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Abstract: To maximize drug targeting to solid tumors, cancer nanomedicines with prolonged circulation times are required. To this end, poly(ethylene glycol) (PEG) has been widely used as a steric shield of nanomedicine surfaces to minimize serum protein absorption (opsonisation) and subsequent recognition and clearance by cells of the mononuclear phagocyte system (MPS). However, PEG also inhibits interactions of nanomedicines with target cancer cells, limiting the effective drug dose that can be reached within the target tumor. To overcome this dilemma, nanomedicines with stimuli-responsive cleavable PEG functionality have been developed. These benefit from both long circulation lifetimes en route to the targeted tumor as well as efficient drug delivery to target cancer cells. In this review, various stimuli-responsive strategies to dePEGylate nanomedicines within the tumor microenvironment will be critically reviewed.

Keywords: nanomedicine, cancer, stimuli responsive, dePEGylation, EPR effect

1. Introduction

In the treatment of cancer, the main challenge is how to deliver cytotoxic drugs to cancer cells while minimizing off-target toxicity in healthy cells and tissue. Patients currently undergoing cancer chemotherapy will typically experience debilitating side effects[1] (*e.g.* impaired immune system, nausea, cardiomyopathy, hair loss), and in many cases, the cumulative lifetime dose of an anti-cancer drug (*e.g.* doxorubicin; 550 mg/kg) must be limited, irrespective of therapeutic success, to avoid permanent bodily damage.[2] Efforts have therefore been made to develop nanomedicines capable of delivering drugs specifically to cancer cells.[3]

Over the past 30 years, two clinically effective *targeted* cancer therapies have emerged: antibody-drug conjugates (ADCs) and nanoparticle-based systems. Currently, 4 ADCs and 7 distinct nanoparticle-based drug delivery systems (DDS), targeted against a variety of human cancers, have received market approval.[4, 5] For ADCs, active targeting of cancer cells is achieved through antibody recognition of (over-)expressed receptors (tumor-associated antigens).[6] Once bound, ADCs are endocytosed, the conjugated drug released and the cell destroyed. Although effective, ADCs are costly to manufacture, can elicit adverse immunogenic responses (limiting repeat dosing) and are largely restricted to the delivery of small molecule (and serum stable) drugs.[7] In the case of nanoparticle-based DDS, drugs are encapsulated within a self-assembled nanoparticle, hidden and protected from the *in vivo* environment. Pharmacokinetic (PK) profiles are dictated by the nanoparticle and, in theory, it is possible to deliver almost any therapeutic cargos, from small molecule drugs to plasmid DNA, to target cells and tissue within the body. An enormous variety of nanoparticle-based DDS have been reported, however the most widely investigated, and the majority approved for clinical application, are liposomes.[8] In the targeted treatment of cancer, all clinically approved nanoparticle-based nanomedicines are liposomes designed to passively target tumors *via* the enhanced permeability and retention (EPR) effect.[9, 10]

1.1 The enhanced permeability and retention (EPR) effect

Following administration to the body, small molecule drugs freely diffuse into tissue and away from the site of injection. In contrast, intravenously (*i.v.*) injected nanoparticles are restricted to the circulating blood flow, unable to cross the tightly packed endothelium due to their larger size. For optimal biodistribution, nanoparticles should be larger than 10 nm in diameter – below which they are filtered from the body *via* the kidneys[11] – and smaller than 200 nm in diameter – above which they are rapidly recognized and phagocytosed by blood resident macrophages (principle cells of the mononuclear phagocyte system, MPS), within the liver and spleen, and are cleared from the body.[12]

The EPR effect is a phenomena characterized by the ill-defined ('leaky') vasculature and poor lymphatic drainage of tumors that arises as a result of rapid angiogenesis (blood vessel growth) within tumor tissue.[13] Circulating nanoparticles circulating through the tumor vasculature can therefore passively diffuse across gaps in the endothelium, accumulate within the tumor and remain there for extended

periods of time. Once within the tumor, nanoparticle encapsulated drugs either passively diffuse from the nanoparticle or an endogenous or exogenous stimulus can be exploited to trigger release.

To maximize passive targeting of nanomedicines to solid tumors *via* the EPR effect, nanoparticles with long circulation lifetimes are sought. Put simply, the more times nanoparticles pass through the tumor vasculature, the more will accumulate there. Care must therefore be taken to minimize drug leakage from the nanoparticle *en route* to the tumor while ensuring therapeutically relevant concentrations of drugs are released once there. In the case of liposome-drug formulations, this involves careful choice of lipid reagents (*e.g.* cholesterol to rigidify fluid lipid membranes) to fine tune drug retention/release profiles while at the same time maximizing circulation lifetimes.[14]

1.2 Polyethylene glycol (PEG)

To achieve long circulation lifetimes, the principal biological barrier a nanoparticle must overcome is recognition and clearance by cells of the mononuclear phagocyte system (MPS).[15] The principle organ of the MPS is the liver where hepatic macrophages – Kupffer cells – are highly proficient at recognizing and removing macromolecular, colloidal and pathogenic waste from circulation.[16, 17] Without any surface modification, up to 99% of systemically administered nanoparticles are cleared by the liver.[18] In most cases, it is believed rapid adsorption of blood proteins to the surface of nanoparticles, (a process known as opsonisation), acts as the recognition beacon for MPS cells.[19] For this reason, sterically shielding nanoparticle surfaces with biocompatible polymers, such as polyethylene glycol (PEG), has been effectively employed to minimize opsonisation and prolong blood circulation times of nanoparticles *in vivo*.[20]

PEG is a synthetic polymer of repeating ethylene glycol units. Used as a reagent or additive in a wide range of biological, chemical and industrial settings,[21, 22] it is commercially available in a range of geometries (linear, branched, star, comb), molecular weights (from 300 Da – 6-7 repeating units – up to 10 MDa - >200,000 repeating units) and can be easily functionalized. PEGylation of nanoparticle surfaces has been shown to decrease serum protein adsorption, reduce nanoparticle uptake in the liver and prolong circulation lifetimes.[23] Recently, reports have emerged to suggest PEG can elicit an immunogenic response in mammals.[24] However, the extent of this response, caused by binding of anti-PEG antibodies, remains unclear.[25] PEG remains an FDA approved polymer and is still the most widely used polymeric coating of nanomedicines, both in academic and industrial research. In terms of cancer nanomedicines, PEGylated liposomal-doxorubicin (Doxil®) has been used clinically for over 20 years in the treatment of select breast and ovarian cancers, multiple myeloma and AIDS-related Kaposi's sarcoma.[22]

1.3 The PEG dilemma

While PEGylation prolongs circulation lifetimes, it also limits the cellular uptake of nanoparticles and therefore effective drug delivery to target cancer cells.[26] This so-called 'PEG dilemma' has proved a major obstacle in the effective delivery of therapeutic cargos to cancer cells, particularly those that must be actively transported across the target cellular membrane (*e.g.* proteins and oligonucleotides).[27] For instance, in the delivery of oligonucleotides (ODNs) or small interfering RNAs (siRNAs), significantly lower transfection/transduction efficiencies were observed for PEGylated vs. non-PEGylated DDS.[28] To overcome this dilemma, strategies have been proposed to trigger the *extracellular* shedding of PEG (*i.e.* dePEGylation) from a nanoparticle surface upon reaching the target tumor. This leads to one of three scenarios (Figure 1): 1) rupture of the nanoparticle and extracellular drug release; 2) cellular uptake (endocytosis) of the intact nanoparticle-drug complex or 3) in the case of liposomes, fusion with the target cellular membrane and drug release directly to the cell cytoplasm, crucially avoiding degradative endocytotic liposome uptake.

In a significant number of reported dePEGylation strategies, it is required that PEGylated nanoparticles are first taken up by target cancer cells, whereupon the low pH, reductive and protease-rich environment of the late endosome/lysosome can be effectively exploited to trigger *intracellular* dePEGylation and drug release. However, these systems *do not* overcome the "PEG dilemma" and the very limited uptake of PEGylated nanoparticles remains a major drawback. As such, these systems will not be further discussed in this review but are included in the comprehensive summary of dePEGylation strategies presented in Table 1.

For strategies involving *extracellular* dePEGylation within the target tumor, a key difference is whether dePEGylation causes destabilization of the nanocarrier and *extracellular* drug release (*i.e.* burst release), or intact nanocarrier internalization by target cancer cells and *intracellular* drug release. In the case of *extracellular* drug release, only drugs able to passively diffuse (or be actively transported) across target cancer cell membranes (*e.g.* membrane permeable doxorubicin) can be used. In the case of *intracellular* drug release, the delivery of membrane impermeable therapeutics (*e.g.* proteins, oligonucelotides) is possible. In either scenario, it is essential cancer cells are exposed to therapeutically relevant doses of cytotoxic drugs if improved therapeutic indices are to be achieved.

2. Physical dePEGylation

Two physical approaches to dePEGylate nanoparticle surfaces within target tissues have been investigated. The first, most relevant for liposomal nanomedicines, relies on the exchange of PEGylated lipids from a liposome membrane to a target membrane sink (*e.g.* target cancer cell membranes).[29] Here, the rate at which exchange occurs is heavily dependent on the lipid anchor tethering PEG to the liposome membrane (*i.e.* how strongly it is held within the liposome membrane).[30] The length and saturation of (phospho)lipid fatty acid (FA) chains determines both the thickness and rigidity of a lipid membrane. FA

chain lengths within biological membranes typically vary between C12 and C30 – the number of carbon atoms.[31] FA chains can be saturated (no double bonds) or unsaturated (1 or more double bond). Saturated FAs pack closely together to form rigid lipid membranes (gel state), whereas unsaturated FAs loosely pack to form fluid membranes liquid crystalline state).[32] In addition, the shorter the FA chains, the more fluid the membrane. This is reflected in the liquid crystalline-to-gel transition temperatures (T_m) of individual (phospho)lipids.

In a study of three different lipid-PEG conjugates, no lipid-PEG exchange was observed for long chain, saturated lipid anchors 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG, C18:0 – 2 x 18 carbon FA chain; no double bonds) whereas exchange occurred in the time frame of hours for shorter saturated lipids 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE-PEG; C14:0) or long chain, unsaturated lipids 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE-PEG; C18:1 – 2 x 18 carbon FA chain; each 1 double bond, ω9).[33] This time frame enabled efficient accumulation of liposomes in tumor sites *via* the EPR effect (prior to dePEGylation) coupled with increased cellular uptake within the tumor (following dePEGylation). Conversely, a similar study found that only in the case of DSPE-PEG were circulation times prolonged enough to see efficient passive accumulation of nanoparticles within the tumor.[34] These conflicting results highlight the fine balance required to achieve efficient passive accumulation within target tumors and subsequent dePEGylation *via* physical desorption of lipid-PEG reagents. The propensity for non-specific PEG exchange with biological membranes *in vivo,* prior to reaching the target tumor, has likely limited the widespread application of these approaches.

The second physical approach relies on non-covalent adsorption of PEG to a nanoparticle surface.[35-39] For example, carboxylate-functionalized PEG adsorbed to a cationic nanoparticle surface.[37] In this case, partial protonation of carboxylate groups within the acidic (pH 6.5-7) extracellular tumor microenvironment leads to dePEGylation and subsequent cellular nanoparticle uptake. While this approach is conceptually simple, the stability of the absorbed PEG corona in serum and the propensity of premature dePEGylation under physiological conditions (*e.g.* high salt) and/or through competition from other serum components has likely limited the widespread investigation of this approach.

3. Chemical dePEGylation strategies

By far the most common method to achieve *extracellular* dePEGylation of nanoparticle surfaces, within the tumor microenvironment, is through chemical approaches. In these cases, PEG is grafted to the nanoparticle *via* a stimuli-responsive covalent chemical bond (Table 1 and 2).[40] Stimuli can be both endogenous and exogenous. In the case of endogenous stimuli, intrinsic differences in the pathophysiology of tumor and healthy tissues are exploited, namely the low pH,[41] reducing[42] and matrix metalloprotease (MMP)-rich environment[43] of certain solid tumors. Exogenous stimuli, including light and heat, have the benefit of being under complete user control in both time and space.[44] In a clinical setting however, these approaches rely on the ability to efficiently deliver stimuli (*e.g.* light) to tissues

often deep within the body. The various stimuli-responsive chemistries commonly used in both the *intra-* and *extracellular* dePEGylation of nanoparticles are summarized in Table 1 and 2.

3.1 pH-sensitive dePEGylation

The mildly acidic (pH 6.5-7.2) extracellular environment of hypoxic tumors – a result of increased glucose catabolism and efflux of H^+ by cancer cells – has been exploited to trigger extracellular dePEGylation of nanoparticle surfaces.[45] For this, chemical functionalities stable at physiological pH (pH 7.4) but labile at lower pH are required. The most commonly used acid labile chemical groups are vinyl ethers,[46-50] hydrazones,[51-61] acetals,[62-69] β-thiopropionates,[70] ortho esters[71-73] and benzoic imines[74-82]. Here however, it is important to differentiate between the mildly acidic *extracellular* pH within the tumor microenvironment (pH 6.5-7) and the strongly acidic *intracellular* pH within late endosomes/lysosomes (pH 4.5-5.5) and to stress that optimal sensitivity (and subsequent dePEGylation efficiency) of these acid-labile functionalities is typically at pH 5-5.5. Therefore all these pH-sensitive systems demonstrate inefficient/sluggish acidolytic dePEGylation within the *extracellular* tumor microenvironment. This can be exploited to achieve prolonged and sustained drug release within the tumor and/or partial dePEGylation may still generate the desired outcome. For example, Gu *et al*. reported pH dependent dePEGylation of polycationic micelles through grafting of PEG, *via* benzoic imine linkages, to poly-L-lysine(PLL)/cholic acid co-polymers.[82] By measuring changes in surface charge (zeta potential), the authors were able to show colloidal stability at physiological pH as well as increasing rates of dePEGylation with decreasing pH (complete acidolyis at pH 5.5 within 10 min). At pH 6.5-7 (*i.e.* pH of the extracellular tumor microenvironment) only partial dePEGylation was observed, however this was accompanied by a significant increase in hemolytic activity suggesting partial dePEGylation was sufficient to endow these particles with the desired function. As this system was not tested in cancer models *in vivo*, it remains to be seen whether this slow rate of acidolysis will adversely affect function and efficacy. Indeed, the individual successes of pH responsive dePEGylation systems ultimately depends on the ability to deliver therapeutically relevant drug doses to cancer cells above and beyond those of the administered free drug alone. It is worth noting, however, these technologies – as with any system exploiting endogenous stimuli – will likely demonstrate significant variations in efficacy due to patient-to-patient heterogeneity of tumor pathologies.[83, 84]

3.2 Redox-sensitive dePEGylation

Glutathione (GSH), is an abundant reducing agent (2-10 mM) in most mammalian cells, including cancer cells.[85] Extracellular GSH concentrations in healthy tissue are approximately 1000x lower (2–20 μM),[86] however this value can increase up to 4-fold (4-80 μM) within the tumor microenvironment.[87] There are conflicting reports as to whether this small differential in extracellular GSH concentrations can indeed be exploited to trigger *extracellular* dePEGylation. While a small number of studies report (partial)

extracellular cleavage of disulfide linked PEG constructs within the tumor microenvironment,[88-92] most exploit GSH as an *intracellular* trigger only.[93-134] In these cases, the very large differential between *extra-* and *intracellular* GSH concentrations is a readily exploitable endogenous trigger. Indeed, for systems designed to exploit *intracellular* GSH levels, *extracellular* stability (*i.e.* very limited reduction) of disulfide-PEG constructs is often reported as a key feature in maintaining colloidal stability of nanoparticles in circulation and *en route* to the target tumor. In our critical opinion, exploiting the marginally elevated *extracellular* GSH levels of the tumor microenvironment is an ineffective strategy to overcome the 'PEG dilemma'.

3.3 Protease-sensitive dePEGylation

Within the tumor microenvironment, there are high levels of *extracellular* matrix metalloproteinases (MMPs). These lytic enzymes are secreted at high levels by tumor cells to degrade the extracellular matrix (ECM) and aid cancer cell migration.[135, 136] Short peptides containing enzyme-consensus sequences, linking PEG to a nanoparticle surface, have been effectively used to dePEGylate nanoparticles within the tumor microenvironment.[137-161] Torchilin *et al.* have reported two elegant examples of MMP-triggered dePEGylation. The first employed a multifunctional liposomal formulation comprising longer, MMP-cleavable lipid-PEG_{3400} constructs and shorter, non-cleavable TAT-functionalised lipid-PEG_{2000} constructs.[141] In the absence of MMPs, longer PEG_{3400} chains effectively shielded the cell penetrating function of the underlying TAT peptide and liposomes were sparingly taken up by cells. Upon MMP-mediated dePEGylation however, the newly revealed TAT-functionalised liposomes were avidly taken up by 4T1 breast cancer cells. Going one step further, the same group reported a similar strategy of exploiting MMP-mediated dePEGylation to reveal newly functional drug polymer micelles.[146] Crucially in this approach, dePEGylation did not destroy the integrity of the underlying drug-filled micelle leading to efficient stimuli responsive, *intracellular* drug delivery to cancer cells, as demonstrated in mice models.

It is worth noting here that both cathepsin B (protease)[162] and esterases[138] have also been exploited to trigger dePEGylation of nanomedicines. However, cathepsin B is only found at high levels within (*intracellular*) cancer cells, while esterases are widely distributed in plasma and healthy tissues and not therefore specific to the tumor microenvironment. In our opinion, MMP-mediated dePEGylation of nanoparticles within the tumor microenvironment represents the most selective and efficient strategy to enhance the efficacy of cancer nanomedicines exploiting *endogenous* stimulus.

3.4 Light-sensitive dePEGylation

Photolabile chemical bonds have been extensively used, in both chemistry and biological contexts, to precisely control where and when new functionality is revealed. Unlike endogenous stimuli such as pH,

redox and enzymatic cleavage, the application of light can be precisely controlled in both time, space and intensity (*i.e.* is user defined) and requires no other reactive species (other than, in some cases, water). In addition, photolysis is generally rapid (few seconds, pulsed laser), quantitative and clean.

For light triggered dePEGylation of potential cancer nanomedicnes, *o*-nitrobenzyl (*o*-Nb),[63, 163-166] platinum-azide complexes[167] and azobenze[168] functionalities have all been explored.[169] In the case of *o*-Nb functionalities, non-hydrolytic photolysis proceeds through a cyclic intermediate followed by the release of the desired alcohol and a nitroso by-product.[170] To increase biological compatibility, methoxy substitution of the aryl ring results in reduced toxicity of nitroso byproducts.[171] We have recently reported two separate strategies in which light triggered dePEGylation was successfully used to initiate efficient drug delivery to target cancer cells. In the first example, we created 100 nm, loose core shell micelles composed exclusively of photolabile doxorubicin-PEG_{2000} reagents.[165] In the absence of light, micelles were stable, non-toxic (*i.e.* not taken up by cells *in vitro*) and no doxorubicin release was observed over time. Upon light (365 nm) activation triggered dePEGylation, micelle destabilisation and subsequent burst drug release resulted in *in vitro* cytotoxicity comparable to free doxorubicin. In addition, we were able to demonstrate precise spatiotemporal control of doxorubicin delivery to cells *in vitro* through light templated activation. We are currently assessing this system *in vivo* to determine circulation lifetimes and tumor accumulation of PEGylated doxorubicin prodrug micelles prior to light triggered dePEGylation.

In the second example, light triggered dePEGylation was used to precisely control, in time and space, the function of a simplified membrane fusion system. This system comprises two complementary peptides – peptide E and K – displayed from opposing lipid membranes (either liposome-liposome or liposome-cell).[163] In this case, PEGylation (*via* a photolabile cholesterol-PEG construct) of one lipid membrane effectively shielded the interaction between complementary peptides. However, upon light triggered dePEGylation regain of fusion function was instantaneous. We have subsequently shown our simplified membrane fusion system can be used to selectively deliver liposome-encapsulated cargos, *via* membrane fusion, to target (xenografted) cancer cells *in vivo* (zebrafish larvae).[172] Extending this approach to include light triggered dePEGylation, to enable precise user control of drug delivery, is the subject of current investigations in the group.

The use of light does, of course, raise valid concerns going forward into the clinic. In all reported examples of light triggered dePEGylation, systems are most sensitive to high energy UV-A light (<400 nm). Short wavelength UV light suffers from poor tissue penetration (100-200 μm) and, following prolonged exposure, can elicit significant photocytotoxicity.[173] Only for polymeric systems containing platinum-azide complexes[167] was photolytic dePEGylation investigated using visible light irradiation. Here, decreasing photolytic efficiency correlated with longer light wavelengths. Here however, it is important to note that photodynamic therapies,[174] combining chemical photosensitizers and light activation, are already widely used in the clinic to treat a range of medical conditions, including acne, atherosclerosis and cancer.[175] Furthermore, advances in fibre optic technologies (to deliver UV light deep

within tissue),[176] the development of photolabile chemical bonds sensitive to longer wavelength light[177] and the optimization of photosensitive chemical functionality to minimize light exposure, will only further the clinical applicability of light. One promising development has been photolabile chemical groups sensitive to two photon light,[178] to not only increasing tissue penetration (>1 cm) of light and minimising induced photocytotoxicity but, by restricting light activation to the focal point of two photon beams, enabling activation volumes in patients of <1 femtolitre. In this vein, we and others have also shown it is possible to cleave *o*-Nb groups using 2-photon light.[179] There are currently no examples of responsive dePEGylation of nanoparticles using alternative external stimuli (*e.g.* heat or ultrasound).

4. Conclusion

Stimuli-responsive dePEGylation is a proven strategy to increase the efficacy of cancer nanomedicines passively targeting solid tumors *via* the EPR effect. This approach has the dual advantage of both extended circulation lifetimes of PEGylated nanoparticles (to enhance passive targeting efficiency to tumors) as well as enhanced drug delivery profiles of non-PEGylated (or ruptured) nanoparticles within the tumor microenvironment. To achieve maximal effect, nanomedicines must remain PEGylated *en route* to the tumor (*i.e.* are serum stable) and be efficiently dePEGylated within the *extracellular* tumor microenvironment. Given the very low cellular uptake of PEGylated nanoparticles, strategies that report stimuli-responsive *intracellular* dePEGylation should not be considered effective. In our view, the most promising stimuli-responsive nanomedicines to date have exploited the MMP-rich microenvironment of solid tumors to trigger targeted and *extracellular* dePEGylation. However, by exploiting endogenous pathophysiological differences between healthy and diseased tissue, such as differences in MMP concentrations, the efficacy of these stimuli-responsive systems in patients will likely vary due to patient-to-tumor tumor heterogeneity.[83] In contrast, dePEGylation triggered by external stimuli, such as light, is exclusively determined by the user. While these approaches negate potential differences in efficacy driven by tumor heterogeneity, the current technological limitations of delivering external stimuli to site specific locations in patients remains a major drawback. However, the continued advance and optimisation of fibre-optic technologies as well more advanced photolabile chemical groups will only promote further the future application of light triggered cancer nanomedicines.

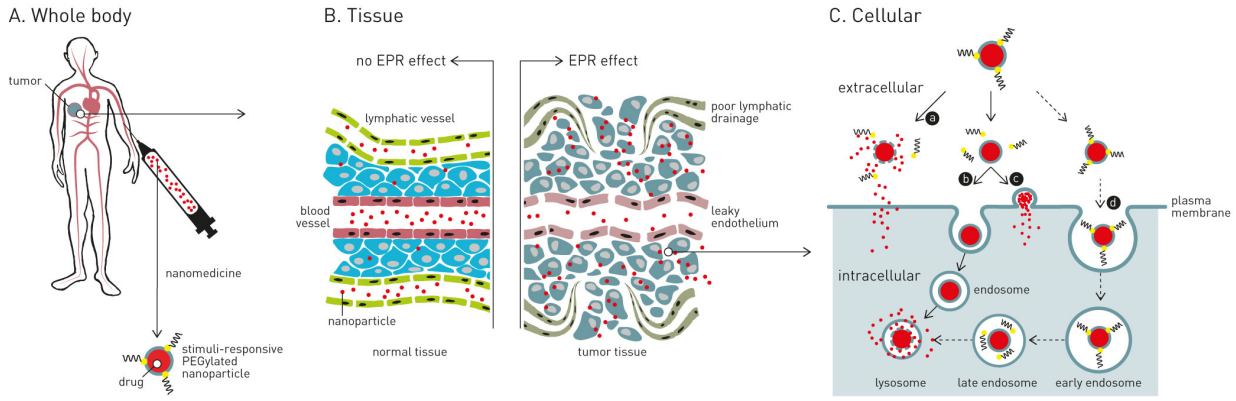


Figure 1. Following passive targeting of solid tumors *via* the enhanced permeability and retention (EPR) effect, stimuli-responsive dePEGylation of cancer nanomedicines can lead to various routes of enhanced drug delivery: **route a** – extracellular dePEGylation, nanocarrier rupture and extracellular drug delivery; **route b** – extracellular dePEGylation, endocytotic nanocarrier uptake and intracellular drug delivery; **route c** – extracellular dePEGylation, nanocarrier fusion with cancer cell membrane and direct cytosolic drug delivery (most relevant for liposomal nanomedicines); **route d*** – endocytotic nanocarrier uptake, intracellular dePEGylation and intracellular drug delivery. * this route *does not* overcome the "PEG dilemma" and the very limited uptake of PEGylated nanoparticles is a major drawback of these systems.

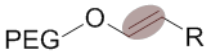
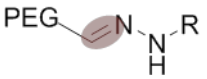
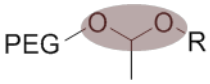
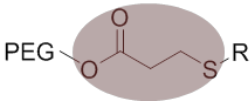
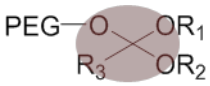
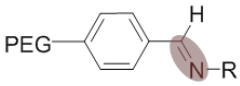
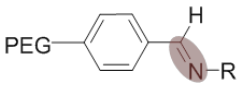
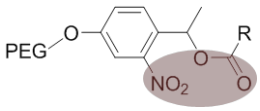
Site of dePEGylation within tumor	Stimulus	Example chemical structure	Nanocarrier	Drug release [*refs*]	
				Intracellular	Extracellular
Intracellular*	**Low pH** Late endosome (pH <6.5) Lysosome (pH 5.5-6.5)	 Vinylether	Liposomes	*[46-49]*	
			Micelles	*[50]*	
		 Hydrazone	Liposomes	*[52-54]*	
			Micelles	*[55-61]*	
		 Acetal	Liposomes	*[62]*	
			Micelles	*[63-69]*	
		 β-thiopropionate	Micelles	*[70]*	
		 Ortho ester	Liposomes	*[71-73]*	
		 Benzoic-imine	Micelles	*[74-76]*	
	Reduction Glutathione (GSH) (2-10 mM)		Liposomes	*[93]*	
			Polymersomes	*[94, 95]*	
			Micelles	*[96-121, 133]*	
			Graphene Oxide	*[122-124]*	
			Mesoporous silica nanoparticles (MSN)	*[125-132]*	
			Magnetic nanoparticles	*[134]*	
	Enzymatic Cathepsin B	 (*peptide consensus sequence*)	Liposomes	*[162]*	

Table 1. Various stimuli responsive chemical functionality used to trigger intracellular dePEGylation of cancer nanomedicines within the tumor microenvironment. *given the very limited uptake of PEGylated nanoparticles, systems reliant on intracellular triggers do not overcome the 'PEG dilemma' and are not further discussed in this review.

Site of dePEGylation within tumor	Stimulus	Example chemical structure	Nanocarrier	Drug release [*refs*]	
				Intracellular	Extracellular
Extracellular	**Low pH** (pH 6.5-7)	 Benzoic-imine	Polymeric Nanoparticles	*[77]*	
			Magnetic nanoparticles		*[78-80]*
			Micelles	*[81]*	*[82]*
	Reduction Glutathione (GSH) (4-80 μM)	 Disulfide	Liposomes		*[88-92]*
	Enzymatic (non-specific) Esterase		Liposomes	*[137, 138]*	
	Enzymatic (specific) Matrix metallo-proteinases (MMPs)	 (*peptide consensus sequence*)	Liposomes	*[139-145]*	
			Quantum dots (QDs)	*[157]*	
			Polymersomes	*[158]*	
			Magnetic nanoparticles	*[159]*	
			Micelles	*[146-156]*	*[160, 161]*
	External Light		Liposomes	*[163]*	
			MSN	*[164]*	
			Micelles	*[168]*	*[63, 165-167, 169]*

353 **Table 2.** Various stimuli responsive chemical functionality used to trigger extracellular dePEGylation of
354 cancer nanomedicines within the tumor microenvironment.

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