Expert Opinion

Photosensitiser delivery for photodynamic therapy. Part 2: systemic carrier platforms

Stéphane A Sibani, Paul A McCarron, A David Woolfson & Ryan F Donnelly†
†*Queen's University Belfast, Medical Biology Centre, School of Pharmacy, 97 Lisburn Road, Belfast BT9 7BL, UK*

Background: The treatment of solid tumours and angiogenic ocular diseases by photodynamic therapy (PDT) requires the injection of a photosensitiser (PS) to destroy target cells through a combination of visible light irradiation and molecular oxygen. There is currently great interest in the development of efficient and specific carrier delivery platforms for systemic PDT. *Objective*: This article aims to review recent developments in systemic carrier delivery platforms for PDT, with an emphasis on target specificity. *Methods*: Recent publications, spanning the last five years, concerning delivery carrier platforms for systemic PDT were reviewed, including PS conjugates, dendrimers, micelles, liposomes and nanoparticles. *Results/conclusion*: PS conjugates and supramolecular delivery platforms can improve PDT selectivity by exploiting cellular and physiological specificities of the targeted tissue. Overexpression of receptors in cancer and angiogenic endothelial cells allows their targeting by affinity-based moieties for the selective uptake of PS conjugates and encapsulating delivery carriers, while the abnormal tumour neovascularisation induces a specific accumulation of heavy weighted PS carriers by enhanced permeability and retention (EPR) effect. In addition, polymeric prodrug delivery platforms triggered by the acidic nature of the tumour environment or the expression of proteases can be designed. Promising results obtained with recent systemic carrier platforms will, in due course, be translated into the clinic for highly efficient and selective PDT protocols.

Keywords: conjugate, dendrimer, EPR effect, liposome, micelle, nanoparticle, photodynamic therapy, polymer, systemic delivery, selective targeting

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1. Photodynamic therapy

Photodynamic therapy (PDT) is a clinical technique based on the generation of eactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), upon photo-irradiation of a non-toxic photosensitiser (PS) with visible light [1]. Irradiation thus initiates the oxidative destruction of targeted tissues and induces apoptosis and autophagy [2]. Due to its dual-specificity, PDT is increasingly used as a cancer treatment, with the advantages of limited side effects and excellent functional and cosmetic outcomes [1]. This is because phototoxicity is limited to sensitised cells in the area illuminated and because PS preferentially accumulate in cancer cells [1,3,4]. Moreover, as ROS travel very short distances, due to their extreme reactivities, PDT-induced photodamage is largely limited to the site of ROS generation [5]. PDT-mediated neovascular targeting is used to treat tumours by destroying their blood supply [6] and is now in common use for the treatment of the choroidal neo-vascularisation (CNV) characteristic of 'wet' age-related macular degeneration [7]. PDT is especially well suited for epithelial tumours that are easy to irradiate with visible

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light, such as those affecting the skin or the female genital tract. For these applications, a number of topical formulations have been developed and are in widespread clinical use [1,8]. However, thanks to the development of fibre optic-based interstitial, intravesical and endoscopic light delivery systems [9-11], it is now possible to treat basal and parenchymal lesions of virtually any part of the body [1]. Such applications clearly require systemic delivery of the PS.

Unfortunately, the extended delocalised aromatic π electron system characterising PS generally makes them highly hydrophobic and thus poorly water soluble and prone to aggregation in aqueous solution [12,13], which decreases their ability to generate ROS efficiently. Moreover, currently clinically approved PS are often plagued by poor bioavailability and unfavourable biodistribution, resulting in a less than ideal tumour specificity and, consequently, undesirable side effects such as prolonged skin phototoxicity and damage to surrounding healthy tissues [1,14].

These drawbacks prompted the development of conjugates and supramolecular carrier platforms like dendrimers [15], polymeric micelles [16,17], liposomes [18,19] and nanoparticles [20] for the systemic delivery of PS. A common characteristic feature of supramolecular drug carriers is that they take advantage of the enhanced permeability and retention (EPR) effect, a phenomenon caused by the abnormal organisation of the tumour neovasculature, the high porosity of the blood vessels comprising these vessels and differences in lymphatic drainage. The EPR effect facilitates both diffusion of heavy-weighted PS delivery carriers into, and their retention within, tumours [21]. Target specificity, cellular uptake and bioavailability of these carrier platforms can be further improved by their conjugation with antibodies [22], peptides [23] and polymers such as poly(ethylene glycol) (PEG), or by the design of prodrugs activated by a specific characteristic of cancer cells or tumour microenvironments [24-26]. Finally, apoptosis being triggered by nuclear, mitochondrial or endoplasmatic reticulum photo-damage, the subcellular localisation of carrier delivery platforms can be targeted to these organelles by conjugation with translocation signals [23]. This review will focus on recent developments in the design and use of carrier platforms for systemic PDT, with an emphasis on strategies used to enhance target specificity.

2. PS conjugates

PS often possess functional groups to which conjugation is possible by esterification or substitution. Numerous PS conjugates have been designed to increase their bioavailability, solubility and target specificity [22-24,27,28]. Discrete sets of membrane-bound proteins, such as receptors and transporters, are often overexpressed at the surface of cancer cells' membrane, and conjugation of PS with sugars, peptides, proteins and antibodies specifically recognising these specific proteins is the rationale to increase target specificity of PDT by receptor-mediated endocytosis [29].

2.1 Saccharide–PS conjugates

Rapidly proliferating cancer cells generally overexpress monosaccharide transporters at the surface of their plasmic membrane to cope with their hyperactive metabolism [30]. This opens up the opportunity to both increase PS solubility and target specificity by conjugation with saccharide moieties [27]. Saccharide–PS conjugates can be synthesised by nucleophilic substitution reactions from available PS, such as fluorinated tetraphenylporphyrin and silicon(IV) phthalocyanine [31]. Alternatively, synthesis of a porphyrin ring starting from glycosylated building blocks can also be performed, although steric hindrance can result in low yields [32,33]. The water solubility of a PS can be improved upon conjugation with a saccharide, but some aggregation can sometimes still be observed, depending on the PS structure. However, conjugation generally does not alter the absorption spectra or the singlet oxygen quantum yield of the PS. Although both passive diffusion and transporter-mediated endocytosis mechanisms have been reported for the cellular uptake of saccharide–PS conjugates (amphiphilicity appears to be important for the passive diffusion) [34,35], some specificity against cancer cells has been reported, with glucose and galactose conjugates of a fluorinated tetraphenylporphyrin [34] obtained by symmetric regioselective substitution with the corresponding acetyl-protected saccharides via thioester bounds followed by deprotection [34]. They found that conjugation of glucose increased the binding to, and the phototoxicity against, a breast cancer cell line compared to galactose-conjugated and un-conjugated fluorinated tetraphenylporphyrin, and that deprotection of the glucose moiety was important for the binding. Interestingly, the binding of the glucose conjugate to a rat fibroblast cell line depended on the degree of malignancy of this cell line. This indicates that saccharide–PS conjugates could potentially be selective towards cancer cells *in vivo*. It is noteworthy that saccharides linked to PS via thioester bound are more stable than O-linked saccharides against acid and enzyme-catalyzed hydrolysis, which could be important for the stability of the conjugate *in vivo*.

2.2 Peptide–PS conjugates

Peptides can be used for their cell-penetrating properties or their affinity to specific receptors. They can be conjugated to PS through an amide bond in between a carboxylic acid group of the PS and a free amine group of the peptide, which can either be the N-terminal portion of the peptide or a lysine residue [23]. Peptides being generally synthesised by Fmoc solid-phase methodology, the PS can be introduced after the last step of peptide synthesis by either the same solid-phase strategy or in liquid phase after the release of the peptide from the resin and the use of a coupling reagent to activate the PS carboxylic acid group (this technique was reported to give higher yields [36]). Alternatively, a lysine residue can be first attached to the PS carboxylic acid group, which is then incorporated into the peptide during the solid-phase synthesis [37]. Peptide–PS conjugates do generally

have similar photophysical properties than the parent PS, although their singlet oxygen quantum yield can be slightly lowered [37,38]. Moreover, peptide–PS conjugates can have improved water solubility [38].

Membrane translocation peptides can be conjugated to PS to enhance their cellular uptake [38,39]. Conjugation of an arginine heptamer peptide to a carboxylic acid functionalised tetraphenylchlorin resulted in a water soluble conjugate with a similar singlet oxygen quantum yield to chlorin e6 (Ce6) [38]. This conjugate was found to greatly enhance cellular uptake and phototoxicity in a human breast carcinoma cell line compared to Ce6, the conjugate being internalised 100 times more than Ce6 after four hours [38]. However, the cell-penetrating properties of membrane translocation peptides depend on the structure of the PS conjugate [40]. In another study, an arginine octamer oligopeptide and a peptide composed of residues 48 – 60 of the HIV1 Tat protein were compared for their ability to enhance the cellular uptake of an hydrophobic or anionic tetraphenylporphyrin (TPP) in the human larynx epidermoid carcinoma cell line Hep2 [40]. These peptides were conjugated to TPP via short PEG linkers to prevent a bend conformation of the peptide over the porphyrin ring. Although conjugation enhanced cellular uptake compared to the hydrophobic TPP, the uptake of the anionic TPP conjugates were much lower than those with the hydrophobic conjugates. Their cellular localisation was also different, the anionic conjugates being found in the lysosome while the hydrophobic conjugates were found in the endoplasmic reticulum [40]. This resulted in drastic phototoxicity differences, the hydrophobic conjugates being phototoxic, while the anionic conjugates were not. Although these experiments indicate that cell-penetrating peptides can indeed enhance PS cellular uptake, this strategy does not seem to be applicable to the prodrug aminolevulinic acid (ALA).

ALA is a naturally occurring precursor of the PS protoporphyrin IX (PpIX) within the haem biosynthetic pathway. ALA-mediated PDT has the advantage of a certain degree of specificity, due to enzymatic imbalances of the haem biosynthetic pathway in cancer cells, resulting in preferential PpIX accumulation in neoplastic cells compared to normal cells [3]. However, ALA is a small charged molecule that may only cross cellular membranes by a slow transporter-mediated mechanism, and conjugation to a cell-penetrating peptide was hoped to enhance ALA cellular uptake and hence PpIX accumulation. Although this conjugate was internalised into transformed murine keratinocyte cells by endocytosis and induced some PpIX accumulation, the magnitude of PpIX formation was found to be half that obtained with ALA and required a long incubation time [39]. This was probably because the conjugate had to escape the endosomal compartment and be degraded by cytoplasmic peptidases for ALA to enter into the haem biosynthetic pathway.

Another use of PS–peptide conjugates resides in their affinity for specific cancer cell receptors to enhance target specificity [23]. This targeting strategy was applied against neovascular endothelial cell overexpressing $\alpha_v\beta_3$ integrin receptors by conjugating a linear or cyclic RGD motif peptide to tetraphenylchlorin (TPC). Conjugation of these peptides greatly enhanced TPC cellular uptake and phototoxicity in the neovascular endothelial cell line HUVEC, the conjugates being internalised 80 – 100 more times than TPC after 24 h. Interestingly, and despite a twofold lowered singlet oxygen yield, the conjugates were phototoxic against the HUVEC cell line, while no phototoxicity was observed with TPC. Importantly, the conjugates exhibited no toxicity towards the non-expressing $\alpha_v\beta_3$ integrin cell line EMT-6, although some non-specific internalisation was observed, possibly due to some degree of conjugate aggregation in the media [37]. An enhanced cellular accumulation was observed with the same RGD peptides conjugated to PpIX in the human cervical cancer cell line SiHa, although the conjugate internalised only twice as much PpIX. Unfortunately, when this conjugate was tested *in vivo* in a mouse tumour model it accumulated in the liver and did not enhance PDT efficiency compared to PpIX [41].

Peptides can be used to target a PS to a specific subcellular compartment, and nuclear localisation sequence (NLS) has been used to target the nucleus. However, although PS–NLS conjugates are generally more potent than PS cell penetrating peptide conjugates [40,42], attempts to detect PS–NLS conjugates in the nucleus have failed thus far.

Peptides can also be used to modulate the pharmacokinetics and distribution of ligand–PS conjugates. In an elegant paper, Stefflova *et al.* [43] conjugated folate to pyropheophorbide a (Pyro) to target cancer cells overexpressing the folate receptor (FR) with or without a small hydrophilic peptide linker and monitored the accumulation of Pyro by near-infrared fluorescence imaging in mice bearing both overexpressing and non-expressing FR cancer cells. As expected, the conjugate with the peptide linker specifically accumulated into the FR overexpressing tumour cells and, importantly, had a much lower accumulation in kidneys and liver than the un-conjugated Pyro or the conjugate without the peptide spacer [43].

One important issue regarding PS–peptide conjugates *in vivo* is their stability in the bloodstream. Injection of a chlorine–peptide conjugate targeting the vascular endothelial cell receptor neuropilin-1 in tumour-bearing mice resulted in a much higher accumulation of the conjugate in the liver than the tumour, where it was degraded by proteases as soon as one hour after injection [44].

2.3 Protein–PS conjugates

Similarly to saccharides and peptides, proteins can be conjugated to PS to increase cancer cell specificity by receptor-mediated targeting. These proteins can either be known ligands binding to cancer-specific receptors, or antibodies raised against these receptors [24,29,45]. Proteins are usually conjugated to PS through amide bonds in between accessible

lysine residues of the protein and carboxylic groups of the PS, the common conjugation strategy being performed by carbodiimine and activated ester chemistry [46-50]. However, the conjugation of proteins to PS is much more delicate than the conjugation of saccharides, peptides and small ligands, as the structure of the protein moiety must be preserved in the process in order to retain its affinity and specificity to the targeted receptor [29,51]. This requires mild conjugation conditions and, hence, the use of relatively water soluble or PEGylated PS or relatively stable proteins. Large proteins usually contain several accessible lysine residues, leading to the conjugation of several PS, which can inhibit its receptor binding affinity, and decrease singlet oxygen quantum yield through PS–PS self-quenching [46,51]. The number and position of lysine residues are thus important factors that should be taken into consideration when selecting a protein in order to maximise the efficiency of the conjugate: the number of lysines should be as high as possible to increase the PS payload, but they must also be well distributed in order to avoid self-quenching of the PS. Although the average number of PS conjugated to a protein can be somewhat controlled, the carbodiimine and activated ester conjugation strategies typically lead to mixtures of conjugates and can cause protein–protein crosslinks. Crosslinking can be avoided by using other conjugation strategies, such as the substitution of a p-bromobenzyl group and the addition of a cysteine residue to the C-terminal of the protein [52] or the use of isothiocyanate-bearing PS [53-56]. Alternatively, crosslinking with the carbodiimine conjugation chemistry can also be avoided by thorough purification of the active ester before the addition of the protein [51].

Overall, the principal advantage of protein–PS conjugates is the strong protein–receptor binding, with affinity constants in the picomolar range. Although protein–PS conjugates are not always as potent as the unconjugated PS [50], they can be highly specific, with no cellular uptake and phototoxicity against cells that do not express the receptor to which the protein binds [48]. However, clean up of PS–protein conjugates can be difficult, especially at high PS/protein ratios, as proteins with hydrophobic regions tend to bind planar hydrophobic porphyrin rings, which can decrease the affinity for the receptor [51]. In addition, long incubation times can be required to achieve *in vitro* phototoxicity in cell culture. When conjugating a highly potent PS to a protein, long-term stability can be an issue [49] and the conjugates have to be strictly stored in the dark to avoid photo-induced degradation.

In addition to receptor targeting, protein–PS conjugates can also be used to target specific subcellular compartments. Conjugation of PS with the STxB subunit of bacterial Shiga toxins and verotoxins can, in addition to targeting the cell surface glycosphingolipid receptor Gb3, overexpressed in ovarian carcinomas and Burkitt's lymphomas [57], also deliver the PS to the endoplasmic reticulum via the retrograde route. This targeting strategy has been applied to chlorin e6 [58] and a glycosylated conjugate of tetraphenylporphyrin [52], which resulted in enhanced photodynamic destruction of cancer cells *in vitro* by factors of 10 and 5, respectively, compared to their un-conjugated counterparts.

Although antibody–PS conjugates do have the disadvantage of a low tumour penetration, especially with monoclonal antibodies, they can be useful to target small early stage tumours, endothelial cells of the tumour vasculature [47] and white blood cells [49], applications for which penetration depth of the conjugate is irrelevant. However, owing to their specificity, the binding of PS–protein conjugates can be compromised in cases of high local secretion of the same protein to which the PS is conjugated [49]. Monoclonal antibody conjugates can have higher PDT efficiency than antibody fragments, due to a higher PS payload and lower clearance rate. Stimulating the expression of a receptor for which a protein–PS conjugate is dressed against can enhance its efficacy [59]. Targeting internalising antigens is more potent than non-internalising antigens [53], and multi-epitope targeting of the same receptor is more potent [50]. ScFv-PS conjugation can enhance PDT as much as 70 times compared to free PS *in vitro*, with similar to better tumour regression *in vivo*, increased tumour to normal tissue ratios and a quicker clearance rate than free PS [46].

2.4 Dendrimers

Dendrimers are highly branched macromolecules composed of repetitive units branched on a multivalent core molecule [15]. PS can be attached at the periphery of the dendrimer branches or be encapsulated as the core of a dendrimer.

Fullerene C_{60} can be used as the core of dendritic architectures to which PS can be attached via a malonate adduct [60-63]. It was found that the number of adducts on the caged C_{60} is important because the generation of singlet oxygen from two pyropheophorbide a (PPa) molecules attached to the same adduct through octyl chains is quenched by photo-induced electron transfer to the fullerene molecule in the mono-adduct. This dendrimer was found to be non-phototoxic. This was not the case for C_{60}, with six symmetrically positioned adduct around the caged C_{60} [60], breaking the conjugation network of the fullerene [61].

The relative positions of PS around a C_{60} core has been found to be important when considering ways to avoid auto-quenching of the PS and, hence, a reduced singlet oxygen quantum yield [61,62].

The latest development of C_{60} hexakis adduct fullerene-PPa dendrimers is the attachment of the monoclonal antibody rituximab (a chimerical murine/human IgG1κ monoclonal antibody (MAb) containing human constant region sequences directed against the CD20 antigen found on the surface of normal and malignant B lymphocyte) to one adduct with ten PPa molecules attached to the remaining five malonate adducts [63]. It was found that this dendritic immunoconjugate was able to specifically bind to cells expressing CD20 with no more dark toxicity than the pure MAb [63].

The photosensitising prodrug ALA has been attached to the periphery of dendrimers through ester links as a means of increasing uptake [64]. It was found that 18 ALA molecules attached to a three-branched second generation polyamidoamine dendrimer resulted in an enhanced PpIX accumulation and PDT efficiency in cell culture models. This indicated that the dendrimer could enter into the cells and that ALA could be released after the action of cellular esterases and, thus, induce PpIX accumulation [65].

Dendrimers can be used to encapsulate hydrophobic PS at the dendrimer core with water soluble dendrimeric branches, thus increasing water solubility and reducing aggregation [66]. A third generation dendrimer with a Zn-porphyrin core and poly(benzyl ether) dendritic branches with neutral or charged ends was synthesised and evaluated in Lewis Lung Carcinoma (LLC) cells [66]. It was found that the cationic dendrimer had an enhanced cellular uptake and PDT efficiency with a lower dark toxicity [66].

2.5 Polymer–PS conjugates

The advantages of using polymer–PS conjugates as PS delivery platforms include selective tumour accumulation by EPR effect, improved solubilisation and decreased aggregation, more favourable bio-distribution and pharmacokinetics, and high PS payload. Moreover, targeting moieties can be included in polymer–PS conjugates to specifically target cancer cells or subcellular organelles [24,28].

Two major common features of polymer–PS conjugates are the possibility to solubilise hydrophobic PS by using water soluble or micelle-forming polymers, and to induce a higher tumour to normal tissues ratio by EPR effect. A typical induction of the EPR effect by polymer–PS conjugates was illustrated by a meta-tetrahydroxyphenylchlorin (mTHPC)–PEG conjugate that was used to treat human mesothelioma tumour xenografts in nude mice and resulted in a much lower skin sensitisation than free mTHPC [67].

Hydrophilic polymers can be used to solubilise highly water insoluble PS, such as fullerene C_{60}, a spherical caged PS with advantageous photophysical properties but plagued by low water solubility [68]. Radical copolymerisation of the non-charged detergent *N*-vinylpyrrolidone (NVP) to fullerene C_{60} allows a very high concentration of 7.8 mm of this fullerene in water to be achieved without aggregation [69]. Hydrophilic cationic polymers such as poly-*S*-lysine [70] and poly-L-lysine [71] can improve the cellular uptake of a PS, possibly due to electrostatic interactions between the negatively charged cellular membrane and the polymer backbone, and target the nucleus. For example, and despite a reduced singlet oxygen quantum yield, the increased cellular uptake and retention of a tri-cationic porphyrin conjugated to poly-*S*-Lysine leads to an enhanced PDT effectiveness in proliferating keratinocytes [70]. This cellular uptake enhancement mechanism could possibly induce some specificity against cancer cells, whose membranes contain more poly(sialic acid) residues than normal cells [71].

Nuclear localisation has been reported for a chlorin e6-poly-L-lysine conjugate in HeLa cells resulting in enhanced PDT efficacy [71]. Another mean of nuclear targeting is to introduce a NLS peptide on a polymer–PS conjugate. This has been demonstrated with mesochlorin e6 monoethylenediamine (Mce6) conjugated to *N*-(2-hydroxypropyl) methacrylamide (HPMA), a cationic NLS peptide enhancing the PDT efficiency compared to a non-NLS containing conjugate by targeting the nucleus [72]. Interestingly, the authors showed that introducing a degradable peptide spacer sensitive to the lysosomal peptidase cathepsin B in between Mce6 and the HPMA backbone enhanced PDT efficiency of both NLS and non-NLS containing conjugates, presumably because of a higher singlet oxygen quantum yield of the free PS compared to the conjugate (HPMA–porphyrin conjugates forms micelle-like structures in aqueous buffers), and a possible redistribution from the lysosome. Moreover, the conjugate containing both NLS and protease-sensitive peptides was reported to have a slightly lower EC_{50} and EC_{90} than free Mce6 [72].

Polymer–PS conjugates containing a magnetic resonance imaging (MRI) contrast enhancer can also be used to improve tumour targeting. They allow the precise position of interstitial tumours to be determined and can provide the focal point for light irradiation in PDT [28]. Importantly, this technique allows the non-invasive monitoring of PS accumulation in tumours by MRI and, hence, permits optimisation of the drug delivery–photoirradiation time interval of the PDT protocol. The MRI contrast enhancer generally used is the paramagnetic gadolinium ion (Gd^{3+}), which is introduced into the polymer–PS as a chelate. This technique has been used with a poly(L-glutamic acid)-mesochlorin e6 conjugate in a human breast carcinoma tumour xenograft model in mice [73]. This allowed determination of the timing for maximal PS accumulation into the tumour neovasculature and into the tumour itself [73]. For a PEG–fullerene conjugate, a good correlation was found between the MRI signal and the PDT effect after injection into fibrosarcoma tumour bearing mice [74].

In addition to the EPR effect, different strategies can be used to increase further the tumour targeting specificity of polymer–PS conjugates, including protease-specific polymer–PS prodrugs and the addition of receptor-specific moieties onto the polymer–PS conjugate. Two strategies are possible in designing protease-specific polymer–PS prodrugs. These depend on the nature of the protease-sensitive component of the polymer–PS conjugate: using a protease-sensitive polymer backbone such as poly-L-lysine [75,76] (first generation), or conjugating the PS to a stable polymer via protease-sensitive peptide spacers [77] (second generation). Several characteristics have to be adjusted in order to optimise the polymeric PS prodrug conjugate regarding its quenching and activation efficiencies, as well as its solubility [76].

Another strategy to enhance cancer cell targeting specificity of polymer–PS conjugates consists of grafting

on receptor-specific peptides. A poly(vinyl alcohol) (PVA)–verteporphin conjugate bearing a peptide recognising the vascular endothelial growth factor receptor (VEGFR-2) was used to treat a laser injury model of subfoveal CNV in the rat and was compared to a non-targeted PVA–verteporphin conjugate and a liposomal formulation [78]. It was found that both verteporphin–PVA and the peptide conjugate enhanced CNV closure compared to the liposomal formulation, due to a decreased clearance rate. Importantly, the peptide conjugate resulted in an improved selectivity and, thus, reduced side effects on normal tissues.

3. Macromolecular delivery platforms

Hydrophobic PS may be solubilised and aggregation avoided by using micelle-forming polymers, dendrimer-based polyion complex micelles, liposomes or nanoparticles. Like polymer–PS conjugates, each of these macromolecular delivery systems is large enough to increase the tumour to normal tissues ratio by EPR effect. They can also be engineered for receptor-mediated targeting.

3.1 Micelles

Micelles are spherical macromolecular complexes that form spontaneously when amphiphilic copolymers are mixed in an aqueous environment above the critical micelle concentration (CMC). The hydrophobic moieties of the polymer coalesce to form the micelle core, while the hydrophilic group forms a hydrated shell around the hydrophobic core [17], thus allowing high water solubility. There are two different types of micelle-forming amphiphilic copolymers, depending on the distribution of the hydrophilic and hydrophobic parts on the polymer: those with two distinct and separate blocks and those for which the hydrophobic and hydrophilic moieties are alternatively distributed all along the copolymer backbone [79-81].

The highly aggregation-prone B-ring benzoporphyrin has been dissolved into methoxyPEG micelles in monomeric form and the PEG molecules coating the surface of these polymeric micelles avoided their clearance by the reticuloendothelial system [81].

Micelles formed with a styrene–maleic acid (SMA) copolymer of 1.56 kDa were found to encapsulate large amounts of the PS zinc protoporphyrin ZnPP (15 – 60% w/w, the highest payload corresponding to 150 mg/ml) without any loss of water solubility for the highest PS payload [82]. Micelles composed of Pluronic® 123 loaded with benzoporphyrin derivatives were found to enhance PDT *in vivo* against M1 rhapdomyosarcoma tumour xenograft in mice compared to free PS, and this enhancement was attributed to an increased transfer of the PS to lipoproteins [83].

Polymeric micelles can enhance PDT efficiency *in vitro* by an increased cellular uptake, as was found for SMA copolymer micelles loaded with ZnPP [84]. Although ZnPP is completely aggregated in SMA micelles [82,84], the release of ZnPP into the cell membranes from highly loaded SMA micelles after endocytosis was found to increase the cellular uptake in Jurka cells fivefold and thus enhance PDT efficiency compared to free ZnPP [84]. An increased cellular uptake in HeLa cells was also found with methoxy poly(ethylene glycol)-b-poly(caprolactone) micelles loaded with PpIX [85], leading to enhanced PDT.

Cationic charges at the surface of polymeric micelles can induce cellular uptake. Uptake into HeLa cells of cationic fullerene C_{60}-loaded PEG block copolymer micelles was found to be correlated with their surface charge density, with an increased zeta potential leading to increased cellular uptake and phototoxicity [86]. Non-charged and anionic micelles were found to be poorly internalised and, thus, devoid of PDT effect.

Although polymeric micelles are generally used to deliver PS by injection, they can also be used for oral delivery. Meso-tetraphenyl porphine-loaded micelles composed of Pluronic F127 and PEG–distearoyl phosphatidylethanolamine were found to have good bioadhesive properties in an everted rat intestine model [87]. An advantage of polymeric micelles is their stability in the bloodstream for sustained bioavailability and also within the formulation itself, allowing storage [16,17]. An advantage of Pluronic P123 micelles compared to liposomes is their stability against hydrolytic and oxidative or photo-oxidative degradation [88]. Fullerene C_{60}-loaded PEG block copolymer micelles were found to be stable at room temperature over four months, in contrast with the liposomal formulation, which, although giving high phototoxicity, was hampered by a low long-term stability [86]. Micelles can also be lyophilised, which is an attractive option for their permanent storage [81]. Ease of preparation is also an advantage of polymeric micelles, as micelles can form spontaneously upon dissolution [16].

Antibodies can be attached to polymeric micelles to increase cancer cell target specificity and PDT efficiency. Antibody can be easily fixed to PEG-based polymeric micelle by adding a small amount of a derivative of the polymer containing a p-nitrophenylcarbonyl group to which the antibody can be conjugated through a carbamate bond [89]. Conjugation of the cancer-specific antinucleosome MAb 2C5 to meso-tetratphenylporphine (mTPP)-loaded PEG-phosphatidyl ethanolamine (PEG–PE) micelles by this technique resulted into an increased PDT efficacy *in vitro* against different cancer cell lines [90] as well as *in vivo* against murine LLC xenografts in mice [91] when compared to non-targeted micelles and free mTPP. Increased cancer cell targeting can also be achieved by conjugation of a ligand to target a cancer-specific receptor. This targeting strategy has been applied to a low-density lipoprotein (LDL) micelle carrier system loaded with silicon phthalocyanine by conjugation of folic acid to the apolipoprotein B-100 (Apo-100) protein [92].

Another way to enhance tumour targeting may be to use magnetically guided micelles and apply a magnet on the

tumour. This technique has been assessed in HeLa cells *in vitro* with diacyllipid-poly(ethylene glycol) polymeric micelle coencapsulating the PS 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a and magnetic nanoparticles. It was found that incorporation of the magnetic nanoparticle, although slightly increasing the micelle size, did not affected the ability of the PS to generate $^1O_2$. Interestingly, and presumably because these micelles can be magnetically attracted, the proximity of a magnet was found to enhance the cellular uptake of the magnetic micelle in HeLa cells [93], which could be an attractive way to target cancer cells *in vivo* without needing to conjugate an antibody.

Another use of polymeric micelles is to improve the delivery of dendrimer porphyrins by the formation of polyion complex micelles. These supramolecular carrier platforms are formed by electrostatic interactions between polyanionic dendrimer porphyrins and polycationic block copolymers. The advantage of polyion complex (PIC) micelles is that the dendrimeric architecture around each PS molecule prevents any PS–PS interaction, and a high PS payload can thus be achieved in the micelle without $^1O_2$ quenching by aggregation or energy transfer. PIC micelles have been engineered with an anionic third generation poly(benzyl ether) zinc porphyrin dendrimer complexed with a cationic poly(ethylene glycol)–poly(L-lysine) block copolymer (PEG-b-PLL), resulting in water soluble micelles characterised by a narrow size distribution and unaltered absorption and fluorescence spectra of the dendrimer porphyrin, which was shown to generate $^1O_2$ upon irradiation [94]. The encapsulation of the dendrimer porphyrin into the PIC micelle was found to greatly enhance PDT efficacy in LLC cells compared to the dendrimer porphyrin, by a factor of 280 – 130 over a 2 – 12 h incubation period, with an increased cellular uptake by a factor of 6 – 8 over the same period of time. Hence, the authors suggest that the increased cellular uptake might not be enough to account for the PDT enhancement, and that the encapsulation of the dendrimer porphyrin into the PIC micelle resulted in its accumulation into sensitive subcellular compartments.

3.2 Liposomes

Liposomes are small unilamellar vesicles composed of a phospholipid bilayer membrane enclosing an inner aqueous environment [18,19]. The phospholipid bilayer of liposomes can dissolve highly hydrophobic PS, such as fullerene C_{60} [95] and maintain aggregation-prone photosensitisers, such as pyropheophorbide-a methyl ester, in monomeric form [96]. This enables the direct generation of $^1O_2$ from the liposome upon photoirradiation. Although liposomes are often used to carry hydrophobic PS, they can also be used to encapsulate charged water soluble molecules, such as the prodrug aminolevulinic acid [97]. Liposome production generally involves the formation of a film after evaporation of the solvent used to dissolve the liposome components, followed by hydration and sonication or extrusion. The properties of liposomes can be altered by addition of different molecules to modify their surface.

Introduction of PEG molecules into a liposome can stabilise the liposome and increase its bioavailability by facilitating evasion of the reticuloendothelial system. PEG is generally conjugated to a phospholipid used to form the liposome bilayer membrane [98-100], but can also be conjugated to the PS that is incorporated into the bilayer membrane [101]. Despite a lysosomal accumulation, PEGylated liposomes loaded with chlorin e6 ester have been reported to enhance PDT efficiency compared to the free PS in gastric cancer cell lines *in vitro* and *in vivo* in gastric cancer xenograft in mice [100]. These effects were linked to the enhanced cellular uptake observed *in vitro*. PEGylated meta-(tetrahydroxyphenyl)chlorin (mTHCP)-loaded liposomes were compared to free mTHCP in a clinical trial to treat spontaneous cutaneous squamous cell carcinoma in cats [98]. Accumulation of mTHCP in normal skin and tumours was monitored. This experiment indicated that the liposomal formulation improved the bioavailability of mTHCP and increased the tumour to normal skin mTHCP accumulation ratio. The maximal tumour concentration of mTHCP occurred at the same time (8 h post-injection) as the highest tumour to normal skin ratio. Four cats out of seven were tumour-free 380 days after treatment. A second clinical trial with an optimised PDT regimen resulted in a complete response in all animals [102].

Coating the liposomal surface with cationic charges can enhance the PDT efficiency of liposomes by electrostatic interaction with negatively charged cell membranes. The cellular uptake and phototoxicity of benzoporphyrin derivative-loaded liposome was found to be enhanced in vascular endothelial cells and human umbilical vein endothelial cells by the introduction of cetylated poly(ethylenimine) (PEI) into the liposome membrane bilayer [103,104]. An increased proportion of PEI leads to an increased PDT efficiency [103]. In addition to enhanced cellular uptake, cationic charges on liposomes can lead to their accumulation in mitochondria and nuclei [104].

In order to increase cancer cell targeting specificity, antibodies, proteins and peptides can be conjugated to a phospholipid composing the liposome. Transferrin has been conjugated to a maleimide group that was added to a distearoyl phosphatidylethanolamine–PEG conjugate to form targeted liposomes [105]. These liposomes were compared to non-targeted liposomes for the delivery of aluminium phthalocyanine tetrasulfonate (AlPcS) to a human bladder transitional cell carcinoma cell line overexpressing the transferrin receptor *in vitro* and to an orthotopic rat bladder tumour model *in vivo*. The transferrin-conjugated liposome was found to be more efficient than both the non-targeted liposome and free AlPcS in terms of PS delivery and photodynamic efficacy upon irradiation.

A PEGylated liposome formulation encapsulating the benzoporphyrin derivative monoacid ring A was found to be

unable to prevent tumour growth in Meth A-sarcoma bearing mice, despite a higher tumour accumulation compared to the non-PEGylated liposome, which could reduce tumour growth [106]. Conjugation of a peptide specific to angiogenic endothelial cells to the PEGylated liposome achieved a strong suppression of tumour growth. Delivery of PS by PEGylated liposomes can also be enhanced by conjugation of cancer-specific antibodies, which was demonstrated *in vitro* and *in vivo* with liposomes bearing the antinucleosome MAb 2C5 attached at the end of the PEG chains [107].

3.3 Nanoparticles

PS can be adsorbed onto the porous matrix of polymeric nanoparticles (NP) composed of poly(lactic acid) (PLA) or poly(lactic-co-glycolic acid) (PLGA) [108,109]. Although PLGA nanoparticles loaded with meso-tetra (p-hydroxyphenyl)porphyrin (p-THPP) could achieve an improved vascular-targeted PDT in a chick embryo chorioallantoic membrane model [110], these NP are limited in their loading capacity. Even at the optimal mTHPP loading (0.03% w/w), there was no increase of PDT efficacy with the PLGA NP and only a twofold increase could be achieved for the PLA NP [109] in an ovarian cancer cell line. Moreover, these NP lose their PS content in aqueous solution at a steady rate. Around 40% of the zinc (II) phthalocyanine content of PLGA NP was lost into the solution after 25 days [108].

To overcome the limitations outlined above, the PS can be embedded into the non-porous core of poly(acrylamide) or sol-gel silica NP [111-117], or be covalently fixed into the matrix of organically modified silica (ORMOSIL) [110,116,118,119]. For these encapsulation methods, the $^1O_2$ generated in the NP matrix upon irradiation must diffuse outside the NP to exercise its cytotoxicity. This was found to be the case for ORMOSIL NP loaded with mTHCP [118] and 2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide (HPPH) [116]. Although a higher encapsulation of methylthioninium chloride in ORMOSIL NP was achieved compared to poly(acrylamide) NP, the generation of $^1O_2$ was found to be higher for the latter [116]. The encapsulation of methylthioninium chloride is of interest because this PS is reduced by plasma reductases into an inactive form, which could be prevented by its embedding into poly(acrylamide) NP [117].

PS can be covalently attached to the surface of NP. Small gold nanoparticles to which phthalocyanine was fixed was found to be able to generate $^1O_2$ and to kill HeLa cells, although the efficacy was only twice as strong as free phthalocyanine [120].

MRI contrast agents can be co-encapsulated into NP along with PS for their non-invasive monitoring. The capacity of such MRI-active NP to generate $^1O_2$ *in vitro* has been demonstrated with sol-gel silica-coated magnetic particles embedding methylthioninium chloride [115] and with Photofrin®-loaded polyacrylamide NP [113,114]. Such particles were able to trigger *in vitro* phototoxicity in gliosarcoma 9L cells [113,114] and in MDA-435 cells [114]. Moreover, injection of 75 mg of such NP into a rat bearing an intracerebral 9L glioma tumour resulted in a reduction and even reversal of tumour growth. However, the tumour did regrow 12 days after treatment [113].

Target cell specificity of NP can be improved by the conjugation of peptides and ligands. This has been shown *in vitro* with the previously described Photofrin-loaded polyacrylamide NP by attaching a RGD peptide on the NP surface to target cancer-specific $\alpha v\beta_3$ integrins. This resulted in specific binding to an $\alpha v\beta_3$ expressing cell line. Interestingly, the efficacy of the same NP against an intracranial glioma model in rats was enhanced by fixation of the tumour vasculature targeting F3 peptide through a PEG linker [114]. Conjugation of folic acid was also found to induce folic acid receptor target cell specificity in human colon cancer cells [121]. Incorporation of a near-infrared transducer into a NP could be useful in the treatment of deep tumours. Such a transducer loaded into a NP could be excited after deep intramuscular injection and co-encapsulation with zinc phthalocyanine could produce $^1O_2$ and kill human colon cancer cells [121].

4. Conclusion

Systemic PDT is a promising clinical modality for the treatment of solid tumours and angiogenic ocular diseases with good efficacy and minimal side effects. The development of water soluble formulations with improved target specificity is highly desirable. Solubilisation of PS can be achieved by conjugation to water soluble targeting molecules and polymers, or by encapsulation into supramolecular delivery vehicles like micelles, liposomes and nanoparticles. In addition to the first level of specificity provided by light delivery, exploiting cellular, microenvironmental and physiological characteristics of the targeted tissues can further enhance systemic PDT target specificity, which is especially useful for locations where precise irradiation is hardly achievable or high specificity is desirable to avoid damage to surrounding healthy tissues. At the cellular level, specific transporters and receptors can be targeted by sugars, peptides and proteins, which can be used to synthesise simple water soluble conjugates, or be used as the targeting unit of a PS–polymer conjugate or an encapsulating delivery vehicle. At the microenvironmental level, the acidity of the tumour extracellular matrix can induce the precipitation of polyion complex micelles, and tumour-associated proteases activate polymeric prodrug platforms. At the physiological level, the EPR can be exploited to target tumours with polymeric PS conjugates and supramolecular delivery vehicles. These recent developments of systemic carrier platforms will probably be translated into the clinic in the near future for highly efficient and selective PDT.

5. Expert opinion

Although PS with improved photo-physical properties such as strong absorption in the near-infrared spectrum, which permits efficient light penetration into deep tumours, and a high $^1O_2$ quantum yield, which improves treatment efficacy, have recently been developed to treat solid tumours and ocular vascularisation diseases by systemic PDT [122], the need for efficient systemic delivery platforms is a prerequisite for their clinical application. The systemic delivery of PS can be improved in terms of efficiency, target specificity and subcellular localisation by their conjugation to solubilising and/or targeting moieties including sugars, peptides, proteins and antibodies, or by their encapsulation into supramolecular delivery carriers such as polymeric micelles, liposomes and nanoparticles. Although simple targeting PS conjugates can achieve these goals, polymer–PS conjugates and supramolecular scarriers do have the advantage of exploiting the EPR effect, increasing the tumour to healthy tissues concentration ratio of the carrier and, thus, reducing possible side effects, notably skin photosensitisation. Moreover, these carriers can be engineered as modular drug delivery systems, with peptide, protein or antibody as addressing unit to trigger cancer-specific receptor-mediated cellular uptake, and a multiplying unit that can be a polymer backbone or a dendrimer. A noted advantage of polymer–PS conjugates and macromolecular carriers is their inherently high PS payload. Polyion complex micelles seem to be promising carriers for this reason and also for the inhibition of PS–PS interaction they provide. Hydrophobic PS such as 2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide can form pure nanocrystals of about 100 nm that can be stably monodispersed in water for at least three months [123]. Although the PS is quenched in its crystalline form, incubation with serum proteins restore its fluorescence, indicating that it might be possible to use these nanocrystals for a more efficient loading of micelles or liposomes. The possibility of a conditional PDT effect triggered by the tumour microenvironment, such as the presence of excreted proteases or increased acidity is also an interesting feature of polymer–PS conjugates and polyion complex micelles. Antibody-mediated cancer cell targeting by PEGylated liposomes can also be engineered to be responsive to acidic pH but this system has not yet been exploited for liposomal delivery.

A number of other techniques used for other applications have yet to be exploited as PS delivery platforms. These include the synthesis of peptidase-resistant peptides with a non-reducible inter-side chain bond [124] and the site-specific PEGylation of proteins on disulfide bridges [125]. Peptidase-resistant peptides could be useful to stabilise peptides used for receptor-mediated targeting and as subcellular localisation signals, especially when endocytosis is the cellular uptake mechanism involved for the cell entry of a PS carrier. Site-specific PEGylation of proteins could be useful to conjugate targeting moieties, such as proteins and antibodies, without impairing their binding. For lysosomal evasion, conjugation to the shiga-like toxin subunit B seems promising, although it was reported that liposomes conjugated with this protein induce an immune response [126]. Triggering of an immune response by alien molecules is always a problem, and this is a potential problem when dealing with peptides, but especially with proteins and antibodies. It is noteworthy that, although virus nanoparticles are currently developed as drug delivery carriers [127-129], they have not been yet used as PS delivery platforms.

Although water soluble PS and simple PS conjugates might be cost-effective compared to more elaborate delivery vehicles, innovative systemic carrier platforms for PDT using different techniques used in separate studies so far might lead to more effective PDT in the near future.

Declaration of interest

This article represents original work and has not been previously published or simultaneously submitted elsewhere for publication. The manuscript has been read and approved by all the authors. The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Brown SB, Brown EA, Walker I. The present and future role of photodynamic therapy in cancer treatment. Lancet Oncol 2004;5:497-508
   - • **Comprehensive review of clinical PDT.**
2. Buytaert E, Dewaele M, Agostinis P. Molecular effectors of multiple cell death pathways initiated by photodynamic therapy. Biochim Biophys Acta (BBA) Rev Cancer 2007;1776:86-107
3. Collaud S, Juzeniene A, Moan J, et al. On the selectivity of 5-aminolevulinic acid-induced protoporphyrin IX formation. Curr Med Chem Anticancer Agents 2004;4:301-16
4. Kessel D. The role of low-density lipoprotein in the biodistribution of photosensitising agents. J Photochem Photobiol B 1992;14:261-2
5. Redmond RW, Kochevar IE. Symposium-in-Print: singlet oxygen; invited review. Photochem Photobiol 2006;82:1178-86
6. Abels C. Targeting of the vascular system of solid tumours by photodynamic therapy (PDT). Photochem Photobiol Sci 2004;3:765-71
   - • **Describes how photosensitisers target the tumour vasculature.**
7. Wormald R, Evans J, Smeeth L, et al. Photodynamic therapy for neovascular age-related macular degeneration. Cochrane database syst rev (Online) 2007;3:CD002030
8. Donnelly RF, McCarron PA, Woolfson AD. Drug delivery for topical photodynamic therapy: difficulties and novel solutions. Trends Cancer Res 2006;2:1-20
   - • **Comprehensive review of drug delivery for topical PDT.**
9. Thompson MS, Andersson-Engels S, Svanberg S, et al. Photodynamic therapy of nodular basal cell carcinoma with multifiber contact light delivery. J Environ Pathol Toxicol Oncol 2006;25:411-24
10. Vesselov LM, Whittington W, Lilge L. Performance evaluation of cylindrical fiber optic light diffusers for biomedical applications. Lasers Surg Med 2004;34:348-51
11. Panjehpour M, Overholt BF, Haydek JM. Light sources and delivery devices for photodynamic therapy in the gastrointestinal tract. Gastrointest endosc clin N Am 2000;10:513-32
    - • **Good overview of gastrointestinal delivery of light and photosensitisers.**
12. Ricchelli F. Photophysical properties of porphyrins in biological membranes. J Photochem Photobiol B 1995;29:109-18
13. Boyle RW, Dolphin D. Structure and biodistribution relationships of photodynamic sensitizers. Photochem Photobiol 1996;64:469-85
14. Moriwaki SI, Misawa J, Yoshinari Y, et al. Analysis of photosensitivity in Japanese cancer-bearing patients receiving photodynamic therapy with porfimer sodium (Photofrin®). Photodermatol Photoimmunol Photomed 2001;17:241-3
15. Wolinsky JB, Grinstaff MW. Therapeutic and diagnostic applications of dendrimers for cancer treatment. Adv Drug Deliv Rev 2008;10:1037-55
16. Lukyanov AN, Torchilin VP. Micelles from lipid derivatives of water-soluble polymers as delivery systems for poorly soluble drugs. Adv Drug Deliv Rev 2004;6:1273-89
17. Van Nostrum CF. Polymeric micelles to deliver photosensitizers for photodynamic therapy. Adv Drug Deliv Rev 2004;56:9-16
18. Derycke AS, De Witte PA. Liposomes for photodynamic therapy. Adv Drug Deliv Rev 2004;56:17-30
19. Chen B, Pogue BW, Hasan T. Liposomal delivery of photosensitising agents. Expert Opin Drug Deliv 2005;2:477-87
20. Koo YE, Fan W, Hah H, et al. Photonic explorers based on multifunctional nanoplatforms for biosensing and photodynamic therapy. Appl Opt 2007;46:1924-30
21. Iyer AK, Khaled G, Fang J, et al. Exploiting the enhanced permeability and retention effect for tumor targeting. Drug Discov Today 2006;11:812-8
    - • **Comprehensive review of the enhanced permeability and retention effect (EPR).**
22. Van Dongen GAMS, Visser GWM, Vrouenraets MB. Photosensitizer-antibody conjugates for detection and therapy of cancer. Adv Drug Deliv Rev 2004;56:31-52
23. Schneider R, Tirand L, Frochot C, et al. Recent improvements in the use of synthetic peptides for a selective photodynamic therapy. Anticancer Agents Med Chem 2006;6:469-88
24. Verma S, Watt GM, Mai Z, et al. Strategies for enhanced photodynamic therapy effects. Photochem Photobiol 2007;83:996-1005
25. Stefflova K, Chen J, Zheng G. Killer beacons for combined cancer imaging and therapy. Curr Med Chem 2007;14:2110-25
26. Cló E, Snyder JW, Ogilby PR, et al. Control and selectivity of photosensitized singlet oxygen production: challenges in complex biological systems. Chembiochem 2007;8:475-81
27. Chen X, Drain CM. Photodynamic therapy using carbohydrate conjugated porphyrins. Drug Des Rev 2004;1:215-34
28. Lu ZR, Ye F, Vaidya A. Polymer platforms for drug delivery and biomedical imaging. Proceedings of the Thirteenth International Symposium on Recent Advances in Drug Delivery Systems; 2007;122:269-77
29. Solban N, Rizvi I, Hasan T. Targeted photodynamic therapy. Lasers Surg Med 2006;38:522-31
30. Macheda ML, Rogers S, Best JD. Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. J Cell Physiol 2005;202:654-62
31. Lee PPS, Lo PC, Chan EYM, et al. Synthesis and in vitro photodynamic activity of novel galactose-containing phthalocyanines. Tetrahedron Lett 2005;46:1551-4
32. Hirohara S, Obata M, Salto A, et al. Cellular Uptake and Photocytotoxicity of Glycoconjugated Porphyrins in Hela Cells. Photochem Photobiol 2004;80:301-8
33. Hirohara S, Obata M, Ogata SI, et al. Sugar-dependent aggregation of glycoconjugated chlorins and its effect on photocytotoxicity in HeLa cells. J Photochem Photobiol B 2006;84:56-63
34. Chen X, Hui L, Foster DA, et al. Efficient synthesis and photodynamic activity of porphyrin-saccharide conjugates: targeting and incapacitating cancer cells. Biochem 2004;43:10918-29
35. Pushpan SK, Venkatraman S, Anand VG, et al. Porphyrins in photodynamic therapy – a search for ideal photosensitizers. Curr Med Chem Anticancer Agents 2002;2:187-207
36. Rahimipour S, Ben-Aroya N, Ziv K, et al. Receptor-mediated targeting of a photosensitizer by its conjugation to

gonadotropin-releasing hormone analogues. J Med Chem 2003;46:3965-74

37. Frochot Cl, Di Stasio B, Vanderesse R, et al. Interest of RGD-containing linear or cyclic peptide targeted tetraphenylchlorin as novel photosensitizers for selective photodynamic activity. Bioorg Chem 2007;35:205-20
38. Choi Y, McCarthy JR, Weissleder R, et al. Conjugation of a photosensitizer to an oligoarginine-based cell-penetrating peptide increases the efficacy of photodynamic therapy. ChemMedChem 2006;1:458-63
39. Dixon MJ, Bourre L, MacRobert AJ, et al. Novel prodrug approach to photodynamic therapy: Fmoc solid-phase synthesis of a cell permeable peptide incorporating 5-aminolaevulinic acid. Bioorg Med Chem Lett 2007;17:4518-22
40. Sibrian-Vazquez M, Jensen TJ, Hammer RP, et al. Peptide-Mediated Cell Transport of Water Soluble Porphyrin Conjugates. J Med Chem 2006;49:1364-72
41. Conway CL, Walker I, Bell A, et al. In vivo and in vitro characterisation of a protoporphyrin IX-cyclic RGD peptide conjugate for use in photodynamic therapy. Photochem Photobiol Sci 2008;7:290-8
42. Sibrian-Vazquez M, Jensen TJ, Vicente MG. Synthesis, characterization and metabolic stability of porphyrin and peptide conjugates bearing bifunctional signaling sequences. J Med Chem 2008;51:2915-23
43. Stefflova K, Li H, Chen J, et al. Peptide-Based Pharmacomodulation of a Cancer-Targeted Optical Imaging and Photodynamic Therapy Agent. Bioconjug Chem 2007;18:379-88
44. Tirand L, Thomas N, Dodeller M, et al. Metabolic profile of a peptide-conjugated chlorin-type photosensitizer targeting neuropilin-1: An in vivo and in vitro study. Drug Metab Dispos 2007;35:806-13
45. Sharman WM, Van Lier JE, Allen CM. Targeted photodynamic therapy via receptor mediated delivery systems. Delivery of photosensitizers in photodynamic therapy. Adv Drug Deliv Rev 2004;56:53-76

•• **Good overview of receptor-mediated targeting of photosensitisers.**

46. Bhatti M, Yahioglu G, Milgrom LR, et al. Targeted photodynamic therapy with multiply-loaded recombinant antibody fragments. Int J Cancer 2008;122:1155-63
47. Fabbrini M, Trachsel E, Soldani P, et al. Selective occlusion of tumor blood vessels by targeted delivery of an antibody-photosensitizer conjugate. Int J Cancer 2006;118:1805-13
48. Kuimova MK, Bhatti M, Deonarain M, et al. Fluorescence characterisation of multiply-loaded anti-HER2 single chain Fv-photosensitizer conjugates suitable for photodynamic therapy. Photochem Photobiol Sci 2007;6:933-9
49. Linares R, Pacheco JR, Good TA. Efficacy of different targeting agents in the photolysis of interleukin-2 receptor bearing cells. J Photochem Photobiol B 2004;77:17-26
50. Savellano MD, Pogue BW, Hoopes PJ, et al. Multiepitope HER2 targeting enhances photoimmunotherapy of HER2-overexpressing cancer cells with pyropheophorbide-a immunoconjugates. Cancer Res 2005;65:6371-9

• **Receptor-mediated targeting of photosensitisers.**

51. Savellano MD, Hasan T. Targeting cells that overexpress the epidermal growth factor receptor with polyethylene glycolated BPD verteporfin photosensitizer immunoconjugates. Photochem Photobiol 2003;77:431-9
52. Amessou M, Carrez D, Patin D, et al. Retrograde delivery of photosensitizer (TPPp-O-beta-GluOH)3 selectively potentiates its photodynamic activity. Bioconjug Chem 2008;19:532-8
53. Hudson R, Carcenac M, Smith K, et al. The development and characterisation of porphyrin isothiocyanate-monoclonal antibody conjugates for photoimmunotherapy. Br J Cancer 2005;92:1442-9
54. Malatesti N, Smith K, Savoie H, et al. Synthesis and in vitro investigation of cationic 5,15-diphenyl porphyrin-monoclonal antibody conjugates as targeted photodynamic sensitisers. Int J Oncol 2006;28:1561-9
55. Staneloudi C, Smith KA, Hudson R, et al. Development and characterization of novel photosensitizer: scFv conjugates for use in photodynamic therapy of cancer. Immunology 2007;120:512-7
56. Sutton JM, Clarke OJ, Fernandez N, et al. Porphyrin, chlorin, and bacteriochlorin isothiocyanates: useful reagents for the synthesis of photoactive bioconjugates. Bioconjug Chem 2002;13:249-63
57. Gariepy J. The use of Shiga-like toxin 1 in cancer therapy. Crit Rev Oncol Hematol 2001;39:99-106
58. Tarrago-Trani MT, Jiang S, Harich KC, et al. Shiga-like toxin subunit B (SLTB)-enhanced delivery of chlorin e6 (Ce6) improves cell killing. Photochem Photobiol 2006;82:527-37

• **Toxin-mediated photosensitiser targeting.**

59. Savellano MD, Hasan T. Photochemical targeting of epidermal growth factor receptor: a mechanistic study. Clin Cancer Res 2005;11:1658-68
60. Ermilov EA, Al-Omari S, Helmreich M, et al. Photophysical properties of fullerene-dendron-pyropheophorbide supramolecules. Chem Phys 2004;301:27-31
61. Ermilov EA, Hackbarth S, Al-Omari S, et al. Trap formation and energy transfer in the hexapyropheophorbide a – fullerene C_{60} hexaadduct molecular system. Opt Commun 2005;250:95-104
62. Rancan F, Helmreich M, Molich A, et al. Intracellular uptake and phototoxicity of $3^1,3^2$-didehydrophytochlorin-fullerene hexaadducts. Photochem Photobiol 2007;83:1330-8
63. Rancan FH, Molich A, Ermilov EA, et al. Synthesis and in vitro testing of a pyropheophorbide-a-fullerene hexakis adduct immunoconjugate for photodynamic therapy. Bioconjug Chem 2007;18:1078-86
64. Fotinos N, Campo MA, Popowycz F, et al. 5-Aminolevulinic acid derivatives in photomedicine: characteristics, application and perspectives. Photochem Photobiol 2006;82:994-1015
65. Battah S, Balaratnam S, Casas A, et al. Macromolecular delivery of 5-aminolaevulinic acid for photodynamic therapy using dendrimer conjugates. Mol Cancer Ther 2007;6:876-85

•• **First use of dendrimers for delivery of photosensitiser precursors.**

66. Nishiyama N, Stapert HR, Zhang GD, et al. Light-harvesting ionic dendrimer porphyrins as new photosensitizers for photodynamic therapy. Bioconjug Chem 2003;14:58-66

•• **First use of dendrimers for delivery of photosensitisers.**

67. Tran N, Krueger T, Pan Y, et al. Correlation of photodynamic activity and fluorescence signaling for free and pegylated mTHPC in mesothelioma xenografts. Lasers Surg Med 2007;39:237-44

68. Nakamura E, Isobe H. Functionalized fullerenes in water. The first 10 years of their chemistry, biology, and nanoscience. Acc Chem Res 2003;36:807-15

69. Iwamoto Y, Yamakoshi Y. A highly water-soluble C60-NVP copolymer: a potential material for photodynamic therapy. Chem Commun 2006;46:4805-7

70. Nuno Silva J, Haigle J, Tome JP, et al. Enhancement of the photodynamic activity of tri-cationic porphyrins towards proliferating keratinocytes by conjugation to poly-S-lysine. Photochem Photobiol Sci 2006;5:126-33

71. Ogura S, Yazaki K, Yamaguchi K, et al. Localization of poly-L-lysine-photosensitizer conjugate in nucleus. J Control Release 2005;103:1-6

72. Tijerina M, Kopeckova P, Kopecek J. Correlation of subcellular compartmentalization of HPMA copolymer-Mce6 conjugates with chemotherapeutic activity in human ovarian carcinoma cells. Pharm Res 2003;20:728-37

73. Vaidya A, Sun Y, Ke T, et al. Contrast enhanced MRI-guided photodynamic therapy for site-specific cancer treatment. Magn Reson Med 2006;56:761-7

74. Liu J, Ohta SI, Sonoda A, et al. Preparation of PEG-conjugated fullerene containing Gd^{3+} ions for photodynamic therapy. J Control Release 2007;117:104-10

• **Use of fullerenes in PDT.**

75. Choi Y, Weissleder R, Tung CH. Selective antitumor effect of novel protease-mediated photodynamic agent. Cancer Res 2006;66:7225-9

• **Use of a protease-triggered PDT effect.**

76. Campo MA, Gabriel D, Kucera P, et al. Polymeric Photosensitizer Prodrugs for Photodynamic Therapy. Photochem Photobiol 2007;83:958-65

77. Gabriel D, Campo MA, Gurny R, et al. Tailoring protease-sensitive photodynamic agents to specific disease-associated enzymes. Bioconjug Chem 2007;18:1070-7

78. Renno RZ, Terada Y, Haddadin MJ, et al. Selective photodynamic therapy by targeted verteporfin delivery to experimental choroidal neovascularization mediated by a homing peptide to vascular endothelial growth factor receptor-2. Arch Ophthalmol 2004;122:1002-11

79. Sezgin Z, Yuksel N, Baykara T. Preparation and characterization of polymeric micelles for solubilization of poorly soluble anticancer drugs. Eur J Pharm Biopharm 2006;64:261-8

80. Sibata MN, Tedesco AC, Marchetti JM. Photophysical and photochemical studies of zinc(II) phthalocyanine in long time circulation micelles for Photodynamic Therapy use. Eur J Pharm Sci 2004;23:131-8

• **Use of long-circulating liposomes in PDT.**

81. Zhang JX, Hansen CB, Allen TM, et al. Lipid-derivatized poly(ethylene glycol) micellar formulations of benzoporphyrin derivatives. J Control Release 2003;86:323-38

82. Iyer AK, Greish K, Fang J, et al. High-loading nanosized micelles of copoly(styrene-maleic acid)-zinc protoporphyrin for targeted delivery of a potent heme oxygenase inhibitor. Biomaterials 2007;28:1871-81

83. Chowdhary RK, Sharif I, Chansarkar N, et al. Correlation of photosensitizer delivery to lipoproteins and efficacy in tumor and arthritis mouse models; comparison of lipid-based and Pluronic P123 formulations. J Pharm Pharm Sci 2003;6:198-204

84. Regehly M, Greish K, Rancan F, et al. Water-soluble polymer conjugates of ZnPP for photodynamic tumor therapy. Bioconjug Chem 2007;18:494-9

85. Li B, Moriyama EH, Li F, et al. Diblock copolymer micelles deliver hydrophobic protoporphyrin IX for photodynamic therapy. Photochem Photobiol 2007;83:1505-12

86. Akiyama M, Ikeda A, Shintani T, et al. Solubilisation of [60] fullerenes using block copolymers and evaluation of their photodynamic activities. Org Biomol Chem 2008;6:1015-9

87. Sezgin Z, Yuksel N, Baykara T. Investigation of pluronic and PEG-PE micelles as carriers of meso-tetraphenyl porphine for oral administration. Int J Pharm 2007;332:161-7

88. Chowdhary RK, Chansarkar N, Sharif I, et al. Formulation of benzoporphyrin derivatives in pluronics. Photochem Photobiol 2003;77:299-303

89. Torchilin VP, Levchenko TS, Lukyanov AN, et al. p-Nitrophenylcarbonyl-PEG-PE-liposomes: fast and simple attachment of specific ligands, including monoclonal antibodies, to distal ends of PEG chains via p-nitrophenylcarbonyl groups. Biochim Biophys Acta Biomembr 2001;1511:397-411

90. Roby A, Erdogan S, Torchilin VP. Solubilization of poorly soluble PDT agent, meso-tetraphenylporphin, in plain or immunotargeted PEG-PE micelles results in dramatically improved cancer cell killing in vitro Eur J Pharm Biopharm 2006;62:235-40

• **Enhanced solubilisation of a poorly water soluble photosensitiser.**

91. Roby A, Erdogan S, Torchilin VP. Enhanced in vivo antitumor efficacy of poorly soluble PDT Agent, meso-tetraphenylporphine, in PEG-PE-based tumor-targeted immunomicelles. Cancer Biol Ther 2007;6:1136-42

92. Zheng G, Chen J, Li H, et al. Rerouting lipoprotein nanoparticles to selected alternate receptors for the targeted delivery of cancer diagnostic and therapeutic agents. Proc Natl Acad Sci USA 2005;102:17757-62

93. Cinteza LO, Ohulchanskyy TY, Sahoo Y, et al. Diacyllipid micelle-based nanocarrier for magnetically guided delivery of drugs in photodynamic therapy. Mol Pharm 2006;3:415-23

•• **Magnetic photosensitiser targeting.**

94. Jang WD, Nishiyama N, Zhang GD, et al. Supramolecular nanocarrier of anionic dendrimer porphyrins with cationic block copolymers modified with polyethylene glycol to enhance intracellular photodynamic efficacy. Angew Chem Intl Ed Eng 2005;44:419-23

95. Ikeda A, Doi Y, Nishiguchi K, et al. Induction of cell death by photodynamic therapy with water-soluble lipid-membrane-incorporated [60] fullerene. Org Biomol Chem 2007;5:1158-60

96. Delanaye L, Bahri MA, Tfibel F, et al. Physical and chemical properties of pyropheophorbide-a methyl ester in ethanol, phosphate buffer and aqueous dispersion of small unilamellar dimyristoyl-L-alpha-phosphatidylcholine vesicles. Photochem Photobiol Sci 2006;5:317-25

97. Kosobe T, Moriyama E, Tokuoka Y, et al. Size and surface charge effect of

5-aminolevulinic acid-containing liposomes on photodynamic therapy for cultivated cancer cells. Drug Dev Ind Pharm 2005;31:623-9

98. Buchholz J, Kaser-Hotz B, Khan T, et al. Optimizing photodynamic therapy: in vivo pharmacokinetics of liposomal meta-(tetrahydroxyphenyl) chlorin in feline squamous cell carcinoma. Clin Cancer Res 2005;11:7538-44
99. Erdogan S, Medarova ZO, Roby A, et al. Enhanced tumor MR imaging with gadolinium-loaded polychelating polymer-containing tumor-targeted liposomes. J Magn Reson Imaging 2008;27:574-80
100. Namiki Y, Namiki T, Date M, et al. Enhanced photodynamic antitumor effect on gastric cancer by a novel photosensitive stealth liposome. Pharm Res 2004;50:65-76
101. Kepczynski M, Nawalany K, Jachimska B, et al. Pegylated tetraarylporphyrin entrapped in liposomal membranes: a possible novel drug-carrier system for photodynamic therapy. Colloids Surf B Biointerfaces 2006;49:22-30
102. Buchholz J, Wergin M, Walt H, et al. Photodynamic therapy of feline cutaneous squamous cell carcinoma using a newly developed liposomal photosensitizer: preliminary results concerning drug safety and efficacy. J Vet Intern Med 2007;21:770-5
103. Takeuchi Y, Kurohane K, Ichikawa K, et al. Polycation liposome enhances the endocytic uptake of photosensitizer into cells in the presence of serum. Bioconjug Chem 2003;14:790-6
104. Takeuchi Y, Ichikawa K, Yonezawa S, et al. Intracellular target for photosensitization in cancer antiangiogenic photodynamic therapy mediated by polycation liposome. J Control Release 2004;97:231-40
105. Gijsens A, Derycke A, Missiaen L, et al. Targeting of the photocytotoxic compound AlPcS4 to Hela cells by transferrin conjugated PEG-liposomes. Int J Cancer 2002;101:78-85
106. Ichikawa K, Hikita T, Maeda N, et al. PEGylation of liposome decreases the susceptibility of liposomal drug in cancer photodynamic therapy. Biol Pharm Bull 2004;27:443-4
107. Elbayoumi TA, Pabba S, Roby A, et al. Antinucleosome antibody-modified liposomes and lipid-core micelles for tumor-targeted delivery of therapeutic and diagnostic agents. J Liposome Res 2007;17:1-14
108. Ricci-Júnior E, Marchetti JM. Zinc(II) phthalocyanine loaded PLGA nanoparticles for photodynamic therapy use. Int J Pharm 2006;310:187-95
109. Zeisser-Labouèbe M, Lange N, Gurny R, et al. Hypericin-loaded nanoparticles for the photodynamic treatment of ovarian cancer. Int J Pharm 2006;326:174-81
110. Vargas A, Pegaz B, Debefve E, et al. Improved photodynamic activity of porphyrin loaded into nanoparticles: an in vivo evaluation using chick embryos. Int J Pharm 2004;286:131-45
111. Gao D, Agayan RR, Xu H, et al. Nanoparticles for two-photon photodynamic therapy in living cells. Nano Lett 2006;6:2383-6
112. Kim S, Ohulchanskyy TY, Pudavar HE, et al. Organically modified silica nanoparticles co-encapsulating photosensitizing drug and aggregation-enhanced two-photon absorbing fluorescent dye aggregates for two-photon photodynamic therapy. J Am Chem Soc 2007;129:2669-75
113. Kopelman R, Lee Koo YE, Philbert M, et al. Multifunctional nanoparticle platforms for in vivo MRI enhancement and photodynamic therapy of a rat brain cancer. J Magn Magn Mater 2005;293:404-10
114. Reddy GR, Bhojani MS, McConville P, et al. Vascular targeted nanoparticles for imaging and treatment of brain tumors. Clin Cancer Res 2006;12:6677-86
115. Tada DB, Vono LR, Duarte EL, et al. Methylene blue-containing silica-coated magnetic particles: a potential magnetic carrier for photodynamic therapy. Langmuir 2007;23:8194-9
116. Tang W, Xu H, Kopelman R, et al. Photodynamic characterization and in vitro application of methylene blue-containing nanoparticle platforms. Photochem Photobiol 2005;81:242-9
117. Tang W, Xu H, Park EJ, et al. Encapsulation of methylene blue in polyacrylamide nanoparticle platforms protects its photodynamic effectiveness. Biochem Biophys Res Commun 2008;369:579-83
118. Ohulchanskyy TY, Roy I, Goswami LN, et al. Organically modified silica nanoparticles with covalently incorporated photosensitizer for photodynamic therapy of cancer. Nano Lett 2007;7:2835-42
119. Roy I, Ohulchanskyy TY, Pudavar HE, et al. Ceramic-based nanoparticles entrapping water-insoluble photosensitizing anticancer drugs: a novel drug-carrier system for photodynamic therapy. J Am Chem Soc 2003;125:7860-5
120. Wieder ME, Hone DC, Cook MJ, et al. Intracellular photodynamic therapy with photosensitizer–nanoparticle conjugates: cancer therapy using a 'Trojan horse'. Photochem Photobiol Sci 2006;5:727-34
121. Chatterjee DK, Yong Z. Upconverting nanoparticles as nanotransducers for photodynamic therapy in cancer cells. Nanomedicine 2008;3:73-82
122. Wainwright M. Photodynamic therapy: the development of new photosensitisers. Anticancer Agents Med Chem 2008;8:280-91
123. Baba K, Pudavar HE, Roy I, et al. New method for delivering a hydrophobic drug for photodynamic therapy using pure nanocrystal form of the drug. Mol Pharm 2007;4:289-97
124. Sako Y, Goto Y, Murakami H, et al. Ribosomal synthesis of peptidase-resistant peptides closed by a nonreducible inter-side-chain bond. ACS Chem Biol 2008;3:241-9
125. Brocchini S, Godwin A, Balan S, et al. Disulfide bridge based PEGylation of proteins. Adv Drug Deliv Rev 2008;60:3-12
126. Uchida T. STX-liposome conjugates as candidate vaccines. Drugs Today 2003;39:673-93
127. Georgens C, Weyermann J, Zimmer A. Recombinant virus like particles as drug delivery system. Curr Pharm Biotechnol 2005;6:49-55
128. Lee LA, Wang Q. Adaptations of nanoscale viruses and other protein cages for medical applications. Nanomedicine 2006;2:137-49
129. Portney N, Gozkan M. Nano-oncology: drug delivery, imaging, and sensing. Anal Bioanal Chem 2006;384:620-30

Affiliation

Stéphane A Sibani[1], Paul A McCarron[2], A David Woolfson[3] & Ryan F Donnelly[†4]
[†]Author for correspondence
[1]PhD Student
Queens University Belfast,
Medical Biology Centre,
School of Pharmacy,
97 Lisburn Road,
Belfast BT9 7BL, UK
[2]University of Ulster,
Department of Pharmacy and
Pharmaceutical Sciences,
Cromore Road, Coleraine,
BT52 1SA, UK
[3]Chair in Pharmaceutics
Queens University Belfast,
Medical Biology Centre,
School of Pharmacy,
97 Lisburn Road,
Belfast BT9 7BL, UK
[4]Lecturer in Pharmaceutics
Queens University Belfast,
Medical Biology Centre,
School of Pharmacy,
97 Lisburn Road,
Belfast BT9 7BL, UK
Tel: +44 0 28 90 972 251;
Fax: +44 0 28 90 247 794;
E-mail: r.donnelly@qub.ac.uk