

# Exploiting the Tumor Phenotype Using Biodegradable Submicron Carriers of Chemotherapeutic Drugs

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**ABSTRACT:** Tumor tissues possess characteristics that distinguish them from healthy tissues and make them attractive targets for submicron carriers of chemotherapeutic drugs (CTX). CTX are generally administered systemically in free form to cancer patients resulting in unwanted cytotoxic effects and placing limitations on the deliverable CTX dose. In an effort to raise the therapeutic index of CTX there are now liposome-based CTX formulations in clinical use that are more tumor specific than the free form of CTX. However, progression to liposome-based chemotherapy in the clinic has been slow and there have been no approved formulations introduced in the last decade. Alternative carrier systems such as those made from the biodegradable polymer poly(lactic-co-glycolic) acid (PLGA) have been investigated in preclinical settings with promising outcomes. Here we review the principle behind biodegradable submicron carriers as CTX delivery vehicles for solid tumors with a specific focus on liposomes and PLGA-based carriers, highlighting the strengths and weaknesses of each system.

**KEY WORDS:** submicron carriers, PLGA, liposomes, chemotherapy, tumor targeting, enhanced permeation and retention effect, EPR, nanoparticles

**ABBREVIATIONS:** CTX: chemotherapeutic drug(s); PLGA: poly(lactic-co-glycolic) acid; EPR: enhanced permeation and retention; MPS: mononuclear phagocyte system; PEG: poly-(ethylene glycol); U.S. FDA: United States Food and Drug Administration; i.v.: intravenous(ly).

## I. INTRODUCTION

There are a number of traits possessed by tumors that distinguish them from healthy tissues, thereby rendering them viable targets for submicron carriers of chemotherapeutic drugs (CTX). One of these traits is the demonstration of the enhanced permeation and retention (EPR) effect for macromolecules and nanoparticles. To explain, the aberrant neovasculature of neoplasms is characterized by a range of structural and functional abnormalities including randomly distributed apertures of up to 1–2  $\mu\text{m}$  in size in the endothelial monolayer, which in turn contributes to a relatively high degree of leakiness.<sup>1</sup> These apertures result from compounding features that include an inadequately formed or discontinuous basement membrane, transendothelial channels, and wide interendothelial junctions.<sup>2</sup> The leakiness results in relative ease of accessibility to macromolecules and

submicron carriers that would normally be excluded, although there is still the barrier of the interstitial fluid pressure, which is generally high in tumors and increases with increasing tumor size.<sup>3</sup> Most tumors lack adequate lymphatic drainage and consequently macromolecules and submicron carriers can become trapped within the tumor microenvironment for significant durations. These combined phenomena (leakiness and inadequate lymphatic drainage) were first recognized in the context of macromolecules by Maeda *et al.* and are known collectively as the EPR effect.<sup>4</sup> It should be noted that discontinuous (or sinusoidal) capillaries possessing large gaps between endothelial cells and discontinuous or absent basement membrane are also found in healthy bone marrow, liver, and spleen.<sup>2</sup> Therefore, these healthy tissues will be susceptible to enhanced permeation with submicron carriers; however, due to effective lymphatic drainage, there should ideally be less accu-

mulation of these carriers in these tissues compared to tumors. Thus, submicron carriers have the potential to target tumors passively using the EPR effect.

Another important trait that distinguishes tumors from healthy tissues is the presence of a rich neovasculature within tumors comprising actively proliferating endothelial cells expressing a range of surface proteins not expressed by, or expressed at lower levels in, quiescent endothelial cells that normally constitute the venules of healthy tissues. These cell surface proteins (e.g.,  $\alpha$ V $\beta$ 3) can act as target receptors for CTX-carrying submicron particles that have been coated with complementary ligands [e.g., the tripeptide arginine-glycine-aspartic acid (RGD)]. This process of active targeting can bias localization of submicron carriers to the tumor over other tissues.

The benefits of using submicron carriers to encapsulate CTX are manifold and extend beyond the EPR effect and active targeting. These benefits include prevention of rapid dissemination of the drug nonspecifically throughout the body and instead promote its delivery to target tissues in a concentrated form, protection from degradative enzymes and agglutinating factors that would otherwise inactivate the drug, and the potential for formulation modifications that can affect pharmacokinetic and biodistribution characteristics.<sup>5</sup> This last point is salient since it is important that the CTX is neither released too quickly nor too slowly and it is also important for the carriers to avoid uptake by the mononuclear phagocyte system (MPS) and remain in circulation long enough for sufficient accumulation at the tumor site.<sup>6</sup>

## II. PHYSICOCHEMICAL PROPERTIES OF SUBMICRON CARRIERS THAT DETERMINE RATE OF CLEARANCE FROM CIRCULATION

The fate of intravenously (i.v.) administered submicron carriers is primarily determined by their size, surface characteristics, and molecular composition.<sup>6,7</sup>

### A. Size

When it comes to delivering submicron carriers to tumors, there are certain limitations that determine optimum size. Generally speaking, carriers less than 10 nm in diameter are not suitable for tumor targeting due to their rapid elimination by the kidneys.<sup>8</sup> When considering the upper size limit of systemically administered submicron carriers, a number of factors may impact tumor targeting efficiency. These include average fenestration size of the tumor, which can vary depending on tumor type or patient. Some tumors may have fenestrations as large as 700 nm but this is not always the case and it is generally accepted that carriers greater than 500 nm would be unsuitable for tumor targeting purposes. Empirically it has been shown, using liposomes (see below), that carriers 70–200 nm in diameter are optimal in terms of rates of clearance from the blood and accumulation in tumor tissues versus other healthy tissues. Carriers greater than 250 nm become more readily cleared by the spleen while carriers in the 10–70 nm range are vulnerable to clearance by tissues with fenestrated capillaries, possessing transendothelial circular openings (40–80 nm), such as the lung, skin, brain, muscle, and connective tissue.<sup>3</sup> Another drawback of carriers that are less than 70 nm in diameter is the higher ratio of surface area to volume thereby limiting the CTX load. In general, the rate of clearance of liposomes increases with increasing size and this is primarily explained by the MPS, particularly for conventional liposomes.

### B. Surface Characteristics

“PEGylation” [coating the carrier surface with the synthetic amphiphilic poly-(ethylene glycol) (PEG)] can alter the kinetics of clearance such that liposomes in the range of 80–250 nm have clearance rates that are less affected by changes in size.<sup>9,10</sup> PEGylation increases the hydrophilicity, and decreases the negative charge, of submicron carriers [liposomes and poly(lactic-co-glycolic) acid (PLGA) based] thereby abrogating aggregation and limiting opsonization.<sup>11</sup> It has been shown that negatively charged liposomes

may be cleared from the circulation more rapidly than neutral liposomes, often through opsonization, while positively charged liposomes have been shown to be toxic and are also rapidly eliminated.<sup>12</sup>

### C. Molecular Composition

The use of carriers made from biodegradable constituents is an attractive option since it avoids the harmful effects that can result from long-term accumulation of nonbiodegradable carriers within the patient. It is also desirable that the degradation of these particles results in the generation of nontoxic and readily eliminated byproducts. Finally, carriers made from compounds that can be modified to influence various pharmacokinetic properties and biodistribution patterns are highly desirable. These characteristics can be found for both liposomes and PLGA-based carriers and have been well researched as candidates for submicron delivery of CTX to cancer patients.

## III. LIPOSOMES

Liposomes are self-assembling colloidal structures that are biocompatible and biodegradable and were originally identified by Bangham *et al.*<sup>13</sup> In terms of colloidal-based drug-delivery systems, liposomes have undergone the most scrutiny. The initial findings in the early 1980s that liposomes were rapidly cleared from the circulation by the MPS instigated some pessimism as to the potential of liposomes as either diagnostic or therapeutic tumor targeting vectors.<sup>14,15</sup> However, by the late 1980s hope had been restored with an accumulation of findings that the phospholipid component of liposomes can affect their systemic clearance rate *in vivo* while both a combination of phospholipid components and cholesterol content can affect the drug-retaining capacity or permeability of the liposome.<sup>16,17</sup> There are a range of phospholipids capable of forming liposomes; examples include phosphatidylcholines, phosphatidylglycerols, phosphatidylserines, and sphingomyelins. Specifically, small unilamellar liposomes containing solid-phase phospholipids, sphingomyelin, or distearoyl phos-

phatidylcholine have longer circulation times than liposomes composed of fluid phospholipids (e.g., egg yolk phosphatidylcholine) due to their increased stability in plasma. Other lipids, such as cholesterol, are often included in the liposome formulation to further stabilize the liposome.<sup>18</sup> Unilamellar liposomes possess an aqueous core suitable for the encapsulation of water-soluble chemotherapeutic drugs and range in size from 50 to 250 nm in diameter. Multilamellar liposomes possess many lipid bilayers, making them particularly suitable as carriers of lipophilic drugs.

As mentioned earlier, to limit uptake by the MPS, liposomes can be PEGylated, and this hydrophilic coating can extend blood circulation times to days as opposed to hours when compared to conventional (uncoated) liposomes. In addition, longer-chained PEGs (e.g., PEG 1900 and PEG 5000) can increase blood residence times by approximately twofold compared to shorter-chained PEGs (e.g., PEG 750 and PEG 120).<sup>19</sup> Two of the liposome formulations to gain approval for clinical use were vectors for doxorubicin, Doxil (for treatment of Kaposi's sarcoma and refractory ovarian cancer) and Myocet (for treatment of metastatic breast cancer).<sup>20–22</sup> The primary difference between the two formulations is that Doxil is PEGylated while Myocet is uncoated and this is reflected in their blood circulation half-lives of 55 and 2.5 h, respectively. Preclinical studies using PEGylated liposomes have demonstrated their capacity for prolonged circulation, extravasation at tumor capillary beds, and improved antitumor activity when encapsulating a CTX compared to administration with the free form of the drug.<sup>14</sup> Specifically, in mice, it was shown that 24 h after i.v. injection of PEGylated liposomes (80–100 nm in diameter), 29% were still circulating in the blood compared to <2% for conventional liposomes. In addition, accumulation of PEGylated liposomes within the liver and spleen was reduced by more than threefold and approximately twofold, respectively, when compared to conventional liposomes. Pharmacokinetic studies indicated that PEGylated liposome clearance is dose independent and obeys single first-order kinetics while clearance of conventional liposomes was found to be dose dependent, suggesting uptake

by the MPS. In a colon carcinoma model, i.v. administered PEGylated liposomes accumulated to a greater extent (2.3-fold) in the tumor compared to conventional liposomes and it was established that the liposomes were capable of extravasating, thereby entering the tumor interstitium. Finally, the therapeutic index of a CTX (epirubicin) was increased when encapsulated into PEGylated liposomes and used to treat tumor-challenged mice (colon carcinoma C-26).<sup>14</sup> In a separate study, non-PEGylated versus PEGylated liposomes were compared for organ uptake rate in non-tumor-challenged mice and it was shown that the former when ~150 nm in diameter were mostly (~50% of injected dose) present in the liver within 6 h while <20% remained in circulation.<sup>23</sup> This was compared to PEGylated liposomes of a similar size which, after 6 h, were still found in circulation in large quantities (>40% of injected dose) while <20% were present in the liver and <5% in the spleen. In the same study, *in vivo* fluorescence microscopy was used to assess the ability of PEGylated liposomes of various sizes (63, 133, 198, and 388 nm) to passively target tumors (C-1300 neuroblastoma in mice).<sup>23</sup> A greater percentage (40–45%) of the injected dose of 133 and 198 nm liposomes remained in the circulation (at  $t = 6$  h) compared to the 63 nm (25%) and 388 nm (25%) liposomes. The reason for the 388 nm liposomes not remaining in circulation to the same extent as those in the 100–200 nm range was primarily due to increased uptake of larger liposomes by the spleen. When fluorescently labeled PEGylated liposomes (size  $126 \pm 35$  nm) were injected into tumor-challenged mice, fluorescence could be detected in the tumor vasculature on the surface of the tumor within 30 min of administration, while interstitial patches of fluorescence were detectable within 6 h. The location of the fluorescence indicated that the liposomes had extravasated to some degree. When liposomes were  $402 \pm 48$  nm, extravasation was not detected. Similar findings with respect to liposome size were reported by others using different tumor models and different liposomal formulations suggesting that such characteristics can possibly be ascribed to PEGylated liposomes in general.<sup>24,25</sup>

#### IV. PLGA

PLGA is a biodegradable synthetic copolymer that has long been approved for a range of medical applications by the U.S. FDA and that can be used to manufacture submicron carriers via a range of techniques.<sup>26</sup> The solvent extraction/evaporation method is most commonly used to prepare submicron particles due to ease of preparation, high reproducibility, and flexibility in being capable of loading hydrophobic or hydrophilic (e.g., doxorubicin) CTX using the single- or double-emulsion method, respectively. Due to their hydrophobic nature, uncoated PLGA carriers are rapidly cleared from the blood making surface modifications essential if longer circulating half-lives are desired. PLGA carriers can be readily modified with PEG, which reduces their surface negative charge as well as surface hydrophobicity. Once injected, i.v. unmodified PLGA carriers (~200 nm in diameter) have been shown to accumulate in various tissues (primarily in the liver > spleen > lungs), probably as a result of the MPS where they degrade at a more rapid rate than their *in vitro* counterparts.<sup>27,28</sup> Although PEGylation of PLGA carriers can decrease clearance rates, these carriers still generally have shorter circulation half-lives than liposome formulations, suggesting that alternative/additional modifications of PLGA carriers are required.<sup>29</sup> One possible explanation for poorer circulation times is that many of the methods used to PEGylate PLGA carriers, such as through amino- or carboxyl-terminated PLGA, adsorption, or incorporation of PLGA polymer conjugates, lead to inadequate surface-coating density of PEG. It has been suggested that significant loss of PEG (or other surface-coated ligands) can occur due to the hydrolysis of PLGA.<sup>30</sup> Recently, coating carriers with biotinylated PEG, or other biotinylated ligands, via avidin has proven to be an efficient method of enhancing ligand density.<sup>31,32</sup> To explain, an avidin-lipid bioconjugate (e.g., avidin-linoleic acid or avidinpalmitic acid) was included in the PLGA carrier formulations. Due to the amphiphilic nature of the bioconjugate, avidin orients itself to the surface of the PLGA carrier and is therefore available for the binding of biotinylated ligands or biotinylated PEG. Treatment of tumor-challenged mice with

doxorubicin-loaded PLGA carriers that had been PEGylated using avidinpalmitic acid resulted in antitumor effects equivalent to free doxorubicin, but with significantly reduced cardiotoxicity.<sup>32</sup>

Surface modifications with heparin, chitosan, poloxamers, and albumin have also been attempted as alternatives to PEG. Specifically, human serum albumin has been proposed as a promising coating agent for many reasons, which include: it is highly abundant, weakly immunogenic, nontoxic, and biodegradable, and has been implicated in playing a role in active targeting through albumin receptors on tumor cells.<sup>33</sup> Treatment of glioma with free doxorubicin is generally ineffective at least partially due to the impediment of the blood-brain barrier. Promising results in terms of crossing the blood-brain barrier have been obtained using surfactant-coated nanocarriers of various CTX.<sup>34–37</sup> Surfactant coating of submicron carriers has been shown to be important if effective delivery to the brain is to be achieved.<sup>38</sup> This is due to apolipoproteins (found in the plasma) adsorbing to the surfactant that then promote receptor-mediated endocytosis of the carriers by brain endothelial cells. One such surfactant is the block copolymer, poloxamer 188 (Pluronic F-68), which was used to coat doxorubicin-loaded PLGA submicron carriers and was shown to have enhanced antitumor activity over free doxorubicin and doxorubicin-loaded PLGA submicron carriers coated with polysorbate 80 (considered the gold standard surfactant for brain delivery).<sup>39</sup> Comparing a number of PLGA-based formulations in a rat glioblastoma model, it was shown that PLGA carriers incorporating lecithin in their core and encapsulating doxorubicin stabilized with human serum albumin and then coated postlyophilization with poloxamer 188 had greater antitumor activity than PLGA carriers that contained no lecithin.<sup>40</sup> It was proposed that the presence of lecithin may have enhanced the efficiency of coating by the poloxamer. Surprisingly, the most effective formulation also had the largest size (468 nm; PDI 0.4) compared to 250 and 380 nm for other formulations. This is an interesting and possibly important observation since based on studies with liposomes (see above), carriers of this size might have been assumed to be ineffective due to

splenic-mediated depletion. Human serum albumin was chosen as a stabilizer over the more commonly used polyvinylalcohol because polyvinylalcohol may be unsafe for parenteral applications.<sup>40</sup> However, the use of human serum albumin resulted in significantly larger PLGA carrier size (>400 nm) compared to when polyvinylalcohol was used (200 nm).<sup>39</sup>

In one study comparing Taxol (a currently U.S. FDA approved formulation of the CTX paclitaxel) with PLGA carriers encapsulating paclitaxel, it was shown that the latter had greater *in vivo* antitumor activity in a murine liver tumor model.<sup>41</sup> These PLGA carriers (112 nm in diameter and a neutral surface charge) were made using a nanoprecipitation method and included in the formulation were PLGA, PLGA-PEG, and the biodegradable diblock copolymer PCLPEG. The nanoprecipitation method used for encapsulating PTX was shown to be better than the single-emulsion method in terms of loading, encapsulation efficiency, and percent recovery of paclitaxel. Thus, PLGA carriers offer a potential alternative to Taxol, which suffers from the disadvantage of having Cremophor EL as part of its formulation (to enhance paclitaxel solubility), which can cause a range of deleterious side effects.<sup>42</sup>

## V. ACTIVE TUMOR TARGETING OF LIPOSOMES AND PLGA-BASED CARRIERS

Endothelial cells in tumors overexpress a range of cell surface molecules responsible for migration, adhesion, proliferation, or survival and these include alphaV-beta3 (possibly the most intensively researched), alphaVbeta5, alpha5beta1, E-selectin, endoglin, endosialin, and vascular endothelial growth factor receptor.<sup>43,44</sup> Active targeting of tumor vasculature is feasible through the differential expression of these and other cell surface markers that are either not expressed or expressed to a lesser extent on quiescent vasculature. Active targeting would include targeting to sites of angiogenesis where CTX may be able to generate an antiangiogenic effect by destroying the proliferating endothelial cells, thereby causing tumor cell death indirectly since malignant tumors are dependent on angiogenesis for growth and

metastases.<sup>45</sup> Targeting of tumor vasculature offers a number of advantages over directly targeting the tumor cells, including (i) ease of access—endothelial cells are readily “seen” by targeting particles and do not need to pass into the tumor interstitium to have an antitumor effect; (ii) attacking the tumor endothelia reduces the possibility of acquired drug resistance; (iii) endothelial cells have a more predictable pattern of homogeneous expression of surface proteins and are less likely to be subject to antigenic mutations often associated with tumor cells, thereby rendering them as less evasive targets. Particles coated with peptides or antibodies specific for these markers have been shown to improve tumor targeting of lipid-based and PLGA-based submicron carriers of CTX. For instance, the peptides NGR (Asn-Gly-Arg) and CDCRGDCFE (RGD-4C) have been shown to target tumor vasculature *in vivo* through the binding of CD13 (aminopeptidase N) and alphaV integrin, respectively.<sup>46–49</sup>

### A. Liposomes

In one study, PEGylated doxorubicin-liposomes coated with the targeting NGR peptide were used to treat SCID (severe combined immunodeficient) mice implanted with an orthotopic neuroblastoma xenograft.<sup>50</sup> Targeted liposomes accumulated within tumors tenfold more than non-targeted liposomes at 24 h post-administration. In addition, it was shown that administration of these targeted liposomes carrying doxorubicin (3 mg/kg doses of doxorubicin) i.v. to tumor-challenged mice resulted in substantial tumor regressions and significantly extended survival times compared to mice treated with non-targeted liposomes that had little antitumor effect. The addition of the targeting peptide made no difference to the degree of accumulation of liposomes in the liver but, inexplicably, resulted in a tenfold to 20-fold increase in the spleen, although no cytotoxicity within the spleen was observed. In a separate study, using a syngeneic murine orthotopic tumor model of pancreatic carcinoma, where PEGylated liposomes (120 nm in diameter) were functionalized with RGD-4C, it was shown that these liposomes localized to

the vasculature of the tumor but not to the adjacent healthy pancreatic tissue.<sup>51</sup> Encouragingly, there was also very little or no detectable accumulation of these targeted liposomes to other tissues such as the heart, liver, spleen, and brain. When treated therapeutically with 1 mg/kg doxorubicin, only actively targeting liposomes possessed detectable antitumor activity compared to free doxorubicin and doxorubicin encapsulated in non-targeting liposomes. Immunohistochemical analysis revealed that sites of necrosis coincided with sites of alphaVbeta3 expression. It was also noted that metastases were more vulnerable to treatment than the primary tumor, possibly because of there being more neovascularization in the metastases.

### B. PLGA-Based Carriers

It is reasonable to assume that, as observed in preclinical studies with liposomes (above), surface functionalization of PLGA-based carriers with targeting ligands for the tumor and/or the tumor vasculature will improve their therapeutic efficacy. The majority of studies demonstrating improved targeting of PLGA-based carriers to date have been performed *in vitro* using a range of targeting ligands to tumor or endothelial cells.<sup>52–55</sup> *In vitro* studies have shown that active targeting of PLGA-based carriers to specific cell types can be achieved by attaching monoclonal antibodies to preformed PLGA-based carriers. For example, cytokeratin-specific monoclonal antibodies specific for breast epithelial cells were adsorbed to the surface of PLGA-based carriers, which rendered them capable of being internalized by breast epithelial cells but not by a different cell type (colon).<sup>56</sup> Of particular interest, it was noted that uncoated particles were internalized by both cell types, thereby indicating that coating of particles with antibodies renders the particles less amenable to nonspecific uptake. Such a phenomenon may be explained by the monoclonal antibody creating a hydrophilic surface, thus repelling nonspecific uptake. Adsorption of monoclonal antibodies (or other targeting ligands) may not be as efficient as other methods of surface functionalization of PLGA particles, partly due to

potentially suboptimal surface coating and also due to the loss of bound antibody through hydrolysis of PLGA. As alluded to earlier, PLGA hydrolysis could also be problematic for antibody directly or indirectly attached to PLGA through covalent linkages such as N-ethyl-N'-(3-(dimethylamino)propyl)carbodiimide-activated carboxy-terminated PLGA polymers or through functionalized coblock polymers (e.g., functionalized PEGylated PLGA), but can be overcome through the use of amphiphilic ligand conjugates.<sup>30,31,57,58</sup>

There is a dearth of *in vivo* studies involving active tumor targeting of PLGA-based submicron carriers. However, of those that have been published there have been some promising outcomes with PLGA particles encapsulating either doxorubicin or paclitaxel, using aptamers, RGD, or folates as targeting ligands independently. The same group that demonstrated the improved antitumor efficacy of paclitaxel in PLGA particles (PTX-PLGA) over Taxol subsequently compared active versus passive targeting of PTX-PLGA in the same murine tumor model.<sup>59</sup> They used the tripeptide RGD as the targeting ligand, which is capable of binding  $\alpha V\beta 3$ , an integrin expressed by active endothelial cells as well as tumor cells.<sup>60</sup> RGD was grafted onto PLGA particles during their manufacture using PCL-PEG-RGD. It was shown that the RGD-functionalized PTX-PLGA targeted tumor endothelium more precisely and displayed significantly more antitumor activity than non-functionalized PTX-PLGA. In a separate study, PEGylated PLGA particles (156 nm diameter; zeta potential = -32.9) loaded with PTX were coated [using N-ethyl-N'-(3-(dimethylamino)propyl)carbodiimide/N-hydroxysuccinimide chemistry] with an aptamer (AS1411) specific for nucleolin, a cell surface protein that is expressed by angiogenic endothelial cells as well as tumor cells.<sup>61</sup> These particles displayed significantly more tumor activity and resulted in prolonged survival of mice challenged with C6 glioma xenografts compared to Taxol or particles not coated with aptamer.

## VI. CONCLUSIONS

For more than three decades, the field of nanomedicine in general is one that has had the scientific community buzzing with excitement at its potential in terms of therapeutic applications; however, the translation from preclinical studies, which have been plentiful and promising, to clinical success and approval for medical practice has been slow and infrequent.<sup>62</sup> There are many factors that may individually or cumulatively explain this slow progress, which include safety concerns, U.S. FDA regulations, prohibitive resources required to instigate clinical trials, patient variability and compliance, and the multiple manufacturing steps often required.<sup>63</sup>

Nevertheless, if cancer patients are to experience greater quality of treatment both in terms of enhanced antitumor efficacy as well as reduced off-target toxicities, then advances in CTX delivery need to be made. Both liposomes and PLGA-based submicron carriers have been intensively researched as vectors for CTX, to an extent that their advantages and disadvantages have been well characterized (Table 1). To date, only a few liposomal formulations (Doxil, Myocet, DaunoXome), and as yet no PLGA-based formulations, carrying CTX are available to cancer patients and they all rely on passive tumor targeting. It is evident from preclinical studies that improvements in tumor therapy are likely to be made using submicron carriers of CTX that are actively, rather than passively, targeting the tumor (the neovasculature of the tumor in particular), and there are concerted efforts by researchers to use materials that have already been approved as safe by the U.S. FDA or are likely to be so in the future. The promising results with PLGA-based submicron carriers showing delivery of CTX across the blood-brain barrier herald a promising noninvasive therapy for glioma patients. Further *in vivo* studies of actively tumor targeting carriers need to be performed to optimize therapeutic approaches and side-by-side comparisons of liposomes versus PLGA-based submicron carriers would be edifying since there is currently a paucity of such studies.

**TABLE 1:** Advantages and disadvantages of chemotherapeutic drug (CTX)-loaded liposomes and poly(lactic-co-glycolic) acid (PLGA)-based submicron carriers

Liposomes		PLGA-based carriers	
Advantages	Disadvantages	Advantages	Disadvantages
Size and charge readily modified	Labile (storage)	Tunable CTX release kinetics <sup>64</sup>	Subject to undesired burst release <sup>65</sup>
Carry hydrophobic and hydrophilic drugs	Vulnerable to complement-mediated lysis <sup>66</sup>	Carry hydrophobic and hydrophilic drugs	Circulation half-life generally shorter than liposomes <sup>29</sup>
Nontoxic	Multiple manufacturing steps	Nontoxic	Poor loading (1%) often observed despite high encapsulation efficiencies <sup>67</sup>
Weakly immunogenic	High production cost	Weakly immunogenic <sup>68</sup>	Relatively high polydispersity indices
Biodegradable		Biodegradable	Upscaling manufacture may be problematic
Readily surface functionalized		Readily surface functionalized	
		Potential to avoid multidrug resistance <sup>69,70</sup>	
		Drug delivery to cytosol (via endocytic pathway) <sup>71</sup>	

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