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PERSPECTIVE

Nanoparticles: their potential use in antibacterial photodynamic therapy†

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Received 29th November 2010, Accepted 19th February 2011
DOI: 10.1039/c0pp00360c

Photodynamic therapy (PDT) has been proposed as a new technique to inactivate microorganisms as it does not lead to the selection of mutant resistant strains; a clear benefit compared to antibiotic treatment. PDT has also attracted the interest of nanotechnology as the effectiveness of the treatment can be greatly enhanced by the use of nanoparticles. In the last decade, different approaches to the combination of nanoparticles and PDT have been investigated in relation to the antimicrobial applications of the technique. One use of the nanoparticles is to improve the delivery of photosensitiser to the bacteria; others use the nanoparticles to improve the inactivation kinetics. A different approach utilises nanoparticles as a photosensitiser. In this review these diverse types of interactions will be described.

1. Introduction

Compounds can absorb the energy associated with an electromagnetic wave promoting electron excitation from the ground to the excited state. This state can experience a great number of processes as such excited states are unstable; the energy absorbed is quickly released and the compound returns to its original ground state. Examples of such processes are (Fig. 1): energy transfer, proton transfer, rearrangement, fluorescence, internal conversion and intersystem crossing. Fluorescence is a radiation process, whilst internal conversion and intersystem crossing are radiationless processes; in internal conversion the molecular spin state remains the same whilst it changes for intersystem crossing.[1] Photosensitisers (PS) are chemicals capable of transferring the energy absorbed to other compounds that, in turn, generate metastable species that are very reactive. These reactive species are responsible for the outcome of the process called photodynamic

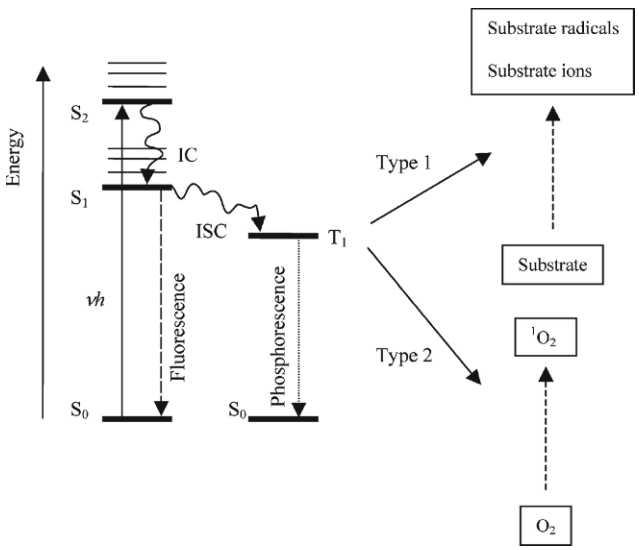

Fig. 1 Schematic representation of processes involved in photodynamic therapy. The photosensitiser in its ground state (S_0) absorbs the energy of a photon and moves to an excited state (S_n), from here, it can release the energy as radiation (fluorescence), can undergo internal conversion (IC) or intersystem crossing (ISC) to the triplet state (T_1). From the triplet state the energy is either released as radiation (phosphorescence) or transferred to the substrate (type 1) or to O_2 (type 2) returning to the ground state. The result is the direct oxidation of the substrate or the production of singlet oxygen that subsequently oxidises the substrate.

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† This article is published as part of a themed issue on immunological aspects and drug delivery technologies in PDT.

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therapy (PDT). The light source is generally a laser at the wavelength corresponding to the absorbance peak of the PS; in some applications, however, white light has been employed.[2,3]

There are two pathways for the reactions associated with PDT; in type 1 reactions, the excited PS reacts directly with the substrate oxidising it. Alternatively, the PS in the excited state can react with oxygen in the ground state (triplet oxygen, $^3\Sigma_g^-$) forming reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet}$) and $OH^{\bullet}$. This mechanism is called type 2 and the ROS are responsible for oxidising the substrate and generating the products of the PDT reaction[4] these two pathways are schematically described in Fig. 1.

There are two different singlet oxygen states, called $^1\Sigma_g^+$ and $^1\Delta_g$; they differ from the spin and orbital occupation of the two electrons in the two degenerate antibonding π_g-orbitals. $^1\Delta_g$ has 23 kcal mol^{-1} higher energy than the ground state and has a longer life time compared to $^1\Sigma_g^+$ that has 37 kcal mol^{-1} higher energy than the ground state; due to the short lifetime of $^1\Sigma_g^+$, only $^1\Delta_g$ is considered to be involved PDT[5] and is denoted $^1O_2$.

The application of PDT to cancer treatment has been widely studied and successfully applied for the treatment of oncological pathologies. Antimicrobial photodynamic therapy (APDT) has been suggested for the treatment of infections because the ROS produced during irradiation of a PS can react with microbes, resulting in their death. Further interest in the antimicrobial use of this technique is generated by the fact that the microbial inactivation is the result of a multi-target process. Cell membrane damage with consequent leakage of cytoplasm material has been reported;[6–11] moreover DNA is also affected by PDT.[12] The possibility of bacteria acquiring resistance has been assumed to be minimal because of these various targets;[13] this hypothesis has also been recently experimentally verified.[14,15]

There is a general consensus that Gram negative bacteria are less susceptible to APDT than Gram positive bacteria; this has been attributed to the different cell wall structures found in these microbes. Other microorganisms such as yeasts,[16] fungi[17,18] and viruses[19] are also vulnerable to APDT; therefore, this technique can be applied to the treatment of various kinds of infectious diseases, not just bacterial infections.

APDT has been used in dental applications such as periodontitis,[20] root canal disinfection,[21] gingivitis,[22] and oral candidosis,[16] probably due to the easy access of the light source to the mouth. Other medical applications of APDT are burn wounds[23,24] and acne.[25] All these applications are possible as the lethality towards human tissues is significantly lower than towards microbes.[26–29] More recently, PDT has been suggested also as an antibiofilm and antibiofouling[30] method; for this type of application a PS is incorporated into a solid substrate and its capacity to generate ROS is retained.

Nanoparticles have been successfully included in PDT to improve the therapy of cancer, through a combination of enhanced drug delivery and light absorption.[31] Furthermore, nanoparticles have been prepared for diagnostic assay based on PDT.[32]

There is a vast literature concerning the application of nanoparticles in cancer treatment[4] but little has been done on the antimicrobial aspects of such interactions. The drive in the development of non-antibiotic-based approaches for treating infectious diseases has been instrumental in expanding the application of antimicrobial techniques such as PDT. The increasing isolation of bacterial species showing resistance to antibiotics is a growing concern for health authorities around the world; great effort has been dedicated to the improvement of the performance of APDT through the design of new PS or using nanoparticles. In this review we describe the different approaches used to enhance the performance of APDT processes in coupling nanotechnology to PDT.

2. Photodynamic therapy and nanoparticles

Nanoparticles have been recently used in APDT to enhance the effectiveness of the treatment. Four different types of interactions between nanoparticles and PS have been reported:

- PS embedded in polymeric nanoparticles
- PS bound to the surface of nanoparticles
- PS alongside nanoparticles
- nanoparticles as the PS

This classification is consistent with that proposed by Chatterjee *et al.*[33] of active (nanoparticle as the PS) and passive nanoparticles (the other three). Active nanoparticles can be divided further in biodegradable (PLA and PLGA, liposomes) and non-biodegradable types (gold, silica). Each of these will be individually described in this review, only for combinations that have been proved to act against microorganisms. Studies where nanoparticle-PS combinations were prepared but their antimicrobial activities were not tested will not be covered.

2.1. PS embedded in polymeric nanoparticles

Nanoparticles loaded with PS have been used as carrier to deliver the PS into microorganisms and improve antimicrobial performance; particular attention has been placed on biocompatible and biodegradable matrixes such as liposomes,[34–38] polylactic-glycolic acid (PLGA)[39] or cyclodextrins,[40] because the application of APDT is likely to be in medical (wounds, surface sterilisation) or environmental situations (food industry, water purification).

Nanoparticles containing PS have several advantages over free PS:[41]

[1] larger concentrations of PS (critical mass) for the production of lethal reactive oxygen species;

[2] reduced ability of the target cell to pump out the PS, hence reducing the possibility of multidrug resistance;

[3] selectivity of treatment by localized delivery agents, achieved through either passive targeting or by active targeting (charging of the nanoparticle surface);

[4] the nanoparticle matrix is non-immunogenic.

Further benefits of the encapsulation of PS in nanoparticles have been proposed:

[5] stopping the PS from dimerising and trimerising as occurs in the free state, these forms are ineffective;[42]

[6] if the PS is not soluble in water, the encapsulation can be used to enhance solubility.[43]

Two mechanisms of action were postulated; in one, the nanoparticles disrupted the three-dimensional organisation of the cell wall increasing its permeability to the PS. In the other, the photo-inactivation occurs directly in the cell wall.[39] The improved penetrability of PS seems more likely as the PS can be recovered from the cells[36,37] and the disruption of the membrane with EDTA

or induction of competence rendered bacteria more vulnerable to APDT.[35]

The surface charge and the fluidity (in physiological conditions) of the liposomes containing the PS are determinant characteristics of the antimicrobial properties of such delivery vehicles.[34,36,37] Monocationic lipid *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methylsulfate (DOTAP), as well as neutral DL-α-dipalmitoyl-phosphatidyl-choline (DPPC) and L-α-dimiristoyl-phosphatidyl-choline (DMPC), have been used to delivery porphyrin-based PS against methicillin-resistant *Staphylococcus aureus* (MRSA) and DOTAP has consistently returned the highest inactivation results. The comparison of the activity against *Escherichia coli* of porphyrins delivered using neutral liposomes (DPPC) was found to be lower than the activity of the free PS.[34,35] Therefore, cationic liposomes are more effective than neutral ones in APDT possibly because their charge is opposite to the negatively charged cell wall. This electrostatic attraction brings the liposomes and the microbial cells close and facilitate the delivery of the PS; for the same reason cationic PS[44] are more effective in APDT than neutral or anionic ones. Using cationic liposomes, also non-cationic PS could be used in APDT[36] as the neutral charge of the PS would be "masked" by the liposome surface charge. The encapsulation of porphyrin in neutral liposomes (DPPC) resulted in a lower amount of PS recovered from the cells, and also made the attachment to bacteria weaker, as the PS was easier to wash out than if delivered as a free solution.[34]

Another example of encapsulation was provided by Tsai *et al.*[45] using hematoporphyrin in liposomes or micelles; the encapsulation prevented the PS aggregation resulting in a higher antimicrobial effect against a series of Gram positive bacteria. The micelles prepared were smaller and resulted in more effective treatments than the liposomes; this was regarded as advantageous as micelles are easier and cheaper to prepare.

Another important parameter of the liposomes used in APDT is the zeta potential of the nanoparticles; if this is close to zero the liposomes tend to aggregate and the antimicrobial properties are reduced.[40] As a counterpoint, if this is too high (>40 mV), dark toxicity is also present.[38] A negative zeta potential results in repulsion between bacterial cells and nanoparticles.

Poly(lactic-glycolic acid) (PLGA)[39] and polyacrilamide (PAA)[46] nanoparticles containing methylene blue (MB) have been prepared and shown to inactivate both planktonic and biofilm cells. Pagonis *et al.*[39] demonstrated how these concentrated on the surface of *Enterococcus faecalis* (Fig. 2), suggesting that the mechanism of action is an increased delivery of PS through the cell wall; similar images were presented by Ferro *et al.*[40] for *E. coli*.

Wu *et al.*[46] showed that MB-loaded particles have antimicrobial properties against a range of both Gram positive and Gram negative bacteria; the latter were also more resistant to the treatment when in the planktonic state but the inactivations were comparable when biofilms were analysed. The PLGA nanoparticles, but not the PAA, had dark toxicity but the microbial inactivation was greater when irradiated with light.

Calcium phosphate, used as the core of nanoparticles functionalised with different polymers containing MB and porphyrin, has been used against *S. aureus* and *Pseudomonas aeruginosa*.[43] Another layer of a different polymer was also applied to these nanoparticles in order to change the surface charge. As for the liposomes, the surface charge was not influential in the inactivation of *S. aureus*, but was a determining factor for *P. aeruginosa* that could only be reduced with positively charged nanoparticles. Another important benefit of this type of encapsulation was that the porphyrin used in this study was effective against *P. aeruginosa* only if on the surface of the nanoparticles and completely ineffective if free in solution. Therefore, the use of calcium phosphate nanoparticles is essential to obtain the phototoxic effect.

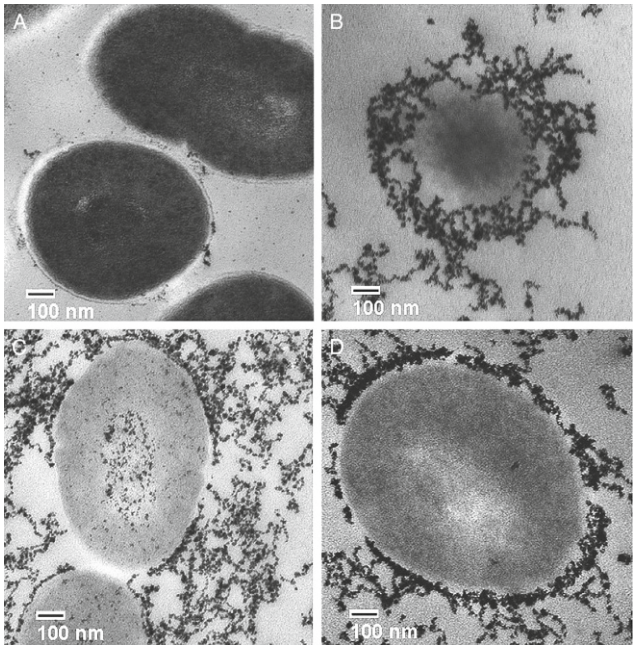

Fig. 2 TEM images of nanoparticles containing MB adhering to the surface of *E. faecalis* after (A) 0 min, (B) 2.5 min, (C) 5 min and (D) 10 min of incubation. Reprinted from T. C. Pagonis, J. Chen, C. R. Fontana, H. Devalapally, K. Ruggiero, X. Song, F. Foschi, J. Dunham, Z. Skobe, H. Yamazaki, R. Kent, A. C. R. Tanner, M. M. Amiji and N. S. Soukos, Nanoparticles-based Endodontic Antimicrobial Photodynamic therapy, *J. Endod.*, **36**(2), 322–328. Copyright 2010, with permission from American Association of Endodontists.

2.2. PS bound to the surface of nanoparticles

PS have been covalently bound to the surface of nanoparticles to prepare what, in essence, is a new PS with better properties than the original PS; this is the main difference of this approach compared to the previous PS encapsulation, which is an improved delivery method. A few reports of antibacterial PS linked to nanoparticles are available: rose bengal (RB) has been linked to glass[47] and polystyrene,[48] toluidine blue (TBO) has been bound to the surface of Au nanoparticles,[49] and porphyrin has been bound to carbon nanotubes.[50]

The first report of nanoparticles immobilised on the surface of a nanoparticle was by Bezman *et al.*,[48] who linked RB to polystyrene particles. They showed that RB-polystyrene particles exposed to white light were capable of inactivating *E. coli*, whilst *S. aureus* has been inactivated with porphyrin–carbon nanotubes, Au-TBO and RB-silica particles have also been shown to be effective against *S. epidemidis*. *E. coli* was killed by RB-polystyrene but not by carbon nanotubes.

In order to bind the PS to the nanoparticle surface, both the PS and the nanoparticle surface have to exhibit some reactive groups where the linking can occur. Generally, nanoparticles do not have such moieties; therefore, the synthesis of such conjugates has been carried out first by functionalising the surface of the nanoparticles with tiopronin,[49] with amine groups,[47] chloromethyl groups[48] or carboxylic groups (acid-functionalisation).[50] The second step is the reaction between the functionalised group on the nanoparticle surface and the PS. In the case of porphyrins, the PS needed to be preliminary functionalised.[50] This way of covalently binding a PS to the surface of the material has been used also in the surface functionalisation of silicone[51] and nylon.[52] The use of tiopronin to functionalise Au nanoparticles is shown in Fig. 3.

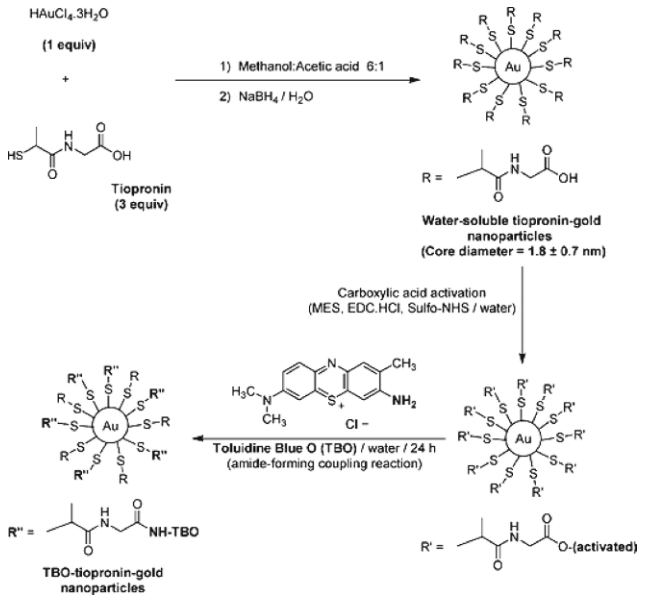

Fig. 3 Synthesis scheme of Au–tiopronin–TBO nanoparticles. J. Gil-Thomas, S. Tubby, I. P. Parkin, N. Narband, L. Dekker, S. P. Nair, M. Wilson and C. Street, *J. Mater. Chem.*, 2007, **17**, 3739–3746. Reproduced by permission of the Royal Society of Chemistry.

The inactivation outcome was greater when the same amount of PS was covalently bound to the particles as free in solution (Fig. 4). Possible explanations for such enhancement are: bacteria can bind the nanoparticles and, therefore, are more easily exposed to the lethal ROS produced by the PS; alternatively, the PS-nanoparticle conjugates generate a greater quantity of ROS. The latter hypothesis was disproved by Guo *et al.*[47] for RB-silica as the quantum yield of singlet oxygen production by pure RB was higher at the beginning of the irradiation than RB-silica (Fig. 5). However, the decay (photobleaching) of RB-silica nanoparticles was slower than RB suggesting that, despite not being initially as effective as free RB, silica nanoparticles coated with RB could provide antimicrobial efficacy for a longer period of time.[47]

It is also interesting to point out that when the PS is linked to the nanoparticles the distribution is not uniform in the medium but is locally concentrated. Consequently, the ROS produced are not homogeneously distributed in the medium either; it is reasonable to speculate that the locally concentrated ROS, caused by PS-nanoparticles, can have a higher lethal effect than in the case of free PS, although at lower overall concentrations.

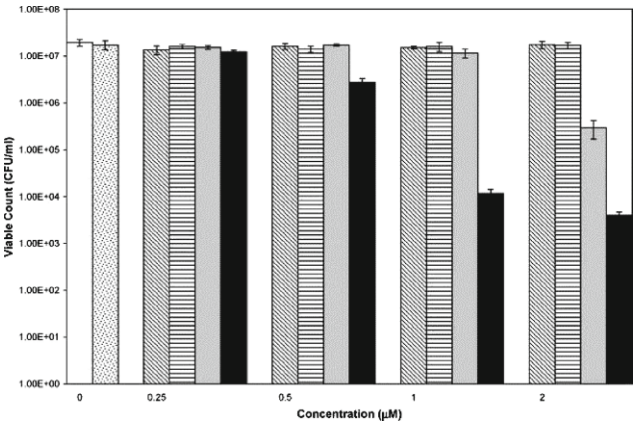

Fig. 4 Effect of TBO (grey bars) and the TBO–tiopronin–gold nanoparticle conjugate (black bars) on viability of *S. aureus* following exposure to 633 nm laser light for 1 min, or incubation in the dark with TBO (diagonal striped bars) or the TBO–tiopronin–gold nanoparticle conjugate (horizontal striped bars). The white bar denotes the viable count of the original bacterial suspension, and the dotted bar represents the viable count of the bacterial suspension after exposure to 633 nm laser light alone. J. Gil-Thomas, S. Tubby, I. P. Parkin, N. Narband, L. Dekker, S. P. Nair, M. Wilson and C. Street, *J. Mater. Chem.*, 2007, **17**, 3739–3746. Reproduced by permission of the Royal Society of Chemistry.

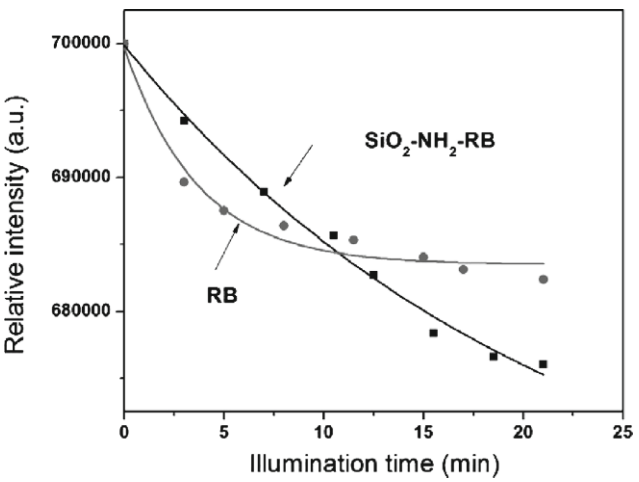

Fig. 5 Fluorescence change of disodium salt of 9,10-anthracenedipropionic acid (ADPA) due to singlet oxygen produced by RB and RB-silica nanoparticles at different irradiation times. Y. Guo, S. Rogelj and P. Zhang, Rose Bengal-decorated silica nanoparticles as photosensitizers for inactivation of gram-positive bacteria, *Nanotechnology*, 2010, **21**, 065102, DOI: 10.1088/0957-4484/21/6/065102. © 2010 IOP Publishing Ltd.

It has been demonstrated that both bacterial membrane disruption and penetration of the PS-nanoparticles through the membrane are highly unlikely to be the reason for the increased antibacterial properties of PS-nanoparticles.[48] This was studied with RB-silica using much larger nanoparticles (200–400 mesh) than for RB-glass (50–80 nm)[47] or Au-TBO (2–3 nm).[49] However, free PS can always cross the bacterial membrane, hence, even if absorption of PS-nanoparticles can occur, this is an improbable cause of lethal photosensitisation of microorganisms.

Interestingly, it has been suggested by Banerjee *et al.*[50] that the binding of insoluble PS to the surface of water-soluble

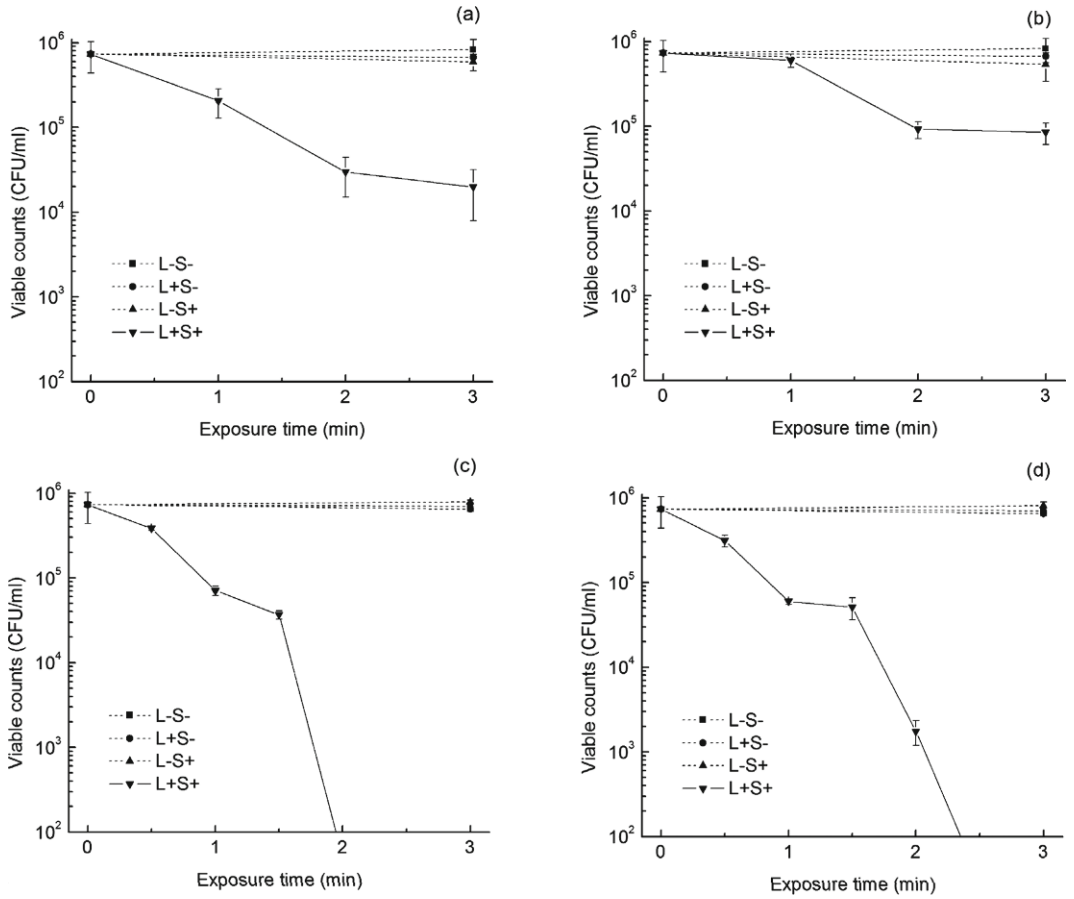

Fig. 6 Inactivation of *E. coli* on silicone containing (a) TBO, (b) TBO + Au nanoparticles, (c) polyurethane containing TBO and (d) TBO + Au nanoparticles (L+ = laser irradiation, L– = no laser, S+ = with TBO, S– = without TBO). S. Perni, P. Prokopovich, C. Piccirillo, J. R. Pratten, I. P. Parkin and M. Wilson, *J. Mater. Chem.*, 2009, **19**(17), 2715–2723. Reproduced by permission of the Royal Society of Chemistry.

nanoparticles can produce a water soluble dye, allowing the use of a wider range of PS for antimicrobial applications.

A different approach, involving the use of a viral protein cage to bind a PS, has been proposed by Suci *et al.*[53] and antimicrobial properties demonstrated against *S. aureus*. They used a genetically modified virus to produce a viral cage presenting cysteines instead of serine, then a ruthenium-based PS, Ru(bpy2)phen-IA, was covalently bound to the cysteine residue. The benefit of using a protein cage structure is the possibility of dual functionalising of the nanoplatform to achieve both selectivity (possibly with antibodies) and more effective antimicrobial properties.

2.3. PS-accompanying nanoparticles

In this kind of interaction the nanoparticles are too big to penetrate the bacterial cell wall, and therefore the possible mechanisms are thought to be physical/chemical interactions between PS and nanoparticles in the microbial surroundings.

Nanoparticles, predominantly Au, in combination with PS have been employed to achieve greater bacterial killing through PDT. Studies have employed TBO or MB in solution or embedded in polymeric matrixes such as silicone and polyurethane.[54–57]

The encapsulation of TBO and Au nanoparticles into polymer matrixes, such as silicone and polyurethane, seems to prevent interaction between the PS and the nanoparticle as the inactivation of both *E. coli* and MRSA deposited onto polymers containing such components is not different from that of the same material containing only the PS[57] (Fig. 6). On the contrary, the antimicrobial properties of MB are improved by Au nanoparticles.[54,56] This could be due to some interaction of the matrix or from the prevention of the adsorption of TBO onto the nanoparticle surface during the encapsulation process.

However, silicone containing MB that went through the same swell encapsulation procedure did show antibacterial characteristics that were improved when Au nanoparticles were added.[56] Therefore, the interaction of the polymeric matrix seems an unlikely explanation; a different behaviour between MB and TBO, despite their close structural similarities, could be the reason for the opposite effect of the Au nanoparticles. Differences between these PS have also been highlighted by the larger amount of MB encapsulated in silicone compared to TBO.

Narband *et al.*[58] demonstrated that adding Au nanoparticles to a solution of TBO improved the inactivation kinetics of *S. aureus* on irradiation. They found that nanoparticles of 2 and 15 nm diameter achieved greater bacterial inactivation depending on the ratio of TBO to nanoparticles. The reasons for such improved lethal properties were speculated to be a higher light capture of the PS when adsorbed on the surface of the nanoparticle and a different decay of PS from the "excited state" to the ground state. This is through a pathway leading to the formation of ROS

(*e.g.* hydroxyl radicals) different from singlet oxygen, the yield of which appeared to be reduced in the presence of Au nanoparticles. The improved light capture hypothesis was demonstrated later by Narband *et al.*;[59] in this study the extinction coefficient of a variety of thiazine cationic dyes (TBO and MB being the main members of such group and these are known antibacterial PS) is greater in the presence of Au nanoparticles. In the same study, other anionic PS did not show such behaviour; as the nanoparticles are positively charged, it appears that adsorption of the PS onto the surface of the nanoparticles, caused by electrostatic attraction, is essential for the enhancement of the PDT outcome.

The possibility that Au nanoparticles interacted as catalysts was proposed by Perni *et al.*;[60] the inactivation of *E. coli* and *S. epidermidis* on silicone containing MB was differently influenced by the presence of Au nanoparticles of different size. Nanoparticles of 2 nm diameter were the only ones demonstrating an improved inactivation process, bigger particles 5, 10 and 20 nm in diameter returned progressively worse antimicrobial outcomes (Fig. 7). As Au nanoparticles of 2 nm are the most effect catalysts of oxidation reaction, Perni *et al.*[60] speculated that Au nanoparticles improve the antibacterial efficacy catalysing the reactions resulting in bacterial death.

Xing *et al.*[61] proposed a different approach to the combination of nanoparticles and PS. They chose an anionic particle (poly-thiophene) to electrostatically bind a cationic porphyrin, and showed an improved antibacterial effect against *E. coli* over that of the free porphyrin. The electrostatic interaction removed the need for a covalent linking step in the preparation; furthermore, poly-thiophene improved the singlet oxygen yield of the porphyrin enhancing the antimicrobial properties.

Quantum dots (QD) made of CdSe/ZnS were used to improve the APDT of TBO;[62] the effect depends on the concentration of quantum dots and is beneficial to the bacterial inactivation only at low quantum dot concentrations. QD increase the photosensitizing ability of TBO through absorbing light with a wavelength less of 488 nm and upshifting this *via* an emission process to approximately 627 nm that is close to the absorption maximum of the TBO. FRET (Förster resonance energy transfer) is the mechanism where excitation energy is transferred to a neighbouring compound in a radiationless process. The fluorescence found for mixtures of QD and TBO was a non-FRET mechanism as the interaction was only possible through a radiation process because the separation between compartments was far greater than that needed in a FRET mechanism.[61] There appears to be another role for quantum dots: they suppress the formation of singlet oxygen from the excited TBO molecules; in the presence of QD more TBO is found in the excited state, but its relaxation to the ground state is not through the formation of singlet oxygen but through other cytotoxic molecular species as such hydroxyl radicals that responsible for the bacterial inactivation observed.

2.4. Nanoparticles as the PS

TiO_2 has been known to be capable of photo-oxidation for a long time. However, the main obstacle to the use of TiO_2 nanoparticles for medical applications was that their absorption is essentially in the UV region of the electromagnetic spectrum. The application of TiO_2 for the disinfection of water contaminated with *E. coli* was shown by Sanabria *et al.*;[63] in this case the light source was the sun, removing the need for a UV lamp and the health and safety risks associated with such radiation in medical applications.

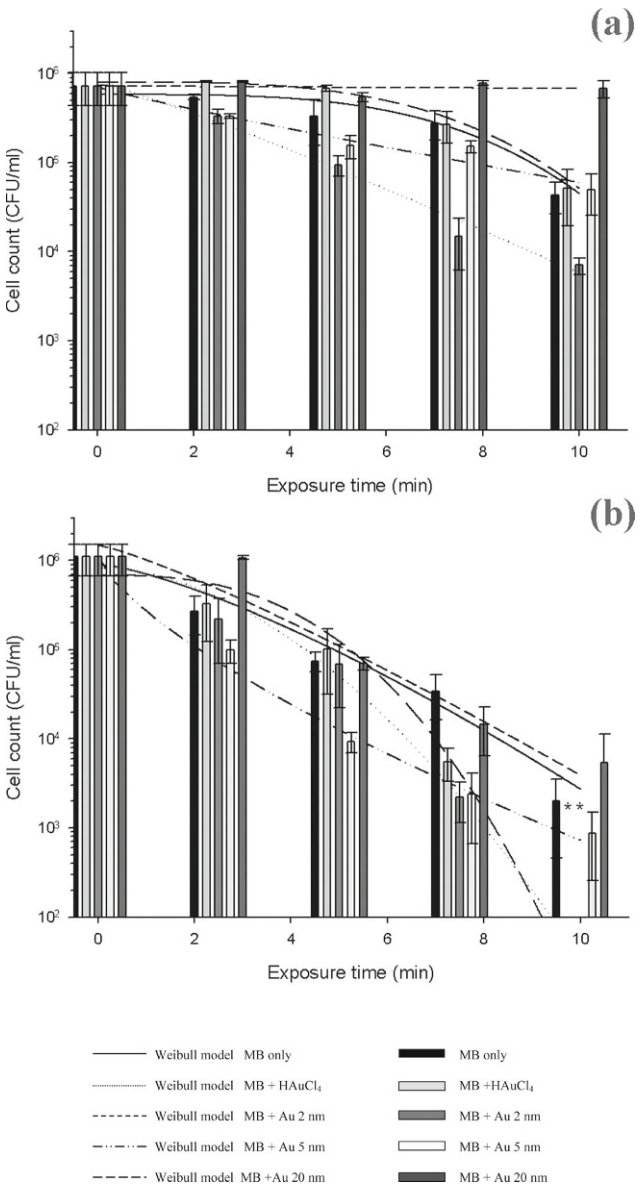

Fig. 7 Inactivation of (a) *E. coli* and (b) *S. epidermidis* on silicone containing MB or MB and Au nanoparticles of different size. With kind permission from Springer Science + Business Media: S. Perni, C. Piccirillo, A. Kafizas, M. Uppal, J. Pratten, M. Wilson and I. P. Parkin, Antibacterial Activity of Light-Activated Silicone Containing Methylene Blue and Gold Nanoparticles of Different Sizes, *J. Cluster Sci.*, 2010, **21**(3), 427–438.

Most of the focus of research has been in shifting the absorbance spectrum of TiO_2 towards the visible region through doping with other elements; Er^{3+} and Yb^{3+} have been used by Wang *et al.*[64] together with Fe^{3+}, the results highlight that TiO_2-Er^{3+}-Yb^3-Fe^{3+} nanopowder was able to inactivate *A. hydrophia* under IR (980 nm), whilst TiO_2-Er^{3+}-Yb^3 did not reduce the number of viable bacteria. Another element used to dope TiO_2 bringing the absorption in the visible part of the spectra was Ag.[65] These nanoparticles had dark toxicity caused by Ag, but under irradiation with white light the microbial reduction was greater than in the dark, demonstrating the photoinduced lethal process.

Fullerenes are the third type of carbon structure; they consist of 60 carbon atoms arranged in a spherical structure. They can absorb light and have been shown to be active PS;[66] they generate different ROS according to the solvent, in polar solvents they produce superoxide and hydroxyl radicals, whilst in non polar solvents they predominately generate singlet oxygen. Many studies have focused on the possible functionalisation of fullerenes making them more soluble in water or other biological fluids.[67]

Tegos *et al.*[68] were the first to show the possibility of APDT with fullerenes functionalised with six different cationic compounds, and examples of the structures of such compounds are shown in Fig. 8. Alcohol functionalised fullerenes were less effective than cationic functionalised fullerenes, however the cationic ones exhibited a higher dark toxicity. Cationic functionalised fullerenes at a concentration low enough to result in minimal dark toxicity were still highly effective PS against both *S. aureus* and *E. coli*. Furthermore, they were more effective than TBO and did not induce photodamage in mammalian cells under the conditions achieving 4–6 $\log_{10}$ bacterial reduction (2 J cm^{-2} under white light).

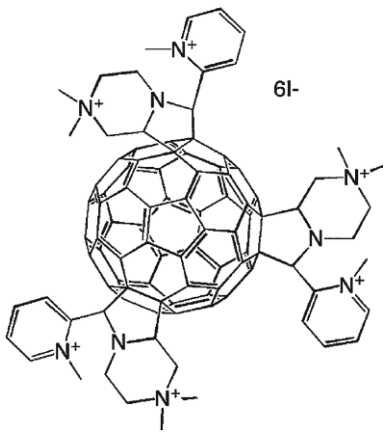

Fig. 8 Structure of fullerene with attached photosensitisers. Reprinted from L. Huang, M. Terakawa, T. Zhiyentayev, Y. Y. Huang, Y. Sawayama, A. Jahnke, G. P. Tegos, T. Wharton and M. R. Hamblin, Innovative cationic fullerenes as broad-spectrum light-activated antmicrobials, *Nanomed.: Nanotechnol., Biol. Med.*, 2010, **6**, 442–452. Copyright 2010, with permission from Elsevier.

A different functionalisation was performed by Spesia *et al.*.[69] They prepared a non-charged *N*-methyl-2-(40-acetamidophenyl)fulleropyrrolidine (MAC_{60}) and a dicationic *N*,*N*-dimethyl-2-(40-*N*,*N*,*N*-trimethylaminophenyl) fulleropyrrolidinium iodide (DTC_{60}^{2+}). They found that DTC_{60}^{2+} reduced *E. coli* about 3.5 $\log_{10}$ after 30 min irradiation under white light.

Recently, additional cationic fullerenes have been synthesised[70] and their antimicrobial properties under light assessed against bacteria and yeasts. All these results also confirmed how the surface charge of the PS is essential for the binding of the PS to the bacterial cells, particularly of Gram negative bacteria, to achieve effective photosensitisation of the target cells. The importance of the proximity of the PS to the target organism in APDT was also shown using viruses.[71]

3. Conclusions

The enhancement of APDT using nanoparticles is a vibrant research area inspired by the need to minimise the use of antibiotics in the treatment of infectious diseases. APDT should not result in resistant mutants because of the multi-target mechanism of killing. Nanoparticles have improved the efficacy of APDT in different ways, enhancing the delivery of PS to microorganisms (encapsulating the PS in nanoparticles) or increasing the $^1O_2$ yield of the PS (covalently binding the PS to the surface of the nanoparticles or simply mixing nanoparticles and PS). Inorganic nanoparticles (TiO_2 and fullerenes) have also been shown capable of photodynamically inactivating microorganisms.

Acknowledgements

JP, MW and IPP thank the BBSRC for supporting this work through grant BB/E012310/1. IPP thanks the Royal Society/Wolfson Trust for a merit award. SP thanks the Wellcome Trust for a ViP Fellowship.

References

1 B. Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, 2001.
2 T. Maisch, R. M. Szeimies, G. Jori and C. Abels, Antibacterial Photodynamic Therapy in Dermatology, *Photochem. Photobiol. Sci.*, 2004, **3**(10), 907–917.
3 M. R. Hamblin and T. Hasan, Photodynamic Therapy: a New Antimicrobial Approach to Infectious Disease?, *Photochem. Photobiol. Sci.*, 2004, **3**(5), 436–450.
4 D. E. J. G. J. Dolmans, D. Fukumura and R. K. Jain, Photodynamic Therapy for Cancer, *Nat. Rev. Cancer*, 2003, **3**, 380–387.
5 M. C. DeRosa and R. J. Crutchley, Photosensitized singlet oxygen and its applications, *Coord. Chem. Rev.*, 2002, **233–234**, 351–371.
6 B. B. Fuchs, G. P. Tegos, M. R. Hamblinand and E. Mylonakis, Susceptibility of *Cryptococcus neoformans* to Photodynamic Inactivation is Associated with Cell Wall Integrity, *Antimicrob. Agents Chemother.*, 2007, **51**(8), 2929–2936.
7 M. Schäfer, C. Schmitz, R. Facius, G. Horneck, B. Milow, K. H. Funken and J. Ortner, Systematic Study of Parameters Influencing the Action of Rose Bengal with Visible Light on Bacterial Cells: Comparison Between the Biological Effect and Singlet-Oxygen Production, *Photochem. Photobiol.*, 2000, **71**(5), 514–523.
8 M. Schäfer, C. Schmitzand and G. Horneck, High Sensitivity of *Deinococcus radiodurans* to Photodynamically Produced Singlet Oxygen, *Int. J. Radiat. Biol.*, 1998, **74**(2), 249–253.
9 S. Ferro, L. Guidolin, G. Tognon, G. Joriand and O. Cappellotti, Mechanisms involved in the photosensitized inactivation of *Acanthamoeba palestinensis* trophozoites, *J. Appl. Microbiol.*, 2009, **107**, 1615–1623.
10 N. A. Romanova, L. Y. Brovko, L. Moore, E. Pometun, A. P. Savatsky, N. N. Ugarova and M. W. Griffith, Assessment of Photodynamic Destruction of *Escherichia coli* O157:H7 and *Listeria monocytogenes* by Using ATP Bioluminescence, *Appl. Environ. Microbiol.*, 2003, **69**(11), 6393–6398.
11 Z. Malik, T. Babushkin, S. Sher, J. Hanania, H. Ladan, Y. Nitzanand and S. Salzberg, Collapse of K + and Ionic Balance During Photodynamic Inactivation of Leukemic-cells, Erythrocytes and *Staphylococcus aureus*, *Int. J. Biochem.*, 1993, **25**(10), 1399–1406.
12 S. Menezes, M. A. M. Capellaand and L. R. Caldas, Photodynamic Action of Methylene blue: Repair and Mutation in *Escherichia coli*, *J. Photochem. Photobiol., B*, 1990, **5**, 505–517.
13 M. Wilson, Lethal Photosensitisation of Oral Bacteria and its Potential Application in the Photodynamic Therapy of Oral Infections, *Photochem. Photobiol. Sci.*, 2004, **3**, 412–418.
14 F. Giuliani, A. C. Martinelli, D. Arbia, L. Fantettiand and G. Roncucci, In Vitro Resistance Selection Studies of RLP068/Cl, a New Zn(II) Phthalocyanine Suitable for Antimicrobial Photodynamic Therapy, *Antimicrob. Agents Chemother.*, 2009, **54**(2), 637–642.
15 A. Tavares, C. M. B. Carvalho, M. A. Faustino, G. P. M. S. Neves, J. P. C. Tomé, A. C. Tomé, J. A. S. Cavaleiro, A. Cunha, N. C. M. Gomes, E. Alves and Almeida, Antimicrobial Photodynamic Therapy: Study of Bacterial Recovery Viability and Potential Development of Resistance after Treatment, *Mar. Drugs*, 2010, **8**, 91–105.

16 E. G. D. Mima, A. C. Pavarina, L. N. Dovigo, C. E. Vergani, C. A. D. Costa, C. Kurachi and V. S. Bagnato, Susceptibility of *Candida albicans* to Photodynamic Therapy in a Murine Model of Oral Candidosis, *Oral Surg., Oral Med., Oral Pathol., Oral Radiol. Endodontol.*, 2010, **109**(3), 392–401.
17 F. P. Gonzales, S. H. da Silva, D. W. Roberts and G. U. L. Braga, Photodynamic Inactivation of Conidia of the Fungi *Metarhizium anisopliae* and *Aspergillus nidulans* with Methylene Blue and Toluidine Blue, *Photochem. Photobiol.*, 2010, **86**(3), 653–661.
18 R. C. Souza, J. C. Junqueira, R. D. Rossoni, C. A. Pereira, E. J. Munin and O. C. Antonio, Comparison of the Photodynamic Fungicidal Efficacy of Methylene Blue, Toluidine Blue, Malachite Green and Low-power Laser Irradiation Alone Against *Candida albicans*, *Lasers Med. Sci.*, 2009, **25**(3), 385–389.
19 T. W. Wong, H. J. Huang, Y. F. Wang, Y. P. Lee, C. C. Huang and C. K. Yu, Methylene Blue Mediated Photodynamic Inactivation as a Novel Disinfectant of Enterovirus 71, *J. Antimicrob. Chemother.*, 2010, **65**(10), 2176–2182.
20 R. Andersen, N. Loebel, D. Hammond and M. Wilson, Treatment of periodontal disease by photodisinfection compared to scaling and root planning, *J. Clin. Dent.*, 2007, **18**, 34–38.
21 A. S. Garcez, M. S. Ribeiro, G. P. Tegos, S. C. Nunez, A. O. C. Jorge and M. R. Hamblin, Antimicrobial Photodynamic Therapy Combined with Conventional Endodontic Treatment to Eliminate Root Canal Biofilm Infection, *Lasers Surg. Med.*, 2007, **39**(1), 59–66.
22 M. Raghavendra, A. Koregol and S. Bhola, Photodynamic Therapy: a Targeted Therapy in Periodontics, *Aust. Dent. J.*, 2009, **54**, S102–S109.
23 T. H. Dai, G. P. Tegos, Z. S. Lu, L. Y. Huang, T. Zhiyentayev, M. J. Franklin, D. G. Baer and M. R. Hamblin, Photodynamic Therapy for *Acinetobacter baumannii* Burn Infections in Mice, *Antimicrob. Agents Chemother.*, 2009, **53**(9), 3929–3934.
24 T. Dai, Y. Y. Huang, S. K. Sharma, J. T. Hashmi, D. B. Kurup and M. R. Hamblin, Topical Antimicrobials for Burn Wound Infections, *Recent Pat. Anti-Infect. Drug Discovery*, 2010, **5**(2), 124–151.
25 K. Degitz, G. Plewig and H. Gollnick, Adjunctive Acne Therapies, *J. Dtsch. Dermatol. Ges.*, 2010, **8**, S75–S80.
26 M. Soncin, C. Fabris, A. Busetti, D. Dei, D. Nistri, G. Roncucci and G. Jori, Approaches to Selectivity in the Zn(II)-Phthalocyanine Photosensitized Inactivation of Wild Type and Antibiotic Resistant *Staphylococcus aureus*, *Photochem. Photobiol. Sci.*, 2002, **1**, 815–819.
27 B. Zeina, J. Greenman, D. Corry and W. M. Purcell, Cytotoxic Effects of Antimicrobial Photodynamic Therapy on Keratinocytes in Vitro, *Br. J. Dermatol.*, 2002, **146**, 568–573.
28 B. Zeina, J. Greenman, D. Corry and W. M. Purcell, Antimicrobial Photodynamic Therapy: Assessment of Genotoxic Effects on Keratinocytes in Vitro, *Br. J. Dermatol.*, 2003, **148**, 229–232.
29 T. Maisch, C. Bosl, R. M. Szeimies, N. Lehn and C. Abels, Photodynamic Effects of Novel XF Porphyrin Derivatives on Prokaryotic and Eukaryotic Cells, *Antimicrob. Agents Chemother.*, 2005, **49**(4), 1542–1552.
30 S. Perni, P. Prokopovich, I. P. Parkin, M. Wilson and J. Pratten, Prevention of Biofilm Accumulation on a Light-activated Antimicrobial Catheter Material, *J. Mater. Chem.*, 2010, **20**(39), 8668–8673.
31 Y. Cheng, A. C. Samia, J. D. Meyers, I. Panagopoulos, B. Fei and C. Burda, Highly Efficient Drug Delivery with Gold Nanoparticle Vectors for in Vivo Photodynamic Therapy of Cancer, *J. Am. Chem. Soc.*, 2008, **130**, 10643–10647.
32 P. K. Jain, X. Huang, I. H. El-Sayed and M. A. El-Sayed, Review of Some Interesting Surface Plasmon Resonance Enhanced Properties of Noble Metal Nanoparticles and Their Applications to Biosystems, *Plasmonics*, 2007, **2**, 107–118.
33 D. K. Chatterjee, L. S. Fong and Y. Zhang, Nanoparticles in Photodynamic Therapy: An Emerging Paradigm, *Adv. Drug Delivery Rev.*, 2008, **60**, 1627–1637.
34 M. Merchant, J. D. Spikes, G. Bertoloni and G. Jori, Studies on the Mechanism of Bacteria Photosensitisation by Meso-substituted Cationic Porphyrins, *J. Photochem. Photobiol., B*, 1996, **35**, 149–157.
35 G. Bertoloni, F. Rossi, G. Valduga, G. Jori and J. van Lier, Photosensitizing Activity of Water- and Lipid- Soluble Phthalocyanines on *Escherichia coli*, *FEMS Microbiol. Lett.*, 1990, **71**(1–2), 149–156.
36 S. Ferro, F. Ricchelli, G. Mancini, G. Tognon and G. Jori, Inactivation of Methicillin-Resistant *Staphylococcus aureus* (MRSA) by Liposome Delivered Photosensitising Agents, *J. Photochem. Photobiol., B*, 2006, **83**, 98–104.
37 S. Ferro, F. Ricchelli, D. Monti, G. Mancini and G. Jori, Efficient Photoinactivation of Methicillin-Resistant *Staphylococcus aureus* by a Novel Porphyrin Incorporated into a Poly-cationic Liposome, *Int. J. Biochem. Cell Biol.*, 2007, **39**, 1026–1034.
38 C. Bombelli, F. Bordi, S. Ferro, L. Giansanti, G. Jori, G. Mancini, C. Mazzuca, D. Monti, F. Ricchelli, S. Sennato and M. Venanzi, New Cationic Liposomes as Vehicles of *m*-Tetrahydroxyphenylchlorin in Photodynamic Therapy of Infectious Diseases, *Mol. Pharmaceutics*, 2008, **5**(4), 672–679.
39 T. C. Pagonis, J. Chen, C. R. Fontana, H. Devalapally, K. Ruggiero, X. Song, F. Foschi, J. Dunham, Z. Skobe, H. Yamazaki, R. Kent, A. C. R. Tanner, M. M. Amiji and N. S. Soukos, Nanoparticle Based Endodontic Antimicrobial Photodynamic Therapy, *J. Endod.*, 2010, **36**(2), 322–328.
40 S. Ferro, G. Jori, S. Sortino, R. Stancanelli, P. Nikolov, G. Tognon, F. Ricchelli and A. Mazzaglia, Inclusion of 5-[4-(1-Dodecanoylpyridinium)]-10,15,20-triphenylporphine in Supramolecular Aggregates of Cationic Amphiphilic Cyclodextrins: Physicochemical Characterization of the Complexes and Strengthening of the Antimicrobial Photosensitizing Activity, *Biomacromolecules*, 2009, **10**, 2592–2600.
41 Y. E. L. Koo, W. Fan, H. Hah, H. Xu, D. Orringer, B. Ross, A. Rehemtulla, M. A. Philbert and R. Kopelman, Photonic explorers based on multifunctional nanoplatforms for biosensing and photodynamic therapy, *Appl. Opt.*, 2007, **46**, 1924–1930.
42 M. N. Usacheva, M. C. Teichert and M. A. Biel, The Role of the Methylene Blue and Toluidine Blue Monomers and Dimers in the Photoinactivation of Bacteria, *J. Photochem. Photobiol., B*, 2003, **71**, 87–98.
43 J. Schwiertz, A. Wiehe, S. Gräfe, B. Gitter and M. Epple, Calcium Phosphate Nanoparticles as Efficient Carriers for Photodynamic Therapy Against Cells and Bacteria, *Biomaterials*, 2009, **30**, 3324–3331.
44 S. Banfi, E. Caruso, L. Buccafurni, V. Battini, S. Zazzaron, P. Barbieri and V. Orlandi, Antibacterial Activity of Tetraaryl-porphyrin Photosensitizers: An in Vitro Study on Gram Negative and Gram Positive Bacteria, *J. Photochem. Photobiol., B*, 2006, **85**, 28–38.
45 T. Tsai, Y. T. Yang, T. H. Wang, H. F. Chien and C. T. Chen, Improved Photodynamic Inactivation of Gram-Positive Bacteria Using Hematoporphyrin Encapsulated in Liposomes and Micelles, *Lasers Surg. Med.*, 2009, **41**(4), 316–322.
46 J. Wu, H. Xu, W. Tang, R. Kopelman, M. A. Philbert and C. Xi, Eradication of Bacteria in Suspension and Biofilms Using Methylene Blue-Loaded Dynamic Nanoplatforms, *Antimicrob. Agents Chemother.*, 2009, **53**(7), 3042–3048.
47 Y. Guo, S. Rogelj and P. Zhang, Rose Bengal Decorated Silica Nanoparticles as Photosensitizers for Inactivation of Gram Positive Bacteria, *Nanotechnology*, 2010, **21**, 065102.
48 S. A. Bezman, P. A. Burtis, P. J. Izod and M. A. Thayer, Photodynamic Inactivation of *E. coli* by Rose Bengal Immobilized on Polystyrene Beads, *Photochem. Photobiol.*, 1978, **28**, 325–329.
49 J. Gil-Thomas, S. Tubby, I. P. Parkin, N. Narband, L. Dekker, S. P. Nair, M. Wilson and C. Street, Lethal Photosensitisation of *Staphylococcus aureus* Using a Toluidine Blue O–Tiopronin–Gold Nanoparticle Conjugate, *J. Mater. Chem.*, 2007, **17**, 3739–3746.
50 I. Banerjee, D. Mondal, J. Martin and R. S. Kane, Photoactivated Antimicrobial Activity of Carbon Nanotube-Porphyrin Conjugates, *Langmuir*, 2010, **26**, 17369–17374.
51 C. Piccirillo, S. Perni, J. Gil-Tomas, P. Prokopovich, M. Wilson, J. Pratten and I. P. Parkin, Antibacterial Activity of Methylene Blue and Toluidine Blue O Covalently Bounded to Modified Silicone Polymer Surface, *J. Mater. Chem.*, 2009, **19**(34), 6167–6171.
52 J. Bozja, J. Sherrill, S. Michielsen and I. Stojiljkovic, Porphyrin Based, Light-Activated Antimicrobial Materials, *J. Polym. Sci., Part A: Polym. Chem.*, 2003, **41**, 2297–2303.
53 P. A. Suci, Z. Varpness, E. Gillitzer, T. Douglas and M. Young, Targeting and Photodynamic Killing of a Microbial Pathogen Using Protein Cage Architectures Functionalized with a Photosensitizer, *Langmuir*, 2007, **23**, 12280–12286.
54 V. Decraene, A. Rampaul, I. P. Parkin, A. Petrie and M. Wilson, Enhancement by Nanogold of the Efficacy of a Light-Activated Antimicrobial Coating, *Curr. Nanosci.*, 2009, **5**(3), 257–261.
55 P. Prokopovich, S. Perni, C. Piccirillo, J. Pratten, I. P. Parkin and M. Wilson, Frictional Properties of Light-Activated Silicone and Polyurethane Against Blood Vessels, *J. Mater. Sci.: Mater. Med.*, 2009, **21**(2), 815–821.

56 S. Perni, C. Piccirillo, J. R. Pratten, P. Prokopovich, W. Chrzanowski, I. P. Parkin and M. Wilson, The Antimicrobial Properties of Light-activated Polymers Containing Methylene Blue and Gold Nanoparticles, *Biomaterials*, 2009, **30**(1), 89–93.
57 S. Perni, P. Prokopovich, C. Piccirillo, J. R. Pratten, I. P. Parkin and M. Wilson, Toluidine Blue-Containing Polymers Exhibit Bactericidal Activity When Irradiated with Red Light, *J. Mater. Chem.*, 2009, **19**(18), 2715–2723.
58 N. Narband, S. Tubby, I. P. Parkin, J. Gil-Tomás, D. Ready, S. P. Nair and M. Wilson, Gold Nanoparticles Enhance the Toluidine Blue-Induced Lethal Photosensitisation of *Staphylococcus aureus*, *Curr. Nanosci.*, 2008, **4**, 409–414.
59 N. Narband, M. Uppal, C. W. Dunnill, G. Hyett, M. Wilson and I. P. Parkin, The Interaction Between Gold Nanoparticles and Cationic and Anionic Dyes: Enhanced UV-Visible Absorption, *Phys. Chem. Chem. Phys.*, 2009, **11**, 10513–10518.
60 S. Perni, C. Piccirillo, A. Kafizas, M. Uppal, J. Pratten, M. Wilson and I. P. Parkin, Antibacterial Activity of Silicone Containing Methylene Blue and Gold Nanoparticles of Various Sizes Under Laser Light Irradiation, *J. Cluster Sci.*, 2010, **21**(3), 427–438.
61 C. Xing, Q. Xu, H. Tang, L. Liu and S. Wang, Conjugated Polymer/Porphyrin Complexes for Efficient Energy Transfer and Improving Light-Activated Antibacterial Activity, *J. Am. Chem. Soc.*, 2009, **131**, 13117–13124.
62 N. Narband, M. Mubarak, D. Ready, I. P. Parkin, S. P. Nair, M. A. Green, A. Beeby and M. Wilson, Quantum Dots as Enhancers of the Efficacy of Bacterial Lethal Photosensitization, *Nanotechnology*, 2008, **19**(44), 445102.
63 J. Sanabria, F. Machuca and C. F. Dierolf, A Comparison of Solar Photocatalytic Inactivation of Waterborne *E. coli* Using tris (2,2′ bipyridineftuthenium(II), Rose Bengal and TiO_2, *J. Sol. Energy Eng.*, 2007, **129**, 135–140.
64 W. Wang, Q. Shang, W. Zheng, H. Yu, X. Feng, Z. Wang, Y. Zhang and G. Li, A Novel Near-Infrared Antibacterial Material Depending on the Upconverting Property of Er3 + -Yb3 + -Fe3 + Tridoped TiO2 Nanopowder, *J. Phys. Chem. C*, 2010, **114**, 13663–13669.
65 T. S. Wu, K. X. Wang, G. D. Li, S. Y. Sun, J. Sun and J. S. Chen, Montmorillonite-Supported Ag/TiO_2 Nanoparticles: An Efficient Visible-Light Bacteria Photodegradation Material, *ACS Appl. Mater. Interfaces*, 2010, **2**, 544–550.
66 Y. Yamakoshi, N. Umezawa, A. Ryu, K. Arakane, N. Miyata, Y. Goda, T. Masumizu and T. Nagano, Active Oxygen Species Generated from Photoexcited fullerene (C-60) as Potential Medicines: O-2(-Center dot) Versus O-1(2), *J. Am. Chem. Soc.*, 2003, **125**, 12803–12809.
67 E. Nakamura and H. Isobe, Functionalized Fullerenes in Water. The first 10 Years of their Chemistry, Biology, and Nanoscience, *Acc. Chem. Res.*, 2003, **36**, 807–815.
68 G. P. Tegos, T. N. Demidova, D. Arcila-Lopez, H. Lee, T. Wharton, H. Gali and M. R. Hamblin, Cationic Fullerenes Are Effective and Selective Antimicrobial Photosensitizers, *Chem. Biol.*, 2005, **12**, 1127–1135.
69 M. B. Spesia, M. E. Milanesio and E. N. Durantini, Synthesis, Properties and Photodynamic Inactivation of *Escherichia coli* by Novel Cationic Fullerene C_{60} Derivatives, *Eur. J. Med. Chem.*, 2008, **43**, 853–861.
70 L. Huang, M. Terakawa, T. Zhiyentayev, Y. Y. Huang, Y. Sawayama, A. Jahnke, G. P. Tegos, T. Wharton and M. R. Hamblin, Innovative Cationic Fullerenes as Broad-Spectrum Light-Activated Antimicrobials, *Nanomed.: Nanotechnol., Biol. Med.*, 2010, **6**, 442–452.
71 E. M. Hotze, A. R. Badireddy, S. Chellam and M. R. Wiesner, Mechanisms of Bacteriophage Inactivation *via* Singlet Oxygen Generation in UV Illuminated Fullerol Suspensions, *Environ. Sci. Technol.*, 2009, **43**, 6639–6645.

