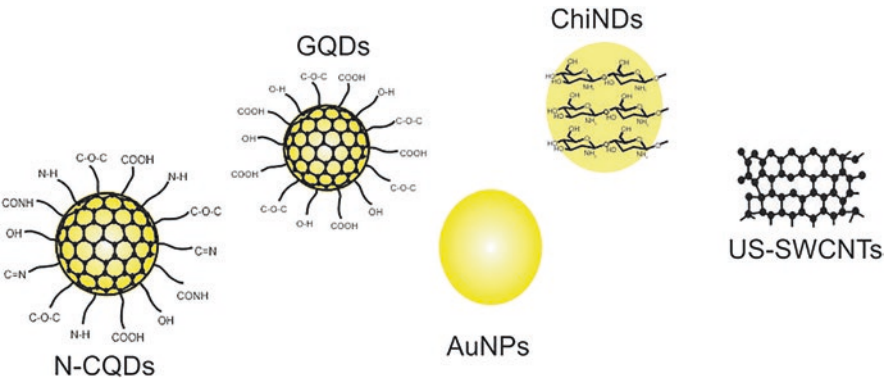

Fig. 2 Photoactive nanoparticles used potentially in APDT

polydimethylsiloxane) doped with different molecules and nanoparticles [porphyrin, methylene blue (MB), crystal violet (CV)/ZnO, Au–MB, CQDs/Ag] eradicate wide range of bacteria [*Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermis*), *Saccharomyces cerevisiae, Escherichia coli* (*E. coli*), *Bacillus subtilis* (*B. subtilis*)] effectively under visible light [12, 24–30].

CQDs and GQDs are zero dimensional carbon nanomaterials with lateral dimension smaller 10 nm. These materials have very interesting properties: high chemical stability, resistance to photo-bleaching, very good solubility in water or organic solvents, high photoluminescence and simple route for high yield synthesis—Fig. 3a, b. Most interesting biomedical property is their ability to generate ROS when

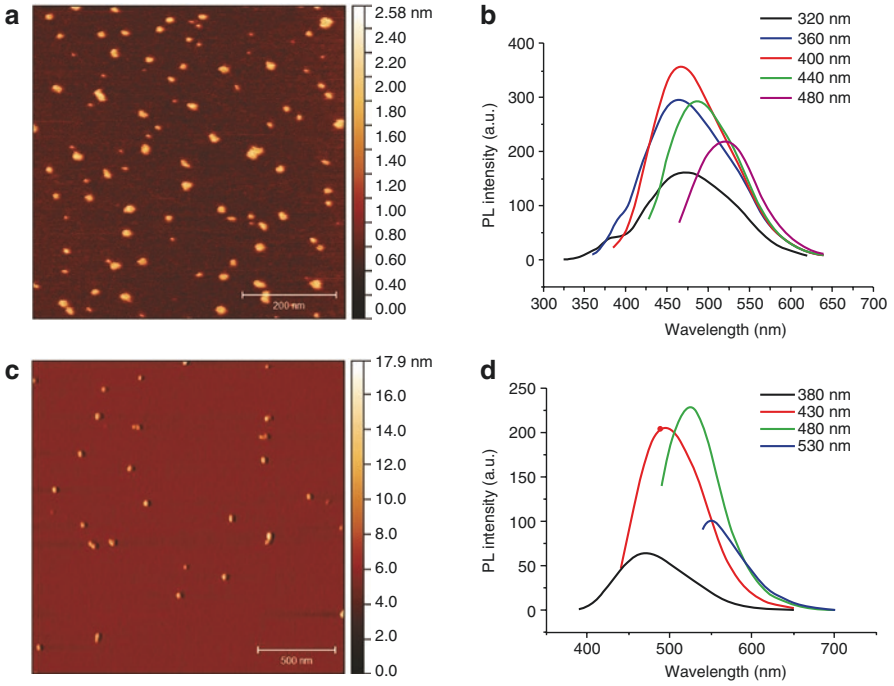

Fig. 3 (**a**) Top view AFM image of CQDs; (**b**) PL spectra of CQDs; (**c**) top view AFM image of ChiNDs; (**d**) PL spectra of ChiNDs

they are triggered by visible light and lack of cytotoxicity. Their functionalization (by different functional groups) and modification (by doping with different heteroatoms for example) contribute to improvement of ROS generation as well as reduction of energy required for triggering of ROS production [31–36]. ChiNDs are novel class of dots with lateral dimension between 20–50 nm, tunable photoluminescence and high chemical stability—Fig. 3c, d. Due to high surface/volume ratio ChiNDs should be more efficient than commercial bulk chitosan in bacteria eradication. There are only few reports on synthesis of ChiNDs by gamma irradiation [37].

AuNPs have been widely studied in biomedicine due to their unique properties and multiple surface functionalities. Spherical AuNPs possess high surface-to-volume ratio, excellent biocompatibility, low toxicity, surface plasmon resonance and ability to quench fluorescence. Hybrids of AuNPs and CQDs produce ROS better than CQD alone [38].

US-SWCNTs are ultrashort 5–10 nm segments of single-walled carbon nanotubes (SWCNTs), with average width of 1 nm and semiconducting nature [39]. They are soluble in polar organic solvents, acids, and water. This high solubility in organic solvents coupled with their short length, should enable these US-SWNTs to be dispersed and incorporated as single tubes into other materials to form

composites. Due to their similarity with CQDs and GQDs, US-SWCNTs should be potent ROS generators triggered by infrared light.

In our earlier investigation we established that pristine and doped CQDs and GQDs can be very toxic against different types of bacteria strains but only under blue light irradiation [34]. By depositing CQDs as very thin films (only 3 nm) on glass and SiO_2 substrates CQDs show good antibacterial activity against *S. aureus* and *E. coli* and moderate antibiofouling effect toward *Bacillus cereus* (*B. cereus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) under blue light [33]. By encapsulating CQDs in polyurethane and polydimethylsiloxane antibacterial activity of these nanocomposites enhances several orders of magnitude [5, 16]. Different authors reported earlier that CQDs/TiO_2, CQDs/Ag or CQDs/ZnO nanostructures as well as CQDs functionalized with (ethylenedioxy)bis(ethylamine)-EDA, N, S doped CQDs and CQDs @hematite composites show good antibacterial potentials against *S. aureus*, *E. coli*, *K. pneumoniae, B. subtilis* [30, 40–44]. CQDs/EDA nanostructures have higher fluorescence quantum yield compared to pristine CQDs and mixed with H_2O_2 show synergistic effect and thus can inhibit bacteria growth in smaller concentrations of each individual chemical [45].

3 Mechanism of Antibacterial Activity CQDs and Their Hybrids

Antibacterial activity of CQDs and their composites with different materials is based on the production of ROS. Generated singlet oxygen attacks bacterial wall membrane and contributes to lipid peroxidation. The bactericidal efficiency of CQDs/polymers depends on the lifetime of generated singlet oxygen [5, 16]. Luminescence method of singlet oxygen production indicates that luminescence of singlet oxygen come from the CQDs located in the interior of polymer matrix. Thus the contribution of the CQDs nearby polymer surface is negligible.

CQDs doping (for example with nitrogen) improves their antibacterial activity by the formation of amide and amino groups. Electrostatic interaction between protonated forms of amines and amides and the lipids of bacterial membrane induces bacterial dead [46].

In the case of CQDs/TiO_2 composites TiO_2 generates ROS-electrons of TiO_2 transfer from valence band to conduction band and thus form holes in the valence band whereas CQDs under visible light emit shorter wavelength and excite TiO_2 again [40]. Antibacterial effect of CQDs@hematite is achieved by electron–hole generation on the surface of this nanocomposite. The electrons in the conduction band react with molecular oxygen and thus produce hydroxyl radicals through an oxidative stress [44].

Agents applied in PDT should have low cytotoxicity. In our previous studies we established that CQDs had low dark cytotoxicity [47]. But it was also reported that cancer cells as well as normal cells might be less sensitive to phototoxicity of GQDs

than bacteria strain due to different level of isocitrate dehydrogenase in the cells. Singlet oxygen affects the level of isocitrate dehydrogenase and the cells with lower level of isocitrate dehydrogenase are more sensitive to death by singlet oxygen [48].

Apart from ROS generation and surface functionalization of CQDs, surface wettability and roughness affect the bacterial death. But the effect of surface roughness is limited by the shape and size of bacteria. Namely, bacteria adhere to surfaces which features correspond to their own diameters [4].

4 Conclusion

In this chapter we discussed new light triggered strategies to combat bacterial infections and possible usage of photoactive polymers for these purposes. Photoactive antibacterial polymers are highly promising solution for novel medical devices. To enable their wise usage for the treatment of urinary infections some changes must be made. For example, the effectiveness of photoactive polymers inside human body can be increased by incorporation of micron sized electronic devices (light emitting diode, light detector, pH sensor, radio frequent device) into polymer matrices. The smart medical device should have multifunctional role: the detection of biofilm formation, the eradication of the formed biofilms by APDT and transferring information to medical staff in real time.

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