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Design considerations of polymeric nanoparticle micelles for chemotherapeutic delivery

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To improve safety and efficacy of anti-cancer therapy, drug-loaded polymeric nanoparticle micelle systems have been designed to target tumour pathophysiology. To accomplish this, nanoparticles take advantage of enhanced permeability and retention (EPR) of macromolecules to target tumours on a tissue level (passive targeting) while conjugated targeting ligands bind cancer surface markers and promote nanoparticle uptake (active targeting). Composition, size, shape, drug loading, and ligand density are all tunable design parameters that impact nanoparticle targeting. Understanding the complex interplay between these parameters and the resulting effects on drug targeting rationalizes adjustments to nanoparticle formulations.

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Introduction

Broad distribution and activity underlie the dose-limiting systemic toxicity associated with anti-cancer drug therapy. Further compounding the problem, promising drug candidates are often bulky and polycyclic hydrophobic compounds with poor aqueous solubility. As a result, they are formulated in mixtures of low molecular weight surfactants and organic solvents, which exert their own non-specific toxicity. Therefore, targeting strategies that replace surfactant-based formulations and deliver a greater portion of the injected dose to cancer cells represent exciting opportunities to significantly enhance treatment safety and efficacy.

To improve selectivity, several unique cancer features have been identified as potential targets, including abnormal vascular structure and pathological overexpression of cell surface receptors. Drug-loaded nanoscale assemblies accumulate selectively in tumour tissue via enhanced permeability and retention (EPR) that results from hyperpermeable tumour vasculature and insufficient lymphatic drainage. Nanoparticles modified with targeting ligands have further demonstrated receptor-specific binding of cancer cells, inducing endocytosis and providing a mechanism for selective drug uptake.

By engineering intelligent biomaterials for these applications, it is possible to develop platform technologies that can be used to target and destroy more cancer cells, and with greater specificity. This review will explore a variety of tunable parameters that can be adjusted when designing polymeric nanoparticle micelles for anti-cancer drug delivery. While some of these design parameters are applicable to liposomal formulations (spherical carriers comprised of lipid bilayers), our focus herein will be exclusively on polymeric micelles (self-assembled amphiphilic polymers). The multifaceted impact these properties may have on tissue and cellular level targeting will be discussed in the context of complex biological systems.

Polymeric nanoparticles and design elements in nanomedicine

Polymeric nanoparticles represent one approach to nanomedicine that takes advantage of hyperpermeable tumour vasculature to improve drug distribution via the EPR effect. Engineering the composition of amphiphilic copolymers gives control over many aspects of the resulting micelles that form in aqueous systems [1•]. Greater selectivity and concomitant reduction in systemic toxicity create opportunities to broaden the therapeutic window and improve the clinical outcomes of cancer treatment [2–4]. By utilizing the bloodstream for distribution, there is also potential to reach both primary and secondary tumours. The polymer can also provide protection against non-specific drug uptake and enzyme mediated drug degradation in the bloodstream [5]. Targeting ligands are also commonly attached to add cell-specific targeting and receptor-mediated uptake [6–8]. Polymeric systems promise flexible chemical modification strategies, simple and tunable self-assembly into ordered structures, and control over physical properties, all through rational design of their composition [1•,9].

Nanoparticles have been produced using a variety of biodegradable polymers as the core forming segment to give a versatile suite of materials for drug delivery. Polyesters (e.g. poly(lactic acid) (PLA)) [10], poly(amino

acids) (e.g. poly(aspartic acid)) [11], and poly(oxypropylene) (e.g. poloxamers or Pluronics) [12] are among the most well studied materials for cancer drug delivery.

Self-assembly and nanoparticle morphology

Nanoparticle micelles can form spontaneously when amphiphilic block or graft copolymers are introduced into aqueous environments [13]. The hydrophobic polymer segments form the micelle core and have the ability to physically load hydrophobic chemotherapeutic drugs [1,14]. Their size and shape influence their pharmacokinetics and biodistribution properties. Nanoparticles under 10 nm can be quickly cleared in capillary beds and lymph nodes, while those above 200 nm are rapidly removed from circulation via splenic filtration [15]. Size and shape also impact particle transport, immune recognition, and cell uptake. Indeed, particle curvature and aspect ratio determine their transport behaviour in the bloodstream [16], and influence cellular internalization processes [17]. In a comparative study of nanoparticle size, poly(methoxypolyethyleneglycol cyanoacrylate-co-n-hexadecyl cyanoacrylate) (PEG-PHDCA) nanoparticles were formulated over a size range of 80-240 nm to investigate the effect of size on tumour targeting and cellular uptake. Smaller particles succeeded in achieving greater circulation and tumour accumulation, but cellular uptake was relatively poor [18].

Size impacts passive targeting because accumulation in tumour tissue via EPR depends on extravasation through gaps in hyperpermeable tumour vasculature, putting restrictions on nanoparticle size. While the ideal size range is a topic of debate, a generally accepted range is 50–150 nm. Nonetheless, several ongoing studies argue that nanocarriers are also useful outside this range. Generally, larger nanoparticles can carry greater drug loads because of the larger available volume for encapsulation [1,19]. However, unless large nanoparticles are flexible and easily compressed, they may encounter difficulty crossing tumour vasculature [20•]. Another balancing consideration is tumour penetration, because intratumoural distribution of large macromolecular assemblies is driven primarily by convection, leaving larger nanoparticles trapped close to blood vessels [21]. Ultrasmall (<10 nm) gold nanoparticles show more uniform tissue distribution because they are able to diffuse through tissue [22], but may experience poor tumour localization due to increased non-specific tissue uptake.

Additionally, nanoparticle geometry impacts transport properties: discs and rod-shaped nanocarriers have shown improved blood circulation properties over spherical particles [16,23,24], leading to increased interest in developing drug carriers that circulate a particular geometry and break into smaller nanocarriers for improved tumour accumulation, penetration, and cell uptake [24,25].

Nanoparticle morphology also influences cellular trafficking processes. Nanoparticle uptake has been shown to behave as a function of size, where diameters greater than 50 nm undergo more rapid receptor mediated endocytosis [26]. Smaller nanoparticles must first form clusters before endocytosis is energetically favoured [27]. Shape also plays a role, where spherical nanoparticles experience faster uptake than rod-shaped nanoparticles, likely due to changes in local curvature or due to binding sites being blocked when the longitudinal edge of the rods are oriented parallel to the cell membrane [28].

Self-assembly is influenced by several factors, including the respective lengths of the core and shell forming blocks, which influence the critical micelle concentration, an important measure of nanoparticle stability [29]. The nanoparticle preparation method also determines the size and shape of the micelles that form, although the resulting drug loading and micelle structure may be kinetically unstable [1•].

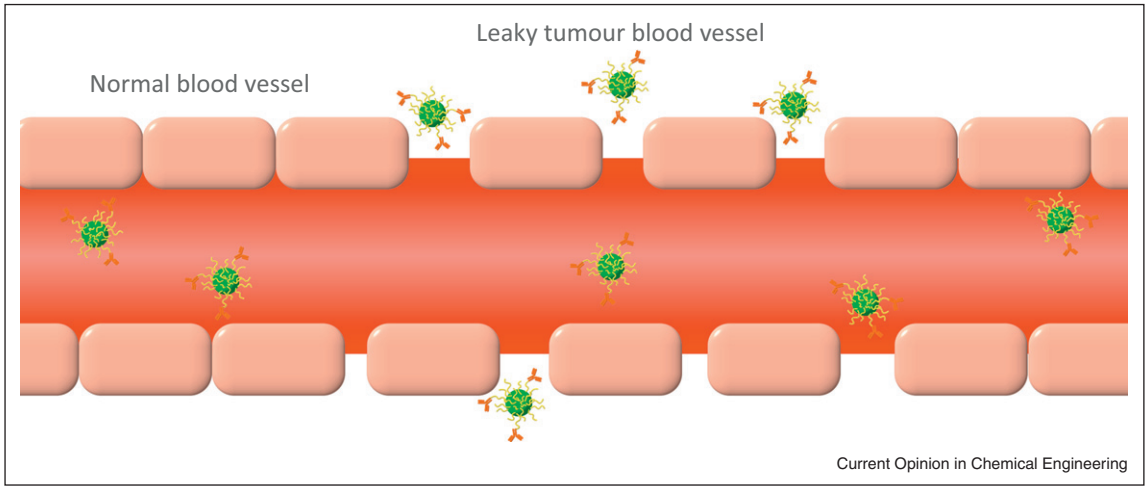
Extended drug circulation and nanoparticle surface properties

A long circulation half life (several hours) is a pre-requisite to tumour accumulation via passive targeting. Multiple passes through hyperpermeable tumour vasculature are required to observe EPR [13,16,30], and this means that drug-loaded nanoparticles must be designed to evade rapid drug degradation and non-specific uptake (Figure 1) [31]. To accomplish this, the most common strategy has been to incorporate poly(ethylene glycol) (PEG) as the hydrophilic block in the copolymers used to prepare nanoparticles [1•,32].

PEG has been shown to be critical design parameter for longer circulation: early particle formulations without PEG demonstrated that particulate drug delivery systems were completely eliminated from circulation within seconds to minutes [33]. The PEGylation strategy has many benefits, including stabilizing nanoparticles against aggregation, providing a neutral surface charge, and limiting adsorption of proteins and opsonins that would invoke clearance by the immune system [34]. Ideally, the length and density of PEG in each micelle would be adequate to create a brush layer, shielding the core from interactions with blood proteins [35•,36]. Polymer nanoparticles are well suited to dense PEGylation because stable incorporation of PEG can be achieved simply by adjusting the hydrophobic segment length in parallel; PEG incorporation is limited in liposomal systems, where high PEG-lipid content tends to form small curved micelles instead of stable membrane structures [36].

While the goal of longer circulation is to achieve greater and selective tumour accumulation, it may also increase systemic exposure and general toxicity because drug activity is not limited to cancer cells [37]. As a result,

Figure 1



Long plasma circulation times promote passive targeting of nanocarriers via EPR by increasing the number of passes through hyperpermeable tumour vasculature. PEGylated nanoparticles are depicted here passing through gaps between disorganized endothelial cells in a tumour blood vessel.

there is a compromise between these opposing factors that requires a balance between high tumour accumulation and low systemic distribution. It's important to realize that, even with PEG-modification, most nanoparticles are taken out of circulation at very short times and are processed by the liver, kidney and spleen — organs which function to remove toxins and waste from circulation.

Drug activity must also be protected in circulation, and metabolic processes are a major concern in conventional drug delivery strategies where the free drug is in direct contact with blood. When drugs are loaded in the nanoparticle core, degradation is inhibited by physically preventing enzymes from accessing the encapsulated material, improving the pharmacokinetic profile [7,38]. The drug is also physically prevented from accessing cells while circulating, limiting non-specific toxicity. All of these benefits have the potential to increase accumulation and specificity of active drug compounds at tumour sites.

Drug loading and micelle stability

An important advantage of incorporating drugs into nanoparticles is that the polymer provides a hydrophobic space to solubilize drug compounds. Typically, poor drug solubility in aqueous media necessitates their formulation in surfactants and organic solvents, which cause side effects of their own [39,40]. Biocompatible polymers that form stable and drug-loaded nanoparticles are therefore an attractive alternative from a formulations perspective [13,41].

Drug loading in polymeric nanoparticles has been achieved in many ways, usually falling into the broad categories of covalent attachment to the polymer, or physical entrapment via hydrophobic interactions in the nanoparticle core. Considering that nanoparticles may represent less than 1% of the total volume in a colloidal suspension, even less of which corresponds to the hydrophobic core, high and stable drug loading is important [1•]. Polymeric nanoparticles have the potential to attain higher drug loading than liposomes, where lipophilic drugs partition primarily into the lipid membrane, further restricting the available space [42]. To enhance drug loading, pH gradients (citrate) have been used to actively load drug compounds through precipitation [43], and alternative core materials have been co-encapsulated into liposomes [44]. However, these approaches are restricted to compounds that are relatively hydrophilic. Many promising drug candidates derive their potency from strong interactions with biological lipid membranes (cells and cell nuclei), which often are accompanied by elevated hydrophobicity [13].

One way to ensure stable drug loading is to covalently bond the drug to the polymer. This polymer-drug conjugate method is useful when using both a polymer and drug that contains easily modified functional groups, such as free amines, carboxylic acids, or hydroxyl groups [45]. This approach has found interesting applications in triggered release, where the polymer-drug conjugate is a prodrug that is cleaved through a labile linker when exposed to specific conditions, such as low pH or enzymatic degradation [45]. Drugs or polymers that are relatively hydrophilic may also be combined using this strategy, where hydrophobic interactions alone would not effectively keep the drug from partitioning out of the core.

Highly hydrophobic drugs do not require chemical modification to be stably incorporated into a hydrophobic nanoparticle core. In these cases, the drug may simply be loaded during nanoparticle preparation and will preferentially partition into the core. This effect may be further increased by integrating components that encourage greater loading, such as covalently attached drug molecules to promote stacking during encapsulation [46•]. With physical drug encapsulation, it is critical that the nanoparticles are stable. Since their disassembly would trigger immediate drug release, intact nanoparticles are vital to targeting strategies. The polymeric nanoparticle requires a low critical micelle concentration (CMC) in serum, which confers thermodynamic stability even under the considerable dilution that occurs immediately on injection into blood, and only intensifies with time as the polymer distributes [47]. Interestingly, some polymer systems have high kinetic stability, especially those with high glass transition temperatures, which means they exhibit slow rates of disassembly even when diluted below their CMC [1•].

High stability and efficient drug loading are both important features of polymeric systems that lead to their utility in targeting applications. However, it is important to keep in mind that the non-specific systemic toxicity associated with many anti-cancer drugs derives from their strong potency. As a result, unnecessarily high drug loading can lead to toxicity in non-target cells under low levels of non-specific particle uptake. Consequently, equal drug doses formulated in different nanoparticle concentrations may behave differently, and the highest possible drug loading is not necessarily the optimal condition.

Bioactive surface modification

In addition to chemotherapeutic agents, nanoparticles can be modified with targeting ligands that selectively recognize and bind to receptors overexpressed on cancer cells. Examples of common ligands include the native ligand to a receptor [48–50], receptor antagonists [51], peptides [52], aptamers [53,54], and antibodies [55••,56] or their fragments [57,58] (Table 1). Targeting ligands may exert their own therapeutic effects, contributing to treatment efficacy beyond their role in targeting and specificity. In selecting an appropriate coupling chemistry, the goal is to achieve high coupling efficiency without sacrificing binding activity and specificity of the ligand [59,60]. Especially where chemical modifications are made on assembled nanoparticles, reactions and processing conditions can disrupt micelle structure or negatively impact drug activity. The required reagents, potential byproducts, temperature, solvent, and necessary purification steps must all be given careful consideration. Ideally, the reaction should proceed under mild conditions, in an aqueous environment, and require minimal post-processing. With desired chemical functional groups in mind, polymers can be chosen or synthesized to provide platforms for simple surface modification protocols. By preserving binding activity, selective nanoparticle uptake by a target cell population is enabled through receptor-mediated endocytosis (Figure 2) [8].

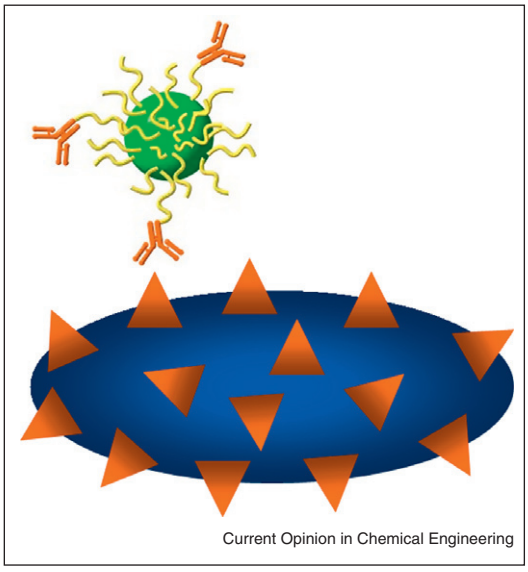
In vitro, actively targeted formulations have a clear advantage over unmodified nanoparticles because greater cell uptake transports greater drug doses to their intracellular targets. However, *in vivo*, functionalizing nanoparticles with targeting ligands often reduces the longer-circulation achieved with PEGylation because the targeting ligands may trigger an immune response [61,62]. Nevertheless, if cellular uptake can compensate for reduced tumour uptake, overall anti-cancer efficacy may improve [63••]. Nanoparticle internalization rates are likely a function of binding strength, which depends on both the intrinsic ligand-target affinity and the ligand density [64••]. This further adds to the debate over the optimal ligand conjugation density because increased uptake and decreased circulation may be linked. One approach is to increase ligand mobility so that they can be recruited to a common local area on the nanoparticle surface in the presence of target cells [61]. In this case, multivalent binding would exponentially increase binding strength through avidity without requiring a high conjugation density [64••].

Table 1

Examples of ligands used to actively target polymeric nanoparticles to cancer cells

Ligand type	Target (ligand)	Ref.
Native receptor ligands	Folate receptor (folate)	[48,49]
	Transferrin receptor (transferrin)	[50]
Receptor antagonists	$\alpha_4\beta_1$ integrin (VLA-4-antagonist peptides)	[51]
Peptides	α_3 integrin (LXY1 cyclic peptide)	[52]
Aptamers	Human epidermal growth factor receptor 2 (S6 aptamer)	[53]
	Prostate-specific membrane antigen (A10 PSMA aptamer)	[54]
Monoclonal antibodies	Human epidermal growth factor receptor 2 (trastuzumab)	[55••]
	Nucleosomes (2C5)	[56]
Antibody fragments	Human epidermal growth factor receptor 2 (trastuzumab Fab)	[57]
	CD19 (HD37 Fab′ or scFv)	[58]

Figure 2



Active recognition and binding of cancer cells can be achieved using targeting ligands against an overexpressed oncogene on the cell surface to provide a means of selective cellular uptake. As an example, an antibody-modified nanoparticle is depicted here binding a cell through an antigen-specific interaction.

However, strong binding raises another obstacle to drug delivery by creating a binding site barrier. Active targeting strategies can inhibit drug penetration to all areas of a tumour, in part due to the slow diffusion of large nanocarriers, but also due to active binding and depletion by the cells closest to an active blood vessel [65–67]. Even the free Herceptin antibody can take up to 24 hours to distribute uniformly in a HER2 overexpressing tumour xenografts [68]. Consequently, accounting for how binding strength influences tumour penetration is another important consideration in nanoparticle design.

Conclusions

Nanoparticle micelles are promising vehicles for targeted drug delivery to solid tumours. However, new formulations are often designed with only a few criteria in mind. Targeting is a multi-stage process, and adjusting a particular design parameter to favour only a narrow set of targeting criteria may not lead to greater efficacy or selectivity overall. In this review, we discussed the current understanding of the effects of common design parameters on multiple aspects of targeting. This discussion highlights the need to account for these complex relationships during the optimization process to achieve optimal results.

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