REVIEW ARTICLE

Liposomes in Active, Passive and Acoustically-Triggered Drug Delivery

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Abstract: Cancer has become one of the most deadly noncommunicable diseases globally. Several modalities used to treat cancer patients exist today yet many have failed to prove high efficacy with low side effects. The most common example of such modalities is the use of chemotherapeutic drugs to treat cancerous cells and deter their uncontrolled proliferation. In addition to the destruction of cancerous tissues, chemotherapy destroys healthy tissues as it lacks the specificity to annihilate cancerous cells only and preferentially, which result in adverse side effects including nausea, hair fall and myocardial infarction. To prevent the side effects of non-selective chemotherapy, cancer therapy research has been focused on the implementation of nanocarrier systems that act as vehicles to encapsulate drugs and selectively transport their agent to the tumor site. In this paper, we shed light on liposomes along with three anticancer drug delivery approaches: passive, active and ultrasound-triggered drug delivery.

1. INTRODUCTION

According to the World Cancer Research Fund International (WCRF), there were an estimated 14.1 million cancer cases around the world, placing cancer amongst the most prominent fatal non-communicable diseases worldwide in 2012 [1]. In another report, published recently by the American Cancer Society, an estimated 1,735,350 new cancer cases were expected to be diagnosed in the US alone in 2018 [2]. Cancer can be treated by one or a combination of the following treatments: surgery, radiotherapy, and systemic therapy. Surgery can be used in an early-stage disease to remove a localized tumor; on the other hand, a condition in a more advanced stage (*i.e.*, metastatic disease) requires the use of systemic therapy such as chemotherapy, hormonal therapy, targeted and immune therapy that uses the bloodstream to reach cancer sites [3]. Chemotherapy is widely used either as a single treatment or as an adjuvant or neoadjuvant treatment along with other treatment modalities [4]. Chemotherapy involves the use of anti-neoplastic agents to treat cancer or relieve its symptoms [5]. Although chemotherapy proved to be effective in destroying cancerous tissues, it can also affect healthy cells, especially those with high turnover such as intestinal epithelial cells, bone marrow, mucous membranes and hair follicles [3, 6]. The cytotoxicity of chemotherapy results in adverse effects that could ultimately be fatal (*e.g.*, cardiac toxicity) [7]. Also, treatment efficacy of chemotherapy may decrease due to lack of drug specificity and hence action [8]. Therefore, it is vital to find a paradigm by which drugs are encapsulated and selectively delivered to the tumor site before getting released.

Research in the area of drug delivery investigated various types of nanoparticles that are effective in transporting potent drugs to diseased tissues [9]. Liposomes, micelles, and solid nanoparticles are three broad categories of these nanoparticles [10]. Nanoparticles can be tailored to acquire specific physical and chemical properties, which can be employed in drug delivery to reduce the adverse effects of cytotoxic agents on normal cells [8]. In this paper, we focus on liposomes as drug nanocarriers in cancer treatment. These carriers allow drugs to be released in a controlled manner, both temporally and spatially [11].

2. LIPOSOMES

Liposomes are vesicle-like structures made of a material similar to the cell membranes (Fig. **1**). They were first described in the mid-60's by the British hematologist Alec D. Bangham and were formally referred to as "Banghasomes" until the name "liposome" was introduced by Weissmann [12, 13]. The word liposome is derived from two Greek words: "Lipo", meaning fat, and "Soma", meaning body [12]. Synthetic and natural liposomes are composed of phospholipids, which are amphipathic molecules made up of a hydrophilic head, a glycerol backbone and two hydrophobic fatty acid tails. The polar head may contain positive and negative charges, and it interacts with water in an aqueous environment, while the hydrophobic tails are sequestered away from the hydrophilic environment in the interior of the lipid bilayer (*i.e.*, between polar heads) [14, 15]. This structure allows liposomes to encapsulate both hydrophilic and lipophilic drugs [16]. Thus, the chemical interaction between different substances can be eliminated by placing them in separate compartments within the liposome [17].
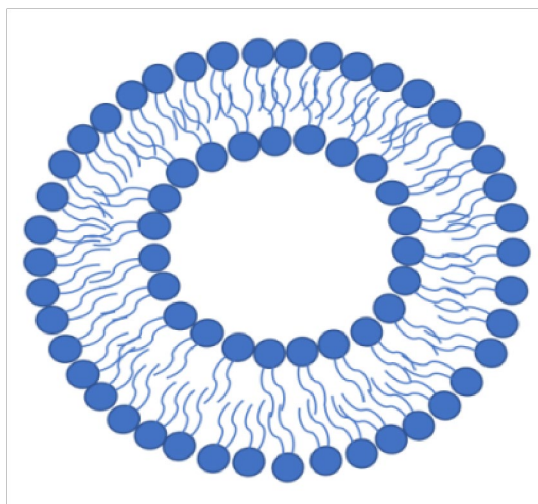

Fig. (1). Structure of a liposome.

2.1. Classification and Stabilization of Liposomes

Liposomes can be classified according to their sensitivity to an external stimulus such as light, pH, ultrasound, *etc*.; however, the most common method of characterization is their size [18]. The diameter of a liposome spans typically between 25 nm to 15 μm, depending on the vesicle structure: unilamellar vesicles (ULV), multilamellar vesicles (MLV) or multivesicular vesicles (MVV) [15, 18]. Both the size and composition of liposomes have vital effects on their functionality. In drug delivery, extended circulation time in the blood is desired. Liposomes are more likely to be recognized as a foreign body once introduced, and hence they are cleared from circulation by the reticuloendothelial system (RES) [19]. Clearance studies showed that the half-life of liposomes can be improved by reducing their size [20, 21]. For example, liposomes that are ~100 nm in diameter possess a long half-life of over 5 hours [22]. Other studies investigated the effect of liposome composition such as liposome charge and lipid saturation along with other factors on the circulation time. Generally speaking, using saturated lipids in the presence of cholesterol extends the circulation time of liposomes [22].

Another approach to increase the half-life of liposomes would be the conjugation of a hydrophilic polymer, such as polyethylene glycol (PEG), to the surface of the liposomes (once formed they are termed stealth liposomes) which creates a protective layer against opsonization, thus making them less susceptible to recognition and clearance by the RES [19]. Moreover, the polymerization of liposomes enhances their stability by reducing the chances of agglomeration and degradation [17]. By comparing the circulation time of stealth liposomes with free drug administration, the former was observed to be superior. For example, the widely used PEGylated liposomal formulation "Doxil" exhibits up to 100-fold longer half-life compared to free doxorubicin [23]. However, the composition of PEG on the surface of liposomes is critical and should be optimized in a way to make it large enough to avoid recognition by the body's immune system, yet not too large as it will hinder the interaction between liposomes and cancer cells, which will eventually affect cellular uptake and internalization [8].

The delivery and the uptake of liposomes into cancerous tissue occur passively, due to the nature of the diseased tissues, or actively, using targeting moieties conjugated to the surface of liposomes. Liposomes can also be designed to be sensitive to an internal or external stimulus to release their cargo. Targeting moieties may also be used to classify liposomes. For example, immunoliposomes have antibodies conjugated to their surface which are selective for specific proteins on the surface of cancer cells [24]. Several liposomal formulations have gained approval for clinical use to treat various medical conditions including sarcomas (especially Kaposi's sarcoma), breast cancers and some forms of lymphoma (Table **1**), while other formulations are still undergoing clinical trials to treat other types of cancer.

Table 1.   Approved liposomal-based formulations for cancer treatment.

Product Name (Approval Year)	Active Agent	Lipid Composition	Indications	Reference
Doxil® (1995)	Doxorubicin	HSPC, PEG$_{2000}$, DSPE and cholesterol	Ovarian cancer, AIDS-related Kaposi's sarcoma and multiple myeloma*	[25-27]
DaunoXome® (1996)	Daunorubicin	DSPC and cholesterol	AIDS-related Kaposi's sarcoma	[28]
Myocet® (2000)	Doxorubicin	EPC and cholesterol	Metastatic breast cancer	[29-31]
Mepact® (2009)	Mifamurtide	DOPS and POPC	Non-metastatic osteosarcoma	[32, 33]
Marqibo® (2012)	Vincristine sulfate	SPH and cholesterol	Acute lymphoblastic leukemia	[34, 35]
Lipodox® (2013)	Doxorubicin	HSPC, PEG$_{2000}$, DSPE and cholesterol	Ovarian/breast cancer, AIDS-related Kaposi's sarcoma and multiple myeloma	[32, 36]

(Table 1) Contd....

Product Name (Approval Year)	Active Agent	Lipid Composition	Indications	Reference
Onivyde® (2015)	Irinotecan	DSPC, MPEG$_{2000}$-DSPE and cholesterol	Metastatic pancreatic cancer	[37, 38]
Vyxeos™ (2017)	Daunorubicin and Cytarabine	DSPC, DSPG and cholesterol	Therapy-realted acute myeloid leukemia (t-AML), AML with myelodysplasia-related changes (AML-MRC)	[39, 40]

*Doxil was first approved in 1995 for AIDS-related Kaposi's sarcoma, then in 1999 for ovarian cancer and later in 2007 for multiple myeloma [27].

Success in clinical trials is limited because there exist multiple factors competing to produce the desired outcome, and those factors are often challenging to control all at once. Some factors are related to the physical properties of ligandconjugated liposomes such as: their size, the method of synthesis, the method used to conjugate the targeting ligands on their surface (*i.e.* the type of linkers used), ligand density as well as the amount of coated PEG used to form stealth liposomes. Such factors highly contribute to the ligand's accessibility to the tumor site and its binding affinity to the target receptor [23]. Other factors; on the other hand, are related to the systemic utilization of liposomes, including the number of nanoparticles that reach tumor sites and the spatiotemporal regulation of drug release [41]. Thus, it is essential to be acquainted with existing advances in this field in order to overcome the aforementioned challenges.

2.2. eLiposomes

Emulsion-containing liposomes, also known as eliposomes, are a type of stealth liposomes that contain nanoemulsions. Nanoemulsions are usually made up of perfluorocarbons (PFCs), especially those with a high vapor pressure, which makes them sensitive to ultrasound (US) waves. PFCs are inert, nontoxic and stable in aqueous environments [42].

In general, liposomes are not highly sensitive to ultrasonic induction unless they contain a gas phase [42, 43]. The presence of air bubbles or other gases in the liposomal vicinity facilitates the release of drug contents, and such liposomes are termed echogenic liposomes [44]. Ultrasound mediated microbubbles were also used in drug delivery where drug extravasation into tumors is facilitated either by the convective flow induced by oscillating bubbles "microstreaming" or by liquid jetting [45]. Liposomes can also be coupled to surface of microbubbles [46]. However, since microbubbles are big in size compared to the pores of the leaky vasculature of cancer tissues, nanoemulsions are getting more attention in drug delivery. eliposomes use nanoemulsions incorporated inside their structures where they change rapidly from liquid to gas upon exposure to US thus shearing the liposomal structure open and releasing their content at the target site [44]. A study conducted by Lattin *et al*. showed that drug release from eliposomes was significantly higher than conventional liposomes [47]. eLiposomes are also small in size which facilitates their passive penetration into cancerous tissue, as opposed to microbubbles.

3. DELIVERY OF NANOCARRIERS TO THE TUMOR

3.1. Passive Targeting

Passive targeting of cancerous tissues is fundamentally based on the inherent physical and chemical properties of tumor cells. The efficacy of passive targeting can be enhanced through the use of nanocarrier systems, which have become the focus of cancer research [48]. Liposomes, micelles, nanoemulsions and polymeric nanoparticles, amongst other nanocarriers, all demonstrate a wide range of advantageous properties that entitle them to be used in drug delivery. Prolonged circulation is one of the useful properties that give nanocarriers the time to reach the tumor site before being opsonized by the patient's immune system. The flexibility in tailoring the size of a nanocarrier is another advantage that allows nanocarriers to exploit the compromised vasculature of cancerous tissues and hence to penetrate their structure. Also, the possibility of conjugating ligands to the surface of nanoparticles allows them to become more target-specific and thus enhancing the intracellular penetration as well as increasing sensitivity to stimuli that trigger drug release [49].

The unique vascular architecture of tumors distinguishes them from healthy cells. As the tumor grows beyond a volume of 2 mm^3, substances needed for its growth and nourishment can no longer be provided by simple diffusion from the blood supply; and the tumor is said to be diffusion limited [50]. To overcome this phenomenon, vascular endothelial growth factor (VEGF), previously known as vascular permeability factor (VPF), is produced which stimulates angiogenesis. Angiogenesis is one of the characteristics of tumors where new blood vessels form from existing ones, and this contributes to the tumor metastatic pathway as it spreads from its primary site into other parts of the body [51]. The fast progression of angiogenesis in cancerous tissues results in the leaky and defective vasculature of the tumor with gap/pore sizes ranging between 100 nm to 2 µm [27]. Hence, the drug enters the tumor site through these fenestrations and accumulates eventually at high concentrations in tumors compared to plasma [52]. Drug retention in tumors results from the poor lymphatic drainage system, which, in normal tissues, is responsible for eliminating waste materials [53]. Subsequently, drug clearance is reduced, and drug toxicity within the tumor vicinity is increased due to prolonged retention [54]. That, combined with hypervascularization, result in the enhanced permeability and retention (EPR) effect which was first discussed by Maeda and Matsumura [54-56].

Many studies were conducted later to examine the EPR phenomenon [27, 57, 58]. For example, a study conducted by Harrington *et al*. showed that the uptake of radiolabeled pegylated liposomes by a tumor is higher than normal tissues by a factor ranging between 1.4 and 16.9 [59]. And even though the EPR phenomenon aids in nanocarrier-based cancer treatment, there exists some problems that hinder its effectiveness. Dams *et al*. [60] studied the effect of frequent injections of radiolabeled pegylated liposomes on their circulation time. They found that a second dose of pegylated liposomes within 5 days and up to 4 weeks after the first injection reduced their circulatory half-life dramatically and increased their uptake in the liver and spleen. This phenomenon was termed the "accelerated blood clearance (ABC) phenomenon", and was further investigated in other studies [61-63].

3.2. Active Targeting

To improve the selectivity of a nanocarrier, ligands can be conjugated to its surface that would specifically bind to the desired receptors overexpressed on tumor cells. The successful application of ligand-conjugated liposomes is highly dependent on their conformation [64], their cellular processing, pharmacodynamics and pharmacokinetics [65]. It has been observed that specific types of receptors are overexpressed on certain cancerous cells, such as transferrin and folate receptors [6, 66]. The amount of drug, therefore, reaching the tumor site and penetrating the diseased tissue can be drastically enhanced by the use of active ligand targeting [67]. Ligands grafted on the surface of a nanocarrier can be antibodies, hormones, aptamers, peptides and others [66]. For instance, the expression of the human epidermal growth factor receptor 2 (HER2) in cancerous cells is 1000fold higher than in normal cells in nearly 25% of breast cancer cases [23]. A study conducted by Park *et al.* proved the

Table 2.   Targeted liposomal formulations in clinical trials.

Treatment Name	Conjugated Ligand	Therapeutic Agent	Condition/Disease	Clinical Status	Reference
MBP-426	Transferrin	Oxaliplatin	Gastric adenocarcinoma, gastroesophageal junction adenocarcinoma, esophageal junction adenocarcinoma	Phase II	[74-76]
MCC-465	F(ab')$_2$ fragment of human monoclonal antibody GAH	Doxorubicin	Metastatic or recurrent stomach cancer	Phase I	[75-77]
MM-302	Antibody fragments	Doxorubicin	HER2-positive metastatic breast cancer	Phase II (stopped)	[75, 78]
SGT-94	Anti-transferrin receptor single chain antibody fragment (TfRscFv)	RB94 gene (plasmid DNA)	Neoplasm	Phase I	[79]

efficient binding and internalization of immunoliposomes (*i.e.*, liposomes with antibodies on their surface) by p185[HER2] overexpressing breast cancer cells (SK-BR-3 and BT-474), which was investigated further in other studies [68, 69]. Hence, the use of targeted liposomes against HER2 receptors could be promising in *in vivo* preclinical trials. Moreover, biligand liposomes that exhibit dual functionality have also been used. Yuan *et al.* conjugated two ligands, transferrin (Tf) and cell-penetrating peptide CPP (TAT), to the surface of liposomes to target melanoma [70]. Liposomes in this study were encapsulated with paclitaxel and doxorubicin which successfully showed a synergistic effect in cytotoxicity studies. Also, *in vitro* cellular uptake studies of dualligand liposomes (Tf/TAT-DOX-LP) showed a higher uptake by B16 cells when compared to single-ligand liposomes (TF-DOX-LP and TAT-DOX-LP) with 8.7 and 2.8-fold increase, accordingly [70]. This could be attributed to the synergistic effect of using both ligands, which is consistent with a study conducted earlier by Tang *et al.* [71]. Bi-ligand liposomes targeting transferrin receptors have also proven their anti-tumor efficacy against lung cancer [72]. In one of the studies, accumulation of bi-ligand liposomes in the brain showed a two-fold increase when compared to single-ligand liposomes and improved penetration through the blood-brain barrier [73]. And despite the extensive research on activeligand targeting of nanocarriers, only few targeted-liposomal formulations made it to clinical trials (Table **2**).

Alongside challenges posed by the intricate nature of developing a new cancer treatment and the long time span it takes to prove its efficacy in clinical trials, pharmaceutical manufacturing costs and government regulations stand as obstacles. One of the solutions to overcome such problems, especially for targeted-liposomes being developed currently, could be through modifying one of the approved nontargeted liposomal formulations listed in Table **1** with the desired ligand as already proposed by a team from the research institute in the University of California (UCSF) and ZoneOne Pharma [80]. Other types of nanocarriers, such as micelles and dendrimers can also be functionalized with conjugated moieties to enhance their accumulation in cancerous tissue and facilitate their cellular uptake through receptormediated endocytosis [81-85].
Both passive and active targeting schemes of nanocarriers showed promising results in cancer treatment according to several studies. The mechanism of drug release also plays an important role in drug delivery where an external or internal trigger can be employed to control the release profile.

3.3. Triggering Drug Delivery

3.3.1. Internal and External Triggers

The main objective of a drug delivery system is to attain specific therapeutic drug levels of cytotoxic agents in the tumor site. This minimizes the side effects arising from chemotherapy which is usually administered at large doses to compensate for its possible clearance from the circulatory system before reaching the desired site [86, 87]. To control the release of a cytotoxic agent encapsulated in a nanocarrier both spatially and temporally, various internal and external stimuli can be used. The type of the stimulus that the nanocarrier will respond to is highly dependent on the structure and composition of the nanocarrier [88]. Internal triggers include pH, temperature, and enzymes, while external triggers include light, electromagnetic waves and ultrasound [44]. It is important to note that external triggers can either directly initiate drug release through a mechanical effect imposed on the nanocarrier or can indirectly cause release through inducing an internal trigger. The choice of one of these techniques is dependent on the type of liposome being employed. For example, heat can be utilized to trigger drug release from thermosensitive liposomes (TSLs). Applying thermal energy results in local hyperthermia at the desired site, where the liposomes accumulate, causing the lipids that constitute the liposomes to change their phase from gel to liquid at their phase transition temperature (T_m) [89]. Pores begin to form in the lipid bilayer of liposomes and the encapsulated drug leaks. Thermal liposomes are stable at normal body temperatures (37°C), which keeps the liposomes intact as they circulate to reach the tumor site. They only begin to disintegrate at a temperature range of 39-43°C, depending on the type of lipids used in their synthesis [44, 87]. Examples of external stimuli that can induce such an internal trigger (heat) are microwaves, radiowaves, and high intensity focused ultrasound [87, 90].

Another type of internal trigger is enzyme activity. The surrounding environment plays a vital role in determining the fate of a liposomal vesicle. Fortunately, cancerous cellular environments happen to be different from healthy cellular environments. One differentiating factor is the type and concentration of enzymes that function in cancerous tissues [91]. In general, liposomes that are sensitive to tissues with a specific microenvironment such as pH, temperature and enzymes are called "bioresponsive" liposomes. During their synthesis, bioresponsive liposomes are internally programmed to undergo changes only when proper conditions are met in order to release their cargo at the diseased tissues. pH-sensitive liposomes have also been studied. These liposomes are made of lipids that are stable at physiological pH (pH 7.4) yet are disrupted under acidic conditions [92]. These are usually synthesized by blending derivatives of phosphatidylethanolamine with compounds having an acidic functional group to stabilize the formulation at neutral pH [24, 93]. It has been observed that pH conditions in tumor sites are acidic and hence trigger a fusogenic response causing liposomes to release their contents [94]. In addition, the use of light to trigger drug release using photosensitive liposomes has also been studied. Those nanocarriers are synthesized using polymerized photoreactive lipids called photoactivable lipids and are modified with sensitizers that respond to light triggers; usually UV light [95]. This poses a potential clinical threat as UV-light exposure can be toxic. Therefore, alternative light sources, like infrared light, are being investigated [18]. Magnetic fields can also be used to trigger drug release. Magnetic field can be used as an indirect external trigger that produces heat and releases drug from TSLs modified with magnetic nanoparticles [96]. Magnetic field can also be used to guide magnetically-responsive liposomes that contain conductive metals to tumor site [96]. The use of ultrasound as an external stimulus has also attracted research in drug delivery, especially that ultrasonic waves can be focused and directed to penetrate deep tissues in a small focal zone, unlike other triggering mechanisms.

3.3.2. Ultrasound

Ultrasound (US) is one example of an external stimulus utilized to trigger drug release after a nanocarrier reaches its target site. US is preferable in drug delivery because it is benign, non-invasive, controllable and causes no pain when applied [97]. It can also be localized with high accuracy in terms of the area it is applied to [10]. The use of US can yield thermal and/or non-thermal effects; both of which contribute to triggering drug release. The thermal effect results from body tissues absorbing thermal energy produced by focused ultrasound (FUS) waves. While the non-thermal effects are mechanical effects that result from the nature of the US waves propagating through the medium [98].

Mild hyperthermia induced by high intensity focused ultrasound (HIFU) was utilized in many studies as a mechanism to release drug from TSLs. To improve the targeting of TSLs and monitor treatment, several studies combined HIFU with magnetic resonance imaging (MRI) in a system known as MR-guided high intensity focused ultrasound (MRHIFU), in order to acquire temperature images to investigate the bioeffects of HIFU. A study conducted by Ranjan *et al.* used the MR-HIFU system to induce hyperthermia between 40-41°C, and they reported 7.6- and 3.4-fold increase of doxorubicin concentration when TSL-MR-HIFU was used compared to free doxorubicin and TSLs alone [99]. Although several *in vitro* and *in vivo* studies were conducted to examine the role of US as a drug releasing mechanism from liposomal formulations, only one study so far has been extended to clinical trials, namely, the ThermoDox® liposomes in the TARDOX Phase I study for treating liver cancer [100]. The study aimed to investigate the release of doxorubicin from thermally sensitive liposomes using focused ultrasound to induce mild hyperthermia. The results of this study were released recently, and concluded that doxorubicin concentration inside tumors showed an average increase of 3.7 times after applying focused ultrasound compared to its concertation immediately after drug infusion [101].

The mechanical effect of US in triggering drug release from nanoparticles utilizes acoustic cavitation, which is defined by the rapid growth and collapse of compressible gas bubbles in response to varying pressure fields caused by US waves. As pressure

passes through a medium, bubbles respond by altering their sizes. They contract at high pressure and expand at the negative pressure phase of the acoustic cycle. If the intensity is low and the resulting change in bubble size is stable, the cavitation is said to be stable and it results in a flowing stream of fluid around the bubbles called "microstreaming", which is proportional to the amplitude of the US waves applied (*i.e.* the intensity of US) [102]. Microstreaming with high velocities may rupture the surrounding cells and synthetic lipid-based nanoparticles if they exist [102]. If the intensity is high and the resulting change in bubble size is unstable, the cavitation is said to be transient.

Therefore, the higher intensity US is associated with more violent bubble collapse and hence a larger shear force that can burst the nanocarriers [103, 104].

Such observations led to further research to determine which levels of US intensities resulted in the most effective drug delivery scheme. Studies by Staples *et al*. conducted on rats models injected with micelle-encapsulated doxorubicin proved that the combination of insonation with an effective drug delivery system reduced tumor growth, regardless of the frequency of US used [105]. However, studies by Ueda *et al*. established that the *in vitro* use of low-frequency US at high intensity is the most effective in releasing drugs from nanocarriers [106].

The mechanical properties of US result in enhanced nanocarrier internalization into cancerous tissue as well as a more efficient drug release process. The different effects of US on the nanocarrier as well as the cell membrane of cancerous tissues are summarized below:

3.3.2.1. Permeabilization of Target Cell Membranes

As bubbles collapse, they produce waves of shear forces and/or sonic jets of liquid that form holes/pores in the cell membrane of the targeted tissues. This increases the cells' permeability to nanocarriers, thus facilitating drug access to cancerous tissue under an acoustic field [104]. In a study conducted by Stringham *et al*., the role of acoustic cavitation in cell membrane permeabilization of rat colon cancer cells was examined [107]. A linear increase in cell membrane permeabilization in correlation with time and intensity of sonication was observed. The subsequent increase in drug uptake into cancerous tissues was recorded. The pores forming at the cell membranes were found to be self-healing at certain US pressures and frequencies where transient pores re-seal through a repairing mechanism as described by Yang *et al*. [108]. The cell membrane permeability is dependent on the US parameters. In one of the studies, it was found that the cell permeability increased with increasing the negative peak pressure, pulse repetition frequency, pulse duration and insonation duration. However, it was also found that cell viability decreased by increasing the parameters above. Therefore US parameters should be optimized to achieve a suitable degree of cell permeation [109].

3.3.2.2. Enhanced Nanocarrier/Drug Transport to Target Cells

The US waves aid in transporting drugs and other nanocarriers in the presence or absence of cavitation. In the absence of bubbles (*i.e*., cavitation), US waves can improve the diffusivity of molecules by inducing a convective motion in the insonated fluid [102]. It can also enhance the targetability of nanocarriers by increasing the ligand/receptor interactions through the convective motion [110]. Also, the presence of bubbles contributes significantly to drug transport through cavitation. The stable oscillation of bubbles when exposed to US generates eddies around them. Such convective motion is termed "microstreaming" and can transport molecules close to the surface of the bubble at high speed [102].

3.3.2.3. Perturbation of Nanocarriers

The effect of oscillating bubbles and shear stress produced by pressure waves acting on the nanocarriers cause them to shear open and release the encapsulated drug at the required site [111]. The bubble activity causing such a violent impact on surrounding vesicles/cells is due to "inertial cavitation". Unlike stable cavitation, inertial cavitation causes bubbles to oscillate in size and eventually collapse during the compression cycle of the wave [112]. This causes shock waves in the surrounding liquid or a micro-jet with high velocity, also known as asymmetric bubble collapse, close to a boundary [112]. A micro-jet can pierce the surrounding structures, thus creating pores that facilitate the passage of drugs. Inertial cavitation is more likely to occur at low US frequencies and high negative peak pressures as predicted from the mechanical index developed by Apfel and Holland [113].

When it comes to ultrasonic liposomal drug delivery, the primary objective is to render these nanocarriers more susceptible to ultrasound. We have previously introduced the concept of eLiposomes [114], which are nanovehicles encapsulating a low boiling point emulsion, capable of acoustic droplet vaporization upon exposure to ultrasound [115, 116]. Although the perfluorocarbon used to synthesize these emulsions has a boiling point lower than 30°C, these eLiposomes are stable at physiological conditions but start to boil at higher temperatures [117, 118]. Our group has also been investigating the multimodal drug delivery of biologically targeted liposomes and acoustic power [119, 120], and comparing it to non-targeted liposomes [121]. We have also reported the concept of acoustically controlling liposomal drug delivery using a model predictive control, so that the concentration of the drug will not drop below therapeutic levels [122].

CONCLUSION

This paper sheds light on a synergistic approach in cancer therapy that involves passive, active and triggered drug delivery. To prevent the potentially toxic effects of nonselective chemotherapy, research has focused on nanocarrier systems that act as vehicles to encapsulate a cytotoxic agent and selectively transport it to the tumor site. Passive targeting is achieved when nanocarriers passively exploit the nature of cancerous tissues that facilitate their site-specific accumulation. The conjugation of target moieties on the surface of liposomes actively enhances their selective internalization into the tumor site through ligand-receptor binding mechanisms. Lastly, to trigger the release of drugs at the tumor site, various external and internal triggers can be utilized including US waves.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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