RESEARCH ARTICLE

Improving the Efficacy of Anticancer Drugs *via* Encapsulation and Acoustic Release

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Abstract: Conventional chemotherapeutics lack the specificity and controllability, thus may poison healthy cells while attempting to kill cancerous ones. Newly developed nano-drug delivery systems have shown promise in delivering anti-tumor agents with enhanced stability, durability and overall performance; especially when used along with targeting and triggering techniques. This work traces back the history of chemotherapy, addressing the main challenges that have encouraged the medical researchers to seek a sanctuary in nanotechnological-based drug delivery systems that are grafted with appropriate targeting techniques and drug release mechanisms. A special focus will be directed to acoustically triggered liposomes encapsulating doxorubicin.

Keywords: Chemotherapy, multi-drug resistance, anthracyclines, doxorubicin, nano-carriers, ultrasound.

1. INTRODUCTION

1.1. Traditional Chemotherapeutic Means

1.1.1. Principle, Environment and Definitions

Cancer is a deadly disease that has threatened humanity for ages and recent statistics show that it is the second leading cause of death in men around the globe [1]. Cancer is defined as the fast-abnormal growth of cells, which occurs due to a genetic change that disrupts the cell life-cycle. Subsequently old or damaged cells lose their ability to undergo apoptosis (programmed cell death). Hence, solid tumors formation becomes of high probability [2]. Such genetic change could be induced through inheritance or due to subjection to a driving factor (*e.g.,* radiation, or the ingestion of pollutant substances, such as the chemicals inhaled with the smoke of Tobacco) [3]. The most dangerous event that could result from cancer proliferation is known as *tumor metastasis*, where malignant cells migrate from the *primary tumor*, to other parts of the body using the bloodstream or the lymphatic system. Subsequently, they settle in the new location and bind to cells and tissues, starting, *secondary malignant tumors* [4]. In either scenarios, both early detection and effective treatment are essential to save patients' lives.

Nevertheless, it is important to emphasize that cancerous tissues have special conditions that make them different from the vicinity of healthy cells and tissues. For instance, due to their fast growth, and as the tumor mass reaches a size of 2 mm^3, deficiency in oxygen and nutrient supply leads to the state of cell hypoxia, which activates various growth factors within the cells (*e.g.,* Vascular Endothelial Growth Factors, VEGF's) [5]. Following this, tumor cells start to recruit new blood vessels in order to supply their demand for more nutrients and oxygen, a process called *tumor angiogenesis*. Unlike the well-organized branching of vascular network in normal tissues, angiogenesis in cancerous sites is not uniform due to the imbalance in pro- and anti-angiogenic signaling within the different parts of cancer. This leads to the development of irregular shaped, tortuous blood vessels between the leaky endothelial cells, with pore sizes of 200800 nm. Moreover, lymphatic vessels in such conditions also become compromised and lose their function of draining interstitial fluids from infected tissues. The process of developing lymphatic nodes with such defects is called *tumor lymph angiogenesis* [6,7].

In addition to the above mentioned irregularities, the tumor metabolism itself changes into anaerobic respiration mode, leading to the build-up of carbon dioxide and acids, decreasing eventually the extracellular pH and turning the site into an acidic one (pH≈ 6.5), with even further drops occurring at the endosomes and lysosomes (pH ≈ 5.0-5.5) [8-10]. Such conditions have inspired medical researchers to develop ways that identify and target cancer locations using appropriate tools and treatment means. In what follows, we present a concise introductory history about a number of conventional cancer treatment approaches before introducing the new promising approaches to treat tumors using nanotherapy.

1.1.2. History of Cancer Treatment

Cancer has afflicted people throughout the world since old ages. In fact, the earliest described cancer incidence was recorded by the ancient Egyptians in 1500 BC, where it was a case that is currently believed to be breast cancer. Back then, it was believed that this disease has no cure, and any medical treatment would only be palliative. This belief was later changed with the

improvement of medical fields; where surface tumors were recorded to be surgically removed. Later, it was discovered that such procedures may result in tumors recurrence in more fierce ways [11]. Hence, they were not encouraged till the invention of anesthesia in the 1800's, as reduction the pain of tumor resection became possible. However, not all the cases were treated successfully. This was later attributed to metastasis in cases with large number of tumors to be removed [12]. Since then, understanding the spreading mechanism of cancer has become a critical factor in understanding the limitations of traditional surgical treatment.

In the early 1900s, with the discovery and invention of tissue imaging techniques through non-invasive techniques, such as sonography, magnetic resonance imaging (MRI) and computed tomography (CT scans), operations became more targeted and effective, though still not optimal [11]. Another leap occurred with the introduction of miniature video cameras and endoscopy; wherein many tumors have been surgically removed through directed tubes without the need for operational incisions. Nowadays, less invasive approaches for eliminating cancerous cells are further studied or applied, including the use of radio- or laser-therapies or freezing tumor cells to death using liquid nitrogen. However, and despite these advances, metastasis has always posed as a significant challenge that requires other means of treatments [11].

During the 20th century, oncologists have developed a new method that is based on non-invasive treatments which rely on chemical drugs; such treatments are currently known as chemotherapy [13, 14].

In the late 1940's, Farber *et al.* first observed an "accelerated leukemic process" on a group of children who were injected with the folic acid conjugates, terpterin and diopterin, to treat anemia that was caused by cancer [15]. Consequently, Farber decided to try treating a group of 16 children, also suffering from acute leukemia, with the folic acid antagonist, 4-aminopteroylglutamicacid (aminopterin). Five children of the group have shown some temporary health improvements despite the high toxicity of the drug and the appearance of different and sometimes fatal side effects [16].

This observation led to many subsequent clinical trials using several antimetabolites or introducing some structural modifications to folic acid antagonists [17-19]. It was then discovered that, despite the high toxicity of these compounds, they had some potential in slowly eradicating leukemia. In 1956, Li and colleagues have tested another folic acid antagonist (methotrexate) on 11 human subjects (6 females and 5 males) suffering from choriocarcinoma or related trophoblastic diseases. Initial results suggested some evidence of marked tumor regression caused by the administered drug [20]. This was the first reported chemotherapeutic "cure" of cancer in history. Later on, data collected from people who passed away after subjection to nitrogen mustard gases during the first and second World Wars, have shown a possible link between atrophied bone marrow and lymph nodes and the ingested gases. This fired up new research on cancer treatment using other alkylating agents such as cyclophosphamide [21]. All these and other clinical trials pointed out that some chemical agents have potentials in treating cancer.

Watson and Crick's work on the DNA structure presented the first hypothesis on the mechanism by which some chemotherapeutics were able to defeat cancer [22]. Other following research was able to conclude that chemotherapeutic compounds kill both healthy and cancerous cells due to their toxicity. However, healthy cells show a rapid multiplication rate after treatment, when compared to the growth of cancer cells. Hence, cancer cells eventually suffocate by the dense network of fast-growing healthy cells. This conclusion has inspired more research in chemotherapy, where new discoveries of other antitumor drugs and their mechanisms of action have begun. An example of such efforts was the work of Skipper and colleagues, who tried to circumvent the side effects of chemotherapy, by instating a fractional cell-kill model.The model suggested that the fraction of cells killed is always fixed, and is dependent on the specific treatment regime [23]. Hence, and based on this model, it was theorized that, in order to completely kill all cancer cells, cycles of treatments were necessary. Accordingly, instead of injecting the patient with high dosages of the toxic drug, it was recommended that the treatment regime is better to involve multiple sessions with enough recovery periods in between, so as to allow healthy cells to grow back before administering another dose. This treatment methodology was expected to completely eliminate tumors. Nevertheless, this behavior was not noticed in reality for multiple reasons that were yet to be discovered.

By the middle of the 1960s, antibiotics (*e.g.,* anthracyclines), antimitotics (*e.g.,* vinca alkaloids), nucleobases (*e.g.,* fluoropyrimidines), cisplatin, etoposide and procarbazine have entered medical trials. The trials primarily focused on the biological effects and attempted to uncover metabolic behaviors that lead to the death of cells. It was during the same period that chemotherapy was recommended as a complementary modality which can be combined with other treatments, such as radiotherapy and surgeries. Moreover, several trials studied the possibility of treating patients with a combination of drugs that have individual action mechanisms. Unfortunately, during this era, the usefulness of antineoplastic agents was dependent on their toxicity levels, as the administration mechanisms followed traditional means [21]. More details on the progress of chemotherapeutic antibiotics, with a special focus on anthracyclines, is given in Section 1.4.

Towards the end of the 1960s, it was discovered that tumor cells have the ability to evolve and develop what is known as drug resistance. This ability is naturally present in all body cells but is more prominent in cancer cells as a line of defense against chemotherapeutic drugs. It was later regarded as the main reason why Skipper's cell death model is offset in reality. A suggested solution to this problem is to begin treatment as soon as possible. However, this suggestion was not supported by the clinical results, as patients would still die either from cancer or drug toxicity [21].

Following these discoveries, the research direction shifted from finding new biological treatments (*e.g.,* searching for more effective antibiotics), towards conducting more technical, clinical research that aims to improve the therapeutic results of discovered chemotherapeutic agents, through careful consideration of tumor cell dynamics. The intensive research in the growth dynamics of cancer cells has led to the development of a model suggested by multiple scientists, based on Gompertzian curves, which indicated that, initially, tumors grow at exponential rates. However, as they expand in size, the growth rate would start to decline [24, 25]. This behaviour is explained by the inconsistent and random structure of tumors that prohibits natural cellular structures, resulting in areas with malnutrition, which increases as the size augments. Nevertheless, the shortage of blood, and thus nutrients supply to the far away parts of the tumor means that the growth rate would slow down in some regions of the tumor while it is faster in others [21].

With such heterogeneous growth rates within a single tumor, treatment dosages become an important factor that affects the treatment efficacy. That is to say, if the treatment dosage was targeted towards slower cells, fast-growing ones might develop drug resistance. Hence, it was suggested that the best practice is to sequentially treat the dominant and faster-growing populations as soon as possible using higher doses. Many researchers have investigated this hypothesis, where only a few have supported it. Randomized trials have suggested that escalating the dose does not necessarily improve the therapeutic outcomes. This suggested that other means are needed [21].

This has led to what we know today as the modern targeted chemotherapy. Discoveries of many biochemical characteristics; such as tumor suppressor genes, signaling pathways, and oncogenes that are related to cancer cells, revolutionized the research ideology [21]. This has led to huge breakthroughs in targeted therapies, with a large volume of published data supporting the effectiveness of such methods of treatment. However, drug resistance remains a major challenge that disrupts the treatment effectiveness. Therefore, considerable effort has been directed towards identifying the main reasons behind the development of the cellular drug resistance and understanding its kinematics, as demonstrated in the next section.

1.1.3. Drug Resistance Kinematics

Multi-drug resistance (MDR) is a type of immune responses, and it occurs when cells develop immunity against a specific type of drugs, while at the same time becoming less responsive to a range of other unrelated drugs [26]. As shown in Fig. (**1**), MDR in cancer cells can be caused due to multiple factors; some of them are more abundant than others. In this work, we give a quick description of each mechanism, and focus on Plasma-drug interaction, as it is considered a leading cause of the MDR problem [26].

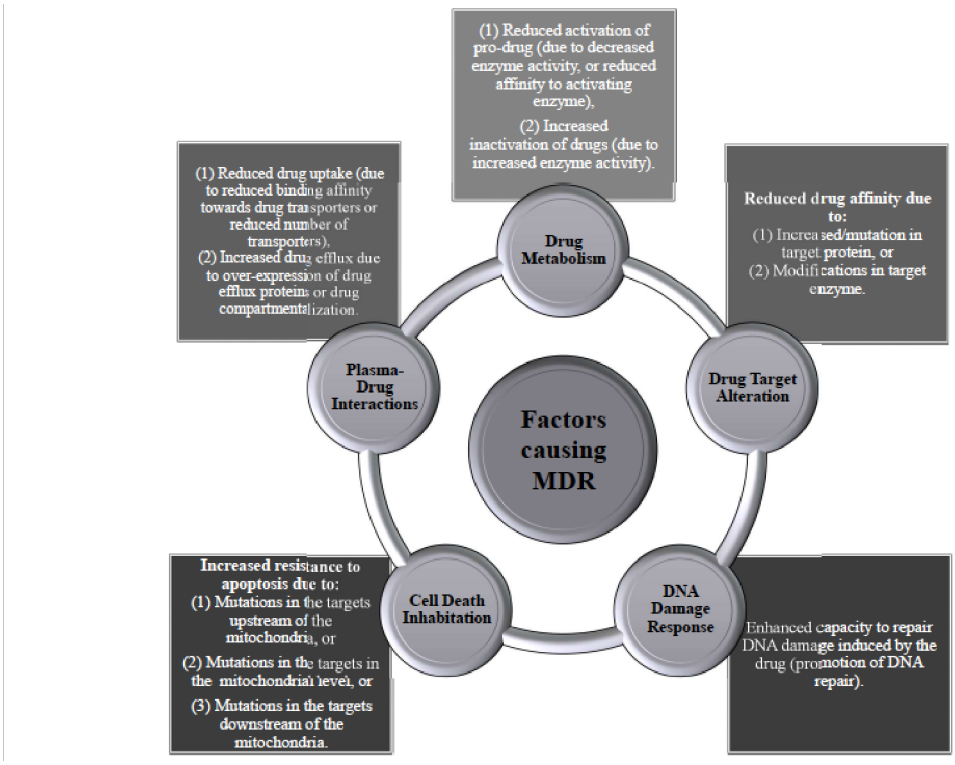

Fig. (1). Factors causing multi-drug resistance (MDR) to chemotherapeutic agents.

1.1.3.1. Drug Metabolism

Drug metabolism (sometimes called drug inactivation) is usually caused by the presence of certain types of enzymes, which are capable of breaking down the administered drug [27]. Cancer cells have the capability of producing certain enzymes that are able to deactivate chemotherapeutic agents, thus developing MDR [27, 28]. Some important examples of drug metabolism enzymes include cytochrome P450 (CYP) system and epoxide hydrolases, which are usually referred to as oxidative metabolism mediators [27, 29].

1.1.3.2. Drug Target Alteration

Another way by which cancer cells can develop drug resistance is through the alteration of the molecular targets used by drugs to identify and bind to cancerous cells. Target alterations can be done through mutations or variation of expression levels. With enough target alterations, cancer cells can become resistant to the drugs. An example of this mechanism is the topoisomerase II-inhibiting drugs. These drugs are designed to attack cells with the proliferation of the enzyme topoisomerase II, where cancer cells became drug resistant through a mutation in the topoisomerase II gene [30, 31].

1.1.3.3. DNA-Damage Response

Although not very popular, some cancer cells can develop a mechanism by which DNA damage can be repaired [32]. Many chemotherapeutic drugs such as cisplatin and doxorubicin (DOX), are known to induce apoptosis by inflecting harmful damage to the DNA of cancer cells. However, with a well-developed arsenal of DNA repair mechanisms, affected cells can have their mutations repaired, leading to them surviving treatment [33]. This form of drug resistance is relatively easy to overcome; as there are wellknown DNA repair inhibitors that can be conjointly used with chemotherapy.

1.1.3.4. Cell Death Inhibition

Completing life cycle of cancerous cells by inducing cell death *via* apoptosis is the ultimate purpose of chemotherapy [34]. Some drugs are designed to promote apoptotic pathways to promote cells death. Two apoptotic pathways are recognized; intrinsic pathways that involve the B-cell lymphoma 2 (BCL-2) family proteins, caspase, and protein kinase B (Akt); and extrinsic pathways that require death receptors on the cell surface [35]. Luckily, many types of cancers that have high expression of intrinsic apoptotic pathways can be targeted with drugs that can cause cell death. However, this research area is still at its infancy and requires further investigation. Clinical trials are inconclusive as some show improvements in chemotherapeutic efficacy while others conclude negative results.

1.1.3.5. Plasma-Drug Interactions

The plasma membrane is the first point of interaction between the drugs and tumor cells. This interaction occurs in many ways and affects the efficacy of drugs. The main goal is for the drug to cross the plasma membrane into the cell, to which it can induce death [36]. One way is through solute carriers. The solute carrier (SLC) family is composed of around 360 transporters which are the resultant of 45 gene families and allow all kinds of chemotherapeutic drugs to enter the cell. Some cancer cells have a low number of SLC transporters present. This reduces cellular uptake into these cells, which in turn reduces the treatment effectiveness.

However, the production of these transporters can be increased through genetic polymorphisms, which, if coupled with chemotherapy, can increase treatment effectiveness. More studies are still needed on SLC to achieve better results [36]. On top of this, drug resistance has been mostly associated with another form of plasma drug transporters, known as "*molecular pumps*". In 1968, Kessel *et al.* have reported an incident of drug resistance when conducting experiments with daunomycin [37]. The results were later elaborated on by Dano and colleagues [38], who associated this drug resistance to an energy-dependent pump. In the parallel work of Juliano and Ling, a molecule called Pglycoprotien (PGP) was discovered to be more densely present on the surface of cancer cells.

Unlike other mechanisms that prevent or reduce drug uptake, PGP pumps have the ability to get the cell to get rid of harmful material by pumping them outside the cell [39]. In 1979, the protein responsible for PGP was identified and was referred to as multi-drug resistant protein-1 (MDR-1). Around the same time, other pumps where identified; such as multi-drug resistance associated protein (MRP), which was discovered in 1992 [40].

Even though the structure of the pump depends on its generating protein, they all have two distinctive features; namely, a nucleotide binding site and a variable transmembrane. When a molecule binds to the transmembrane domain using energy from ATP hydrolysis in the nucleotide binding sites, the pump starts pushing the molecule against the gradient and outside the cell. This action is usually referred to as the "*efflux action*". The most well-known type of *efflux pumps* developed as part of the drug resistance action belong to a family of transporters known as ATP-binding cassette (ABC) [41]. In fact, there are more than 48

known members of this family. While ABC transporters are abundant in all cells, in cancer cells they can be modified and designed so as to have substrate specificity that enables them to transport anti-neoplastic drugs (*e.g.,* vinca alkaloids, anthracyclines, taxanes, and cisplatin). This ability is what makes them very efficient drug resistance mechanisms, and that is why they have been the focus of many studies for the past few years [30].

1.1.4. Antimicrobial Chemotherapy

1.1.4.1. History, Principal and Therapeutic Applications

Anthracyclines are considered the first antitumor antibiotics to get approved by the US Food and Drug Administration (FDA). They are among the most popular and powerful chemotherapeutic drugs used; as they are capable of achieving short-time cancer progression and high response and survival rates [42, 43]. They are well-characterized, and are mostly composed of an aglycone and sugar (called daunosamine). The characterizing tetracyclic ring of these agents usually carries quinone-hydroquinone groups, a methoxy substituent and a short side chain. The activity band of these antibiotics is directly affected by the end group present in the side chain [44, 45].

As early as the 1960's, DOX (also called Adriamycin) and daunorubicin (DNR) have become the first semisynthetic anthracyclines to be isolated from the red pigmentproducing soil bacterium; *Streptomyces peucetius* via mutagenic treatment [42, 46, 47]. Both compounds have inherited fluorescence properties, which render them attractive as useful imaging/theranostic tools [48]. What's more is that the aqueous solubilities of these molecules can be further increased through sugar amino-groups protonation of the agents, forming hence DOX- or DNR-hydrochlorides (DOX-HCl and DNR-HCl, respectively) [48].

As depicted in Fig. (**2**), DOX and DNR share the same general structure of anthracyclines. The only difference between them is the type of the side chain. That is a primary alcohol group terminates the acetylic chain in the case of DOX, while a methyl group ends DNR side chain [44]. This small difference gives DOX the broadest activity spectrum, where it is well-known for treating acute leukemia, breast, bladder stomach, lung, ovarian and thyroid cancers, gastric and bronchogenic carcinoma, neuroblastoma, malignant lymphoma, childhood solid tumors, soft tissue and bone sarcomas, Wilms' tumor, Hodgkin's disease and non-
Hodgkin aggressive lymphomas. DNR, on the other hand, is primarily used against some types of leukemias (*e.g.,* acute myeloid and lymphocytic leukemias) [47-50].

Only two other discovered antibiotic treatments of this family have been approved for clinical practice. The first is idarubicin; which is the analogue of DNR that has increased cellular uptake as a result of the absence of the methoxy group present in the latter. And the second is valrubicin; the analogue of DOX that is often used in the treatment of bladder cancer. Other earlier anthracyclines of microbial origin include actinomycin A and actinomycin C. The severe and chronic side effects, in addition to the limited tolerated dose, restrict the use of these compounds [43, 45, 47].

1.1.4.2. Mechanism of Action

Anthracyclines in general, have well-studied and documented mechanisms of action. They diffuse and permeate into cellular membranes, then intercalate between adjacent DNA strands before killing the cell by apoptosis. This is achieved as they induce elevated torsional stress and produce reactive oxygen species while interacting with the cells, thus poisoning topoisomerase I and/or II enzymes [42, 48, 51]. It should be mentioned that the binding abilities of anthracyclines towards cellular membranes and proteins are mainly owed to their amphiphilic and amphoteric nature, as a result of having both, acidic phenolic and basic amino groups [48].

Such simple mechanism looks intriguing for killing diseased cells and achieving tumor regression. Nevertheless, it involves the high risk of interacting with healthy cells, thus causing multidirectional undesirable side effects, including vomiting, hair loss, nausea, anemia or alopecia, in addition to myelosuppression, heart failure resulting from systematic cardiomyopathy and other drug-specific side effects that occurs in major organs (heart, brain, liver and kidneys) [49, 52, 53] . These effects become even more severe if MDR against the active agent was developed, or if the treated case was a paediatric cancer or required increased multiple dosing schedules [54-56]. Not to forget the presence of other common chemotherapeutic challenges, such as tumor heterogeneity, lack of stem cells and metastatic tumors specificity, deficiency of active monitoring modalities and the inability of bypassing certain physical and biological barriers [6, 57, 58]. Throughout the years, several strategies have been investigated in order to increase the patient's quality of life and overcome such constraints facing traditional chemotherapy. Next section details one of these strategies, with the main objective of combining successful anti-tumor treatments with new delivery systems that are developed by capitalizing on utilizing recent advances in nanotechnology [59].

2. NANOTECHNOLOGY AND CURRENT DEVELOPMENTS IN ANTICANCER DRUGS

The last third of the twentieth century marked the dawning of enormous development in modern drug discovery [60]. Naturally, this has fueled the ambition to develop new strategies that are able to overcome the drawbacks of conventional chemotherapy. One turning point occurred upon the birth of modern "nanotechnology" and the suggestion of using "bottom-up" approaches to build nanostructures. This is widely attributed to the lecture titled "There's Plenty of Room at the Bottom", presented by the American physicist and Nobel laureate; Richard Feynman, in 1959 [61].

Nowadays, nanomaterials are defined as miniaturized objects that have small sizes; typically less than 100 nm, or commonly up to several hundreds of nanometers [62, 63]. Synthetic nanomaterials can be produced using either a "*Bottom-Up"* or "*Top-Down"* approaches. The *Bottom-Up* method involves the use of appropriate solutions to dissolve small molecules followed by building nano-objects using super-critical fluid technologies (*e.g.,* chemical routes, vapor-liquid-solid technique, vapor phase deposition methods, and Liquid-phase techniques)[64]. On the contrary, *Top-Down* methods break macro-molecules into miniaturized nanomaterials using chemical, hydrothermal, high-energy electromechanical or ultra-fast light assisted techniques (*e.g.,* homogenization, milling and Lithography techniques) [62, 65]. Bio-nano-materials, however, have existed in nature from early on. This includes self-assembled viruses, bacteria and several other biological and chemical entities, which exist in complex 3-Dimensional arrangements [66].

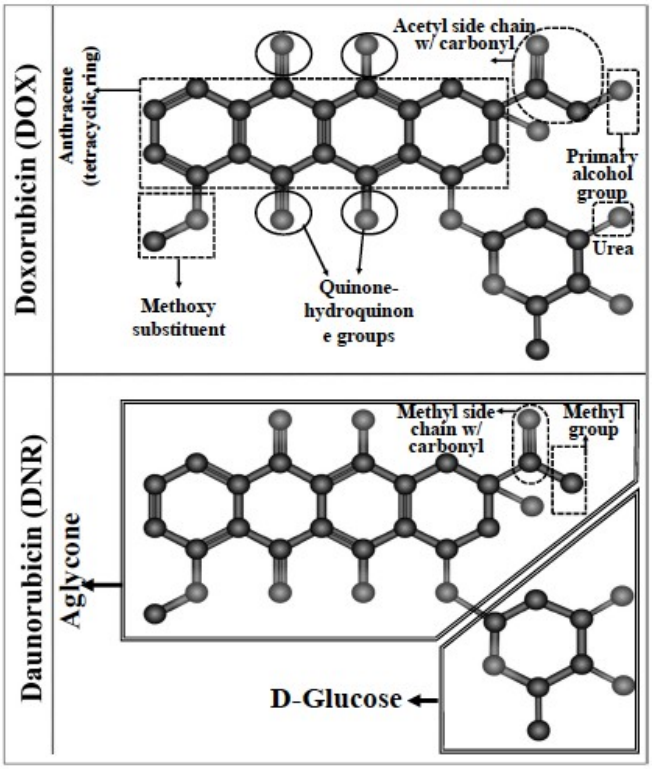

Fig. (2). Molecular structures of DOX and DNR; showing aglyconic and sugar moieties and their adjacent groups.

Nanomaterials can be manufactured from organic (*e.g.,* lipids, polymers, acids) or inorganic materials that often exist or are formed into nanocrystals (*e.g.,* metal oxides, gold nanoparticles and fullerenes) [67, 68]. They can be built into zero-dimensional (0-D) (>100 nm in all directions, *e.g.,* nanoparticles), one-dimensional (1-D) (<100 nm in one direction, *e.g.,* nanotubes, nanorods) or two-dimensional (2D) structures (<100 nm in two directions, *e.g.,* nanoplates) [64]. Several techniques can then be used to characterize the formed material. For instance; the size of nano-objects can be verified using high-angle light scattering, single-particle optical sensing, separation methods (*e.g.,* hydrodynamic fractionation or filtration), ultrasonic spectroscopy, turbidimetry or nuclear magnetic resonance (NMR). Surface morphology can also be studied using electron microscopy techniques (*e.g.,* transmission or scanning electron microscopy (SEM)) or atomic force microscopy. Likewise, interior topography can be determined using freeze-fracture SEM, differential scanning calorimetry, X-ray diffraction, NMR. Several other techniques can also be used to obtain other physical properties of nanomaterials (*e.g.,* fluorescence microscopy, ultraviolet-visible and infrared spectrophotometry, and multiple other chromatography techniques) [64, 65, 69].

All the qualities mentioned above, along with the unique physicochemical and biological properties, have given nanomaterials their popularity and variety of uses in a wide range of interdisciplinary fields; including electronics and devices, medicine and biotechnology, engineering and environmental research [63, 64].

From a medical point of view, nano-materials are considered very attractive. Their high surface area to volume ratios give them a distinctive quantum mechanical nature, which renders them highly soluble and reactive, and able to adsorb, encapsulate, bind or bond to other molecules and compounds. The properties of these entities vary enormously and can be tailored to fit the desired medical application. This includes applications in the areas of drug and macromolecules delivery, *in vitro* and *in vivo* diagnostics, imaging, tissue engineering, nutriceuticals and bio-materials [67, 70].

2.1. Nanotechnology and Drug Delivery

Drug delivery is a direct beneficiary of nanotechnology; where the latter has been acting as an enabling tool that has opened new horizons for the development of unique drug systems [65]. Nanoparticles have shown promise in delivering higher doses of drugs and molecules safely and economically [69]. They were capable of protecting encapsulated or attached agents from biodegradation and biorecognition, thus delaying their elimination from the bloodstream and overcoming the shortcomings associated with conventional therapeutic means [71]. Moreover, they have shown enhanced stabilities, biocompatibilities and overall performances (*e.g.,* functionalities, biodistribution, pharmacokinetics, pharmacodynamics, therapeutic indices, and uptake and release profiles) when compared to currently used delivery systems [67]. Tempted by all these qualities, oncology researchers have been encouraged to consider transporting several hydrophobic, hydrophilic and lipophilic drugs (*e.g.,* DOX, 5-fluoroucacil and curcumin) using a variety of nanotechnological platforms [72]. Liposomes are considered among the most promising and the first marketed nano-platforms which have been extensively used for chemotherapy, thus, are detailed in this work as an example of nano-scale delivery vehicles. A brief description of the history, properties and applications of these carriers is given in the next section, followed by a revisit to the relation between nano-formulations and tumors microenvironment. Finally, the approaches used to improve the transportation of antineoplastic agents utilizing passive, active and triggering techniques are described, with more focus brought to the encapsulation of DOX within a number of nano-entities.

2.2. Liposomes: History, Properties and Applications

Liposomes (Fig. **3**) are nano-sized spherical structures (20nm-1μm in diameter) that were first discovered in the 1960's by Bangham *et al*. [73]. Liposomes are composed of phospholipid bilayers enclosing an aqueous compartment, therefore, they are similar to cell membranes in structure. This feature rendered them good candidates for studying cell membrane functions, like cell fusion and membrane pumps. Several years later, scientists discovered that liposomes were effective carriers for active drugs to treat a number of diseases. Drug encapsulation within these carriers mainly depends on the drug solubility; whereas water-soluble drugs are carried in the internal hydrophilic aqueous core, while lipid-soluble ones are carried in the hydrophobic membranes.

In vivo studies in the 1970's provided a deep understanding of liposomal behaviour inside the living animals. In 1981, three liposome-based companies were founded in the USA. This important development resulted in significant progress in large-scale technology [74].

Liposomes based drug delivery was developed to improve drug pharmacokinetics and biodistribution, and to achieve a controlled drug release rate [75]. Liposomes main application includes: (1) providing an effective delivery system for insoluble drugs like the antifungal "Amphotericin B", (2) attaching targeted agents to the surface of the liposomes and/or making these nanovehicles sensitive towards certain stimuli which enables them to target and bind to specific receptors on biological cells (targeted liposomes) before releasing the loaded drug inside the cells when triggered to free the agents, and (3) chemotherapeutic drugs like DOX are known for their systematic toxicity. Hence, grafting these toxic drugs within the liposomes significantly reduces their toxicity, as explained in next sections.

2.3. Cancer Microenvironment and Passive Targeting

Researchers aspire to revolutionize cancer treatments and develop personalized therapeutic or even precision theranostic systems, which can act as diagnostic, therapeutic and/or post-medication monitoring tools that have the potency to vanquish and completely destroy tumors [4, 76, 77].

One way to develop such strategies is by exploiting the irregular conditions of cancer microenvironment, using nanostructures as delivery vehicles. For instance; making use of tumor angiogenesis, nanocarriers that have much smaller sizes than leaky tumorous vasculatures, can first be loaded or attached to antineoplastic drugs, then easily pass through the

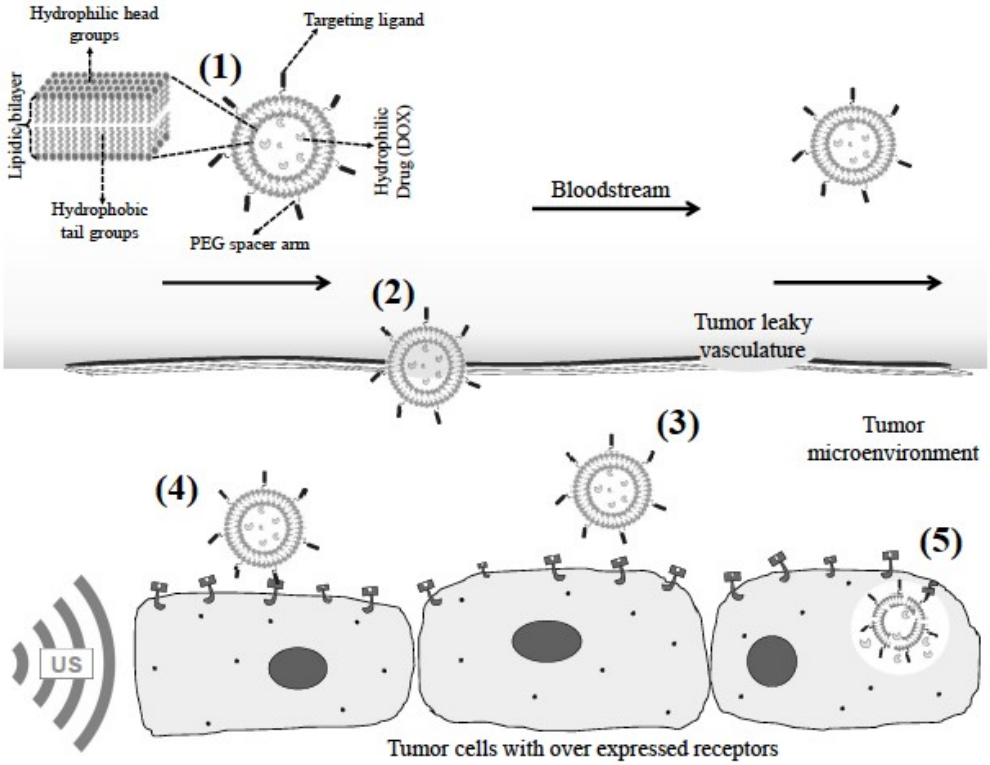

Fig. (3). Targeted PEGylated liposomes encapsulating DOX; since entering the bloodstream till acoustically triggered, releasing the drug as US is applied. (**1**) A liposome encapsulating DOX homing in the bloodstream. (**2**) A liposome extravasating through defected cancerous blood vessels *via* EPR effect. (**3**) A liposome binding to a receptor overexpressed in the surface of a cancer cell. (4) A liposome interacting with a receptor prior to a receptor-mediated endocytosis. (**5**) A liposome internalized into a tumorous cell, prior to releasing DOX as US is activated.

defective blood vessels, hence extravasating into malignant tissues. As they reach desired areas, active ingredients can later be released from the transporting carriers, then stay in the tumor interstitium for extended periods of time, taking advantage of tumor lymph angiogenesis [78, 79]. This phenomenon is known as the enhanced permeation and retention (EPR) effect, and is considered as a passive targeting technique in cancer drug delivery [10, 80]. This effect was first described in 1986 by Matsumura and Maeda [81], where the researchers observed that the injected proteins were found more concentrated in tumor tissues than in the blood, and suggested it was because of the hyper vasculature and poor lymphatic drainage at the tumor site.

EPR has already been utilized in designing different types of liposomes that transport different chemotherapeutic drugs (*e.g.,* Doxil®, Myocet® and DaunoXome®), and have already been marketed and prescribed to patients in clinical treatments, and several other nano-systems are similarly being developed in laboratories or clinical trials [78, 82].

Other passive delivery strategies also exist, and are mainly concerned with the suitable selection of formulation parameters of nanocarriers; *e.g.* particles shapes, sizes, composting materials, surface charges and hydrophilicities [72, 83]. For instance, nanocarriers solubility can be manipulated by decorating the outer surface of the delivering body with poly(ethylene glycol) (PEG) molecules. This technique is known as PEGylation, and has been used in clinical sessions since the early 1990's [84]. It essentially depends on creating a hydrated layer on the surface of nanoformulations, which acts as a steric barrier that prevents the agglomeration and discovery of the nanoparticles by the reticuloendothelial system (RES). PEGylation has been used for shielding a wide range of molecules and nano-systems, including different types of proteins, peptides, enzymatic and antibody fragments, short DNA/RNA molecules and small synthetic drugs [10, 80].

Examples of PEG-pro-drug conjugates include PEGcamptothecin, PEG-irinotecan, PEG-SN38, PEG-paclitaxel, PEG-docetaxel, PEG-DOX, PEG-methotrexate and PEGcurcumin [85]. Following this strategy, delayed chemical/enzymatic degradation, prolonged circulation time and extended *in vivo* half-lives are granted for PEGylated substances, with even better results achieved as molecular weights and formulation sizes are increased [86-90]. That is, when molecular weights of PEG are increased (within the window of 800 kiloDaltons), dense PEG coatings secure better shielding of the hydrophilic layer, thus prevent "*opsonization*"; *i.e.* the detection and elimination of foreign species from the bloodstream [91]. Likewise, larger sizes of nano-formulations are considered adequate for protein adsorption [92]. For instance, PEG-PHDCA nanoparticles, with sizes of 80 nm, 170 nm, and 240 nm, when incubated with serum protein for 2 h, showed protein surface adsorption rates of the 240 nm particles five times more than that of the 80 nm particles [91]. More design-related considerations are mentioned in the next sections.

2.4. Active Targeting Techniques and Controlled Release Mechanisms

The delivery of nanoparticles to carcinoma through the EPR effect is resulting in an increase of 20-30% only in delivery compared with normal organs [93]. Passive delivery techniques are also not enough by themselves to guarantee even distribution and entrapment of active agents within desired locations [69]. For this reason, active targeting techniques are introduced. These techniques primarily aim to enhance the targetability of bioactive ingredients, along with macromolecular drug accumulation, and intracellular release and uptake profiles. Means like receptor-mediated endocytosis and nanoparticles internalization into tumorous cells and tissues are exploited for this purpose [87].

Active targeting techniques primarily rely on decorating multifunctional nano-scale formulations and/or biomolecules with surface moieties (also referred to as ligands, homing devices) that are able to recognize and bind towards certain cellular receptors/components, which are overexpressed or only expressed on cancerous sites. Based on this, tumor receptors (*e.g.,* transferrin receptors [94], folate receptors [95], and Epidermal Growth Factor Receptor (EGFR) [96]) act as anchors for the attachment to the ligands [97, 98], whereas the latter act in a similar way to the "magic bullets" that were first proposed by, Paul Ehrlich; the German Nobel laureate and the father of chemotherapy, in the early 1900's [99].

Moreover, actively targeted nanoparticles can be designed to target the tumor microenvironment itself instead of the tumor cells. One way to achieve this endeavor is by targeting VEGF's (which helps in tumor angiogenesis and neovascularization) and their receptors [100]. Another method is to target αvβ3 integrin, which is an endothelial cell receptor for extracellular matrix proteins. It is highly expressed on neovascular endothelial cells and poorly expressed in resting endothelial cells [101]. Cyclic or linear derivatives of RGD (Arg-Gly-Asp) oligopeptides are the most studied peptides which bind to this type of integrins [102].

Examples of successful active targeting ligands towards cell receptors and/or the tumor microenvironment include antibodies/antibody fragments, small molecules (*e.g.,* folic acid, vitamins, carbohydrates), antibodies or their fragments (*e.g.,* herceptin), proteins (*e.g.,* albumin), peptides and cell targeting aptamers (*e.g.,* siRNA) [103-112], whereas examples of actively targeted nano-vehicles include micelles, liposomes, polymeric nanoparticles, dendrimers and solid lipid nanoparticles [113-118].

Similar to passive targeting, there are many challenges that are yet to be defined in order to effectively deliver the active nanocarrier to the tumor site. It is essential that the conjugated ligand will specifically bind to the receptors which are overexpressed in the tumor cells or vasculature rather than the ones expressed in healthy cells. For this to happen, concentrations of receptors on the tumor cells should be much higher than on the healthy cells, keeping in mind that tumor cells may also alter their receptor expression over time due to genetic mutations, which will have a direct impact on the active targeting [119].

Additionally, tumor penetration and accumulation of the targeted nanocarriers on the periphery of the tumor might be hindered due to the presence of a "binding-site barrier" [120, 121].

Furthermore, there is always the threat of developing unwanted immune response within the cancer cells due to the usage of specific antibody ligands, which hence prevents their usage for repeated administration [104].

Molecular ligands, on the other hand, should also be chosen or designed with the intention of promoting receptor mediated endocytosis, without interfering with stealth ability of the carriers or causing significant activity reduction of the delivered components [68]. This implies the cautious choice of ligands-related design factors, including but not limited to: ligands sizes and conformation, linkers types, chemical activities and affinities, types of reactions, loading methods and ligands surface concentrations [87, 122].

2.5. Controlled (Triggered) Release Mechanisms

Controlled drug release techniques ultimately aim to deliver the active agent to the desired sites of action while minimizing its interactions with other places in the body, and also to provide regular drug distribution over the cancer areas while having control over the time of drug exposure [69].

Drug release can be controlled by applying certain external or internal triggers. External triggers include temperature, magnetic fields, ultrasound (US) and light, while internal triggering involves inducing changes in pH, pressure, temperature, enzymatic and/or redox conditions. A number of these techniques have been extensively reviewed elsewhere [123].

Here we will briefly describe some of the most applied triggers, whereas triggering using US is explained in more details in Section 3.

2.5.1. Magnetic Field Triggering

In this approach, magnetic nanoparticles/molecules are encapsulated/attached to a nanocarrier along with the antineoplastic drug. Once the delivery vehicle reaches the targeted site, the drug is then released as a certain magnetic field is applied. In most cases, the magnetic field acts as a controller that directs the nano-formulations to the targeted site, then, once accumulated at the desired rate, drug release is induced; either by simple diffusion or *via* mechanisms that involve an enzymatic activity or changes under physiological conditions (*e.g.,* pH and temperature).

Several magnetic triggering examples are available in the literature. For instance, Babincová *et al.* [124] reported a method for utilizing magnetic field to release drugs from liposomes, with superparamagnetic particles embedded within their lipid bilayer. The group explained that, with the aid of an alternating magnetic field, the magnetic nanoparticles started to vibrate, hence inducing heat to the liposomes *via* Néel relaxation. Upon exceeding the transition temperature of the lipids, the carriers were disrupted, and the drug was released. One advantage of using this method is that it prevents the surrounding tissue from heating up as a result of using the alternating magnetic current.

P. Pradhan and co-workers [125] successfully developed a temperature-sensitive folate-targeted DOX-containing magnetic liposome. They encapsulated the magnetic iron oxide nanoparticles into the folate-attached liposomes. *In vitro* cell studies have shown higher uptake of DOX with the application of an alternating magnetic field.

Another example was presented in the work of Lina Pradhan *et al.* [126], where the group reported developing a lipid layer encapsulating mesoporous magnetite nanoassemblies. The delivery system was designed with dual triggering mechanisms (pH- and temperature-sensitive lipids), and was able to simultaneously transport two anticancer drugs under an external alternating magnetic field. In their experiments, release kinetics and the stability of the magnetic liposomes were also tuned by varying the composition and concentration of the magnetic nanoparticles [127].

2.5.2. Light Triggering

This triggering mechanism is non-invasive, and can be applied to the skin surface using electromagnetic radiation (*i.e.,* ultra-violet (UV), visible and near infra-red (IR) light)within the range of 200-1000 nm. The depth of penetration of these waves into the body depends on the radiation frequency. UV and such high-frequency waves (wavelength less than 700nm) are more scattered, hence not able to penetrate into the tissue for more than 1 cm. Nevertheless, they dissipate their energies into heat causing local heating [128].

Several mechanisms exist for inducing nanocarriers to release their loads. This includes photo-thermal mechanisms, light-driven isomerization, oxidation, light-induced hydrophobicity changes, polymer backbone photo-fragmentation and photo-driven de-crosslinking [129, 130]

Tatu Lajunen *et al*. [131] developed light-sensitive liposomes that sequester gold nanorods and nano-stars. The former has a light absorption maximum at 650nm, while the latter has broader peaks of absorption (between 700-900nm). Triggering mechanism of this system is based on the conversion of absorbed light into heat energy. This energy raises lipidic bilayers temperature until it exceeds their transition temperature which renders liposomes leaky and causes them to release their drug loads.

Moreover, carbon nanotubes (CNT) have the ability to absorb near-IR light, then convert it into heat energy. This ability has been used to develop nanocarriers, in which CNTs were coated with chitosan and encapsulated within a nanogel, along with DOX.It was reported that when near-IR was applied, a faster rate of DOX release was observed [132].

Additionally, polymerisable methacrylate; a photopolymerizing polymer was also incorporated within the lipid bilayer of liposomes. When polymerization was initiated as UV radiation was applied, the inner vesicles of liposomes fused together, thereby releasing encapsulated drugs [133]. Drug release was also achieved by incorporating degradable or photocleavable lipids within nano-carriers [134, 135].

2.5.3. pH-Based Triggering

As mentioned before, tumor microenvironment is acidic compared to surrounding healthy cells [136]. Accordingly, pH-sensitive nanocarriers are good candidates for delivering the drug into tumor sites. Nevertheless, such vehicles should be stable at physiological pH, then disassemble or transform to release their payloads upon reaching targeted sites that have lower pH levels. Compounds containing acid-sensitive bonds are grafted in such designs, where acidic sensitivities are introduced *via* either protonatable groups (*e.g.,* amines or carboxylic acids) or acid-labile bonds [137]. When a protonatable group accepts or donates protons, it undergoes pH-dependent changes within its physiochemical properties, resulting thus in releasing the incorporated drugs. Whereas when using acid-labile linkers (*e.g.,* acetal, orthoester hydrazine and imine), they degrade or hydrolyze when reaching the acidic medium, thus destabilizing the nanocarrier [138].

It worth mentioning that recently, multiple pH-sensitive nanocarriers have been developed to exploit the acidic nature of tumor sites. One of these are the polymeric micelles [139,140]; in which the inner hydrophobic scores are converted into hydrophilicones through ionization of the pH sensitive groups. This leads to the event of *demicellization*, which results in a rapid release of encapsulated hydrophobic drugs [141]. Other nano-carriers used in this area include dendrimers [142-146] and liposomes [147-151].

2.5.4. Temperature Triggering

Mild hyperthermia can trigger drug release from thermoresponsive nanoparticles. The most common thermally responsive carriers described in the literature are temperature sensitive liposomes (TSL). For example, Thermodox® are temperature sensitive-liposomes encapsulating DOX [152]. Bilayer phospholipids used in liposomes preparation (lipid shell) can exist in either a liquid or a solid phase (gel phase). Each phospholipid is characterised by a specific temperature, at which it undergoes a transition (melt) from the gel to the liquid phase. The use of thermosensitive liposomes that contain phospholipids with a low

transition temperature, generally improves the release of the entrapped DOX, as reported by a number of studies [153-155]. TSL can also be produced by adding leucine-zipper to the membrane of the liposomes [156].

2.6. Optimal Design of Loaded Nanoparticles

As mentioned previously, foreign species that evade detection by macrophages in the bloodstream have to overcome specific physical barriers. These include the spleen, the mechanical sieving by the liver, and glomerular filtration by kidneys. The latter are capable of rapidly filtering minute molecules, possessing hydrodynamic radii less than 6 nm, out of the body, *via* renal filtration and urinary excretion. Particles with sizes above 8 nm are not typically filtered out, whereas particles within the range of 6-8 nm are either filtered out or retained, depending on their surface charges [157, 158]. Additionally, rigid-spherical particles with diameters above 5.0 μm and 0.2 nm are trapped by the lungs and the spleen, respectively [159].

Accordingly, it can be deduced that the preferable shapes of nanostructures are the linear, then the semi-flexible spherical designs [57, 69]. A work by Chan *et al*. reported that spherically shaped nanoparticles improved cell internalization more than rod-shaped nanoparticles [160].

Moreover, using sizes less than 200 nm is preferable; as they enable minute particles to pass freely through the physical barriers and pore sizes of the tumor vascular, hence, enabling them to reach and accumulate inside diseased tissues. It is preferable to use sizes greater than 20 nm; in order to avoid clearance by kidneys [161, 162]. It was also observed that particles with too small sizes often diffuse out from the tumor interstitium [163]. As for biological barriers caused by the RES, where hydrophobic or highly positive charged particles become more prone to opsonization; nanoformulations can be protected via grafting surface modifications using PEG chains and brushes [164-166].

The easiest and most commonly used technique to achieve optimum functionality of therapeutic or theranostic nanocarrier; is by reacting proper *reactive groups* (*e.g.,* amine, hydroxyl, thiol) that are conjugated to a nano-scale cargo via *spacers* (linkers, *e.g.,* PEG); with suitable *ligands* that are evenly distributed in the nanoparticle [69, 87]. A linker with a proper length and high stability will enable the nano-vehicle to extravasate and easily interact with cell receptors while having improved solubility and a reduced chance of losing ligands activity [167, 168]. Cleavable biodegradable linkers can also provide the opportunity of devising controlled drug release in case they were sensitive towards certain physicochemical triggers [77, 169]. The active agents can be entrapped in/attached to the "smart" nanovehicle (polymer matrix or nanocapsule) before or after forming the carrier and decorating it with the homing devices. The nature of molecules, preparation methods and types of reactions and interactions are the main factors that determine the best loading techniques to be used, where nano-carriers transferring drugs should also have reasonable loading capacities ([weight of drug in nanocarrier/ weight of carrier]*100) and entrapment efficiencies ([experimental drug loading/ nominal drug loading]*100). Generally speaking, it is known that nano-formulations have considerably less capacities than regular macro- and micro-devices. Nevertheless, some carriers (namely, dendrimers, micelles, nanostructured lipid carriers and carbon and silica framework structures) are especially attractive as they have very high drug loading percentages that can reach to above 90%. Techniques such as physical entrapment/incubation/ homogenization, covalent, noncovalent or electrostatic interactions, nano-precipitation, emulsification/solvent evaporation, dialysis, or surface absorption are typically used to load the antineoplastic agents in the nanovehicles mentioned above [61, 168, 170-172].

2.7. DOX and Nanoparticles

As mentioned earlier, cardiotoxicity and MDR are the main drawbacks that limit the use of DOX in particular and anthracyclines in general as first-line drugs against cancer [52]. DOX has a significant systemic toxicity, which considerably limits its application in oncology (upper accumulative dose allowed is 450-550 mg/m^2) [173].

Early efforts to solve this issues focused on lowering required drug doses, destabilizing nucleosomes or achieving synergism. This was achieved by applying a combinations of DOX (or its less toxic analogues (*e.g.,* epirubicin, idarubicin)) and other anti-neoplastic agents (*e.g.,* alkyl lysophospholipid perifosine, edelfosine) instead of using one drug, or by seeking to prolong DOX infusion schedules so as to encourage the growth of healthy cells between chemotherapeutic sessions [43, 45, 52, 55, 174]. Other attempts introduced cardioprotective and anti-apoptotic medications (*e.g.,* angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor antagonists, β-blockers, statins, dexrazoxane). Some of these approaches were successful in decreasing *in vivo* toxicity or the oxidative stress of DOX in the treatment of several cancers [56, 175]. Nevertheless, more clinical data and safety profiles of DOX analogues, antioxidants and stains are needed, careful screening of the patients receiving such treatments is essential, and further cytotoxicity reduction and late-onset drug complications are required, especially when treating children-related carcinomas [43, 44, 175].

As early as the 1970's, the attractive properties of nanoparticles have encouraged researchers to consider sequestering or conjugating anthracyclines and other agents within different types of nano-based delivery systems, while continuously trying to introduce targeting elements and triggering techniques [176].

PEGylated liposomes, of sizes around 100 nm, encapsulating DOX-HCl, were the first to be considered and modified for such purposes [42, 177, 178]. Historically speaking, a successful "first in man" clinical trial using this system was conducted

by Gabizon and Barenholz, in 1991-1992. The results of this trial were then published in 1994. Shortly after that, the FDA officially approved the use of this system as an anti-cancer treatment in 1995. It is worth mentioning that the name "Doxil®" is essentially abbreviated from the words "**DOX**urubicin**i**n **L**iposomes"[179,180]. Doxil® nowadays is used to treat ovarian cancer, AIDS-related Kaposi sarcoma, and multiple myeloma, in patients who showed no significant improvement following treatment with other lesspoisoning anticancer drugs [80].

In order to achieve high loading of liposomes, the drug is remotely loaded into the carriers by means of transmembrane ammonium-sulfate [$(NH_4)_2SO_4$] gradient. This process is a base-exchange of the amphipathic weak-base drug with the ammonium ions. The loading is obtained under conditions that [$(NH_4)_2SO_4$] inside the liposomes $\gg$ [$(NH_4)_2SO_4$] in the external medium [179].

Several micellar (*e.g.,* NK-911®) and polymerconjugations (*e.g.,* PK1 and PK2) have been developed and entered different phases of clinical trials since the success of DOXIL® [47, 181]. Recently developed targeted DOXconjugates utilize carriers like polymers (*e.g.,* polyisobutylcyanoacrylate, dendrimers, gold/ silver nanoparticles, carbon nano-formulations, proteins/peptides and vitamins (*e.g.,* folic acid), whereas DOX-encapsulating systems use several nano-particles; such as micelles, liposomes, solid-lipid nanoparticles, polymersomes, nanoemulsions, nanogels, nanocrystals and silica and inorganic nanoparticles. Several sources in literature have extensively reviewed such DOXcombinations, where it was stated that in order for such systems to expand their use in oncology, they should achieve improved curative effects and lower costs [42, 47, 57, 174, 182].

3. ACOUSTICALLY ACTIVE NANOCARRIERS

US is defined as mechanical longitudinal pressure waves which, like sound waves, propagate through various media. It is transmitted as an alternation series of compressions (zones of high pressure) and refraction (zones of low pressure), and is considered a very effective non-invasive tool to attain spatiotemporal control of drug release from nanocarriers [183]. Due to its ability to penetrate into tissues in a tunable way without adversely affecting these tissues, the applications of US in the medical field are very appealing. Triggered drug release from a variety of nanocarriers can be achieved utilizing US, as the latter produces thermal and/or mechanical effects (by cavitation phenomena) or radiation forces. Liposomes and micelles are the most studied used nanocarriers that can release their load under the influence of US [184]. Such triggering approach will be discussed in the next sections after giving a brief introduction about US.

3.1. US Physics and Mechanisms

The main parameters of US waves are the frequency and intensity. The frequency is defined as the number of sinusoidal cycles per second (Hertz, Hz), and the intensity represents the power density, which is the power carried per cross-sectional area of the US beam (W/cm^2). The wavelength and frequency of US are inversely related, i.e., US of high frequency has a short wavelength and vice versa. Unlike sound waves, the frequency of the acoustic waves is higher than the audible range of humans (above 20 kHz). Like other forms of wave energy, these ultrasonic waves can be reflected, refracted (bent), focused, and absorbed [183].

While propagating through a medium, US waves transfer energy to the particles. This does not lead to moving the particles, but instead, they just oscillate in place (move back and forth in a regular rhythm) while the energy is transferred, thus propagating the pressure wave. US waves propagating through a liquid media create what is known as "*acoustic cavitation*" (Fig. **4**), which is defined as the formation of gaseous cavities (gas bubbles) in a medium upon US exposure. The energy from the US waves forces the bubbles to oscillate; expanding at low pressure and contracting at high pressure without collapsing. This slow oscillatory motion of a bubble is called "*stable cavitation*" which occurs at low US intensity [185]. As the intensity of US increases, the amplitude of bubble oscillation increases, until it reaches a point in which the inward moving wall of fluid has sufficient inertia that it cannot reverse direction when the acoustic pressure reverses, but rather continues to compress the gas in the bubble. Consequently, enormous growth and oscillation events occur until the bubble eventually collapses. This is known as "*inertial" or "collapse cavitation*" [184], where the intensity of inertial cavitation increases at higher US intensities and lower frequencies [186].

3.2. Medical Applications of US

US has been used widely in the medical field. It received wide acceptance as a non-ionizing form of energy, which is applied non-invasively. US waves are generated through a transducer (probe) which produces pressure waves when a voltage is applied to it [187]. The pressure waves are then transmitted into fluids or tissues through a gel contacting the transducer [10]. Once produced, the ultrasonic energy is suitably focused into a narrow beam, which is then directed into the tissues. Along its path, the beam interacts with the tissues through various processes, including reflection, refraction, absorption, and scattering of the beam energy. Depending on frequency, intensity (or power density) and length of exposure time, the US waves can be focused on and absorbed by tissues in several ways, so as to achieve different effects for a specific purpose. The choice of frequency used in any application is based on the consideration of wave absorption, adequate penetration and, in diagnostic application, adequate resolution [188].

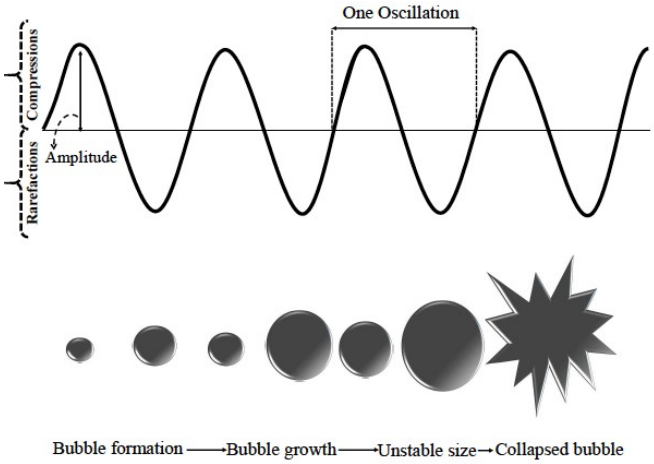

Fig. (4). Formation, growth and collapse of bubbles in acoustic cavitation process (inertial cavitation).

Table **1** shows the different medical applications of US and the suitable intensity/frequency for each application.

Table 1. Medical application of US and the suitable intensity/ frequency for each application.

Medial application	Properties of the US used
Diagnostic	Low intensity (1-50 mW/cm^2) to avoid tissue heating). High frequency (3 and 5 MHz).
Surgical	Very high intensities (above 8 W/cm^2) for tissue destruction. Very low frequencies (20-60 kHz).
Therapeutic (physiotherapy)	Intensity of (0.5 to 3 W/cm^2). Frequencies from (0.7 to 3 MHz).

US is used widely in the medical field for imaging. A transducer produces pulses of US waves into the body, which penetrate the different tissues in their way. Some are reflected back to the transducer (echo signals),while others continue to penetrate deeper. The echo signals returned from many sequential coplanar pulses are processed and combined to generate an image [189].

Furthermore, US can be applied in pulses. In fact, pulsed US output is the preferred mode for medical treatment. During the pulsed US, the machine will produce acoustic waves of controlled wavelength and intensity (*On* period), followed by a pause period where no US is produced (*Off* period). Typical pulse ratios are 1:1 (*e.g.,* 2 mins *On*/ 2 mins *Off*) and 1:4 (*e.g.,* 2 mins *On*/ 8 mins *Off*), although other ratios are also used. The effect of varying the pulse ratio is illustrated in Fig. (**5**).

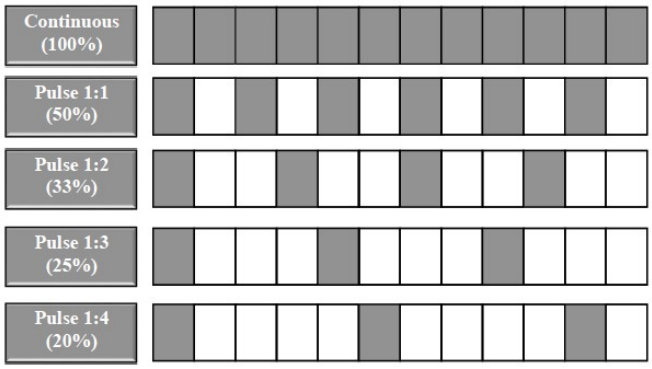

Fig. (5). Different ratios of the US pulses used in medical treatment.

The therapeutic effects of US are divided into thermal and non-thermal. The non-thermal or mechanical effect is caused by the acoustic cavitation, which is used for example in medical imaging. For instance, in thermal therapy, highintensity focused US (HIFU) in the continuous mode is used to generate pressure waves. When an ultrasonic wave propagates through the body,

it is attenuated by the contact with different tissues through the effects of absorption and scattering. The absorption of these waves causes an increase of tissue temperature that is inversely proportional to the attenuation of US [190]. HIFU is capable of inducing "*coagulation necrosis*", which is the death and clotting of the cells. HIFU is non-invasive; where the US waves are focused and, therefore, no damage to the tissue in the path of the US beam occurs.

The good depth-focus capability of the HIFU could be used as an additional therapeutic technique in acoustically accessible tumors [191]. Thermal therapy through hyperthermia (above 43˚C) can be used to destroy cancerous cells, like prostate, pancreatic,breast and liver cancer [192-195]. Lower power densities will result into smaller temperature rises (mild hypothermia); usually to 40-45°C, initiating a series of subcellular events, rendering the cells susceptible to various forms of damage, leading to subsequent cell death or apoptosis [196].

US has been also used in drug delivery due to the ability of US to produce cavitation activity. The collapse of cavitation bubbles leads to the formation of shock waves or highvelocity micro-jets that are capable of generating transient or permanent pores in the walls of blood vessels, hence, enhancing extravascular delivery of therapeutics in the desired site [197]. This process, known as "*sonoporation*" is a promising drug delivery technique.

3.3. US-responsive Nanocarriers

A number of nano-vehicles have been or are currently being developed to show responsiveness towards US. The most famous two examples include liposomes and micelles. Other relatively new examples include nano-emulsions and solid nanoparticles, as will be discussed in this section.

3.3.1. Micelles

Micelles are nano-scale colloidal particles that have sizes ranging between 5-100 nm [198]. In aqueous media, they are self-assembled into stable formations that are composed of hydrophobic cores and hydrophilic coronas. Hydrophobic drugs can be sequestered within their inner cores, where such combinations have long circulation time in the blood due to their small sizes. Micelles can also be decorated with a wide spectrum of targeting moieties once used for chemotherapy, such as antibodies, folic acid, epidermal growth factors and transferrin [199]. They can also respond to multiple internal and external triggers, such as pH conditions, temperature triggering and US [198-200]. Table **2** and Table **3** below give examples of research and modeling studies that investigated acoustically triggered micelles loaded with DOX.

3.3.2. Nano-Emulsion

Nano-emulsions are biphasic dispersion of two insoluble liquids, with the "continuous phase" surrounding the "dispersed phase" and stabilized by an amphiphilic surfactant. Stabilization prevents the dispersed phase from coalescing, while the drug is often carried in the non-aqueous dispersed phase of the emulsion [232]. As USis applied and affects nanobubbles or emulsions, they are destroyed due to cavitation events generating micro-jets, resulting in the formation of pores in cell membranes. Due to these pores, the drug released will have an easy path into the tumor cells.

Acoustic nano-droplets are phase-shift nanoparticles that undergo a change in their phase from liquid to gas under the influence of external stimuli, such as the US. The strategy of using acoustic droplets for therapeutic and diagnostic application was recently proposed by Kripfgans and coworkers in 2000 [233]. These nano-droplets consist of phasechange perfluorocarbon (PFC) material with a lipid/cholesterol coating. During US radiation, the PFC droplets inside the lipid coating undergo a phase transition from a liquid into gas bubbles; a process termed "*acoustic droplet vaporization*" (ADV). When the droplet changes its phase, the size is increased by several orders of magnitude,and the lipid coating is disrupted. Tumor targeting can be achieved by passive or active methods. Various receptors like folic acid [234] and aptamers [235] have been attached to the nano-droplets, with DOX encapsulated to target varioustumors. Wang*et al*. [236] incorporated DOX along with superparamagnetic iron oxide nanoparticles into the droplets to assist the carrier towards the tumor site by an external magnetic field. Due to the expansion of the droplets to form gas bubbles, the lipid coating is disrupted, resulting in rapid release of the drug content. The rapid expansion can also cause physical disruption at the tumor site. In a recent work by Yi-Ju Ho *et al*. [237], the group used DOX-encapsulated nano-droplets to disrupt the tumor vascular through US stimulation and, thereby, improve the drug extravasation and immune response.

3.3.3. Solid Nanoparticles

As the name indicates and unlike nano-emulsion,solid nanoparticles are miniaturized spherical nano-entities that have solid cores. Presently, there are several types of solid nanoparticles used in drug delivery, one of the most common

Table 2. Examples of publications on US-guided DOX delivery using polymeric micelles.

Study	Cancer Type	*In Vivo*/*In Vitro*	US Parameters	Micelles	Effect

Rapoport, 2004 [201]	Ovarian carcinoma A2780 and MDR cells	*In vitro*	US pulses/continuous (1, 3 MHz) and (20 kHz-3 MHz) power densities (0.058 W/cm^2, 6 W/cm^2 and 0-0.2 W/cm^2)	Pluronic® P-105	US releases drug from micelles and enhances the intracellular uptake of both released and encapsulated drug.
Nelson *et al*., 2002 [202]	Colon tumor (DHD/K12/TRb)	*In vivo*	20 kHz and 70 kHz (0.048 W/cm^2 , 1 W/cm^2 and 2 W/cm^2)	Stabilized Pluronic micelles	US releases DOX at the tumor site and reduces tumor volume compared with noninsonated tumors.
Husseini *et al*., 2000 [203]	-	*In vitro*	Pulses/continuous (20 to 90 kHz) (0 to 3 W/cm^2)	Pluronic® P-105	The shock waves produced by transient cavitation events disrupt micelles and release the drug into aqueous environment.
Marin *et al*., 2001 [204]	HL-60 Human Leukemia cells	*In vitro*	30 min sonication at 20 kHz. Power densities (1.4 mW/cm^2, 14 mW/cm^2 and 33 mW/cm^2	Pluronic® P-105	US increases the intracellular drug uptake from Pluronic® micelles.
Gao *et al*., 2005 [205]	Ovarian carcinoma A2780 cells	*In vivo*	30s of US (1-MHz or 3MHz), (3.4 W/cm^2)	Pluronic® P-105 with PEG2000-DSPE	DOX uptake by tumor cells was significantly enhanced by tumor sonication.
Husseini *et al*., 2007 [206]	-	*In vitro*	70 kHz, (0.27 W/cm^2)	Stabilized/ unstabilized Pluronic® P-105	Micellar drug release under the influence of US is caused by the shearing phenomena associated with cavitation events.
Husseini *et al*., 2005 [207]	-	*In vitro*	70 kHz, (0.28 W/cm^2)	Pluronic® P-105	The onset of drug release from unstabilized micelles corresponds to the emergence of a subharmonic peak in the acoustic frequency spectra.
Rapoport *et al*., 2004 [201]	Ovarian carcinoma A2780 cells	*In vivo*	1 MHz, (1.2 W/cm^2)	Pluronic® P-105	US applied to the tumor substantially enhanced the intracellular drug uptake by tumor cells while decreasing drug uptake by other organs including the heart.
Staples *et al*., 2010 [208]	DHD/K12/TRb colorectal epithelial cancer	*In vitro*	20-kHz, continuous wave mode with an intensity of 1.0 W/cm^2	Pluronic® P-105	US increased the average drug concentration by about 50% in the tumor around the 30-minute time point post-treatment.
Munshi *et al*., 1997 [209]	HL-60 human leukemia cells	*In vitro*	80 KHz	Pluronic® P-105	DOX IC_{50} was lowered from 2.35 to 0.19 mg/ml.
Hasanzadeh *et a*l., 2011 [210]	Breast adenocarcinoma tumors	*In vivo*	3 MHz, (2 W/cm^2); 28 kHz, (0.04 W/cm^2)	Pluronic® P-105	Dual frequency sonication improved drug release from mi-celles and increased drug uptake by tumors due to sonoporation.
Pruitt and Pitt, 2002 [211]	HL-60 Human leukemia cells	*In vitro*	70 kHz, (1.5 W/cm^2)	Stabilized/ unstabilized Pluronic® p-105	US resulted in a synergistic killing effect with DOX and low concentrations of either stabilized or unstabilized Pluronic®P-105.

(Table 2) contd….

Study	Cancer Type	*In Vivo*/*In Vitro*	US Parameters	Micelles	Effect
Staples *et al*., 2009 [212]	DHD/K12/TRb colorectal epithelial cell line	*In vivo*	20, 467 kHz	Pluronic® P-105	Tumors treated with drug and US displayed, on average, slower growth rates than noninsonatedtumors.
Husseini *et al*., 2013 [213]; Husseini *et al*., 2015 [214]	-	*In vitro*	70 kHz, (0.53 W/cm^2-3 W/cm^2)	Pluronic® P-105 with a folate moiety attached	Percent drug release increases as the power intensity of US increases. The maximum amount of release (14%) was measured at 5.4 W/cm^2. Release follows a zeroorder model, re-encapsulation follows a first-order model.
Husseini *et al*., 2000 [215]	HL-60 human leukemia cells	*In vitro*	70 kHz, (1.3 W/cm^2)	Pluronic® P-105	The application of US causes the release of DOX from micelles or causes the HL-60 cells to take up the micelle encapsulated DOX.
Husseini *et al*., 2002 [216]	HL-60 human leukemia cells	*In vitro*	70 kHz, (1.2 W/cm^2)	Pluronic® P-105	The increase in drug accumulation in the cells as a result of ultrasonication is not due to an increase in endocytosisdue to ultrasonication.
Husseini *et al.* 2005 [217]	HL-60 human leukemia cells	*In vitro*	70 kHz, (1.3 W/cm^2)	Pluronic® P-105	Apoptosis is the mode of cell death in acoustically-activated drug delivery using polymeric micelles.

Table 3. Examples of modeling studies of US-guided DOX delivery using polymeric micelles.

Study	US Parameters	Micelles	Effect
Wadi *et al*., 2017 [218]	70 kHz (3.54 W/cm^2 5.43 W/cm^2)	Pluronic® P-105	The MLE-optimized filters proved to outperform the other estimators in predicting micellar release using US.
Martins *et al*., 2016 [219]	70 kHz (1.009 W/cm^2 to 5.914 W/cm^2)	Folate-targeted Pluronic® P-105	The model took into consideration that the drug release increased with increasing power density. The micelle reassembly also increased with increasing power density.
Husseini *et al*., 2011 [220]	70 kHz (0.76 W/cm^2)	Stabilized/unstabilized Pluronic® p-105	DOX release from stabilized micelles was significantly lower. No statistically significant difference between re-encapsulation rate constants for stabilized and unstabilized micelles.
Abusara *et al*., 2018 [221]	70 kHz	Pluronic® P-105	Kalman filter was used to estimate the acoustic release and reduce the uncertainty of release measurements
Stevenson-Abouelnasr *et al*., 2007 [222]	20 kHz, (0.058 W/cm^2)	Unstabilized Pluronic® p105	A mathematical model is consistent with the assumption that US stimulates DOX release from micelles. The release and recapsulation profiles were fit using a 4-parameter model.
Husseini *et al*., 2007 [223]	20 kHz and 70 kHz, (0.015 W/cm^2 and 0.38 W/cm^2)	Pluronic® P-105	DOX release is not a strong function of temperature, suggesting that thermal effects do not play a major role in the physical mechanism involved.

Husseini *et al*., 2011 [224]	20 kHz, (0.033 W/cm^2, 0.048 W/cm^2 and 0.058 W/cm^2)	Stabilized/unstabilized Pluronic® p-105	NN-MPC was shown to be an effective tool to model, optimize, and control the release of DOX from P-105 micelles under different ultrasonic power densities at 20 kHz.
Husseini *et al*., 2009 [225]	70kHz and 476 kHz	Stabilized Pluronic® p-105	USis capable of disrupting the covalent network of the stabilized micelles. No significant difference in degradation rates when employing the two frequencies in question at the same mechanical indexwas observed.

(Table 3) contd….

Study	US Parameters	Micelles	Effect
Husseini *et al*., 2010 [226]	70 kHz, (0.58 W/cm^2, 0.675 W/cm^2 and 0.765 W/cm^2)	Pluronic® P-105	Modelling showed the good prediction of the release and reencapsulation of DOX at three different temperatures. The rate of micellar destruction increases with temperature, and their re-assembly decreases with temperature.
Abdel-Hafez and Husseini, 2015 [227]	70 kHz, (3.54 W/cm^2 to 5.91 W/cm^2)	Pluronic® P-105 and folate-targeted Pluronic® P105	The optimal release estimate is obtained by probabilistically adding the estimates from the hypothesized Kalman filter estimates and was able to account for the uncertainty in the system.
Diaz de la Rosa *et al*., 2013 [228]	70 kHz, (0.1 W/cm^2 to 0.8 W/cm^2)	Pluronic® P-105	The dynamic model was used to predict the release behaviour of doxorubicin from the polymeric micelles.
Diaz de la Rosa *et al*., 2013 [229]	70 kHz and 476 kHz	Pluronic® P-105	The paper compared the mechanism of Dox release from micelles at 70-kHz and at 500 kHz ultrasound.
Husseini *et al*., 2009 [230]	20 kHz, (0.058 W/cm^2, 0.047 W/cm^2 and 0.033 W/cm^2)	Pluronic® P-105	ANN can be used to capture the highly nonlinear dynamics of acoustically activated drug release from polymeric micelles.
Husseini *et al*., 2002 [231]	20 kHz, (58 mW/cm^2)	Pluronic® P-105	The model of zero-order release with first-order reencapsulation appears to represent the release data of this polymeric system better than other kinetic models.

is silica nanoparticle. Paris *et al*. [238] have developed a nanocarrier based on mesoporous nano-silica particle with gates that allows the encapsulation and release of drugs. This was achieved by functionalizing mesoporous silica with a p(MEO_2MA)-coTHPMA copolymer that is able to open and close the gates of the carrier pores. Upon US irradiation, the coil-like conformation of the polymer is changed, opening the gates of the mesoporous of the carrier and allowing the entrapped cargo to be released. Song *et al*. [239] were able to encapsulate two drugs inside PLGA/ mesoporous silica fibers, and also succeeded in releasing them under the influence of US. Other examples of solid nanoparticle include silica nanotubes [240], polystyrene nanoparticles [241], and lipid nanoparticles [242]. US irradiation in combination with the nanoparticle significantly improved the therapeutic index of the treatment.

3.4. The Acoustic Release of DOX from Liposomes

3.4.1. Enhanced Acoustic Release Mechanism

The primary limitation of Doxil® is the slow release of the agent from liposomes, as well as, the limited penetration of these liposomes deep into the tumor [190]. The future of nanomedicines in oncology requires more than just the passive drug accumulation in tumors through the EPR effect. Once DOX-liposomes are injected into the bloodstream, it is vital that drug release is achieved in an efficient and controlled manner. The release can be controlled via chemical or physical means, like hyperthermia and pH strategies [243, 244].This review focuseson drug release triggered by physical means; precisely, low-frequency US (LFUS).

LFUS is used in drug delivery due to its ability to enhance the permeability of biological membranes for drug and gene delivery [245, 246]. As liposomes have a similar structure to that of biological membranes, applying LFUS increases the permeability of these systems to release their contents of the entrapped DOX in a well-controlled manner.

US exposure of tumor tissue comprising sono-sensitive liposomes may, not only, induce drug release from these carriers, but also increase intracellular drug uptake [247]. Earlier studies have shown that applying LFUS (20 kHz) on liposomes encapsulating calcein [248, 249], and DOX [247, 250] has enhanced the controlled release of the encapsulated drug from liposomes in a more efficient manner, when compared to high-frequency US up to 3 MHz [251]. Animal studies have reported a therapeutic benefit of combining US and liposomal cytostatic in tumor treatment, showing the potential of using this strategy within cancer therapy [252, 253]. The targeted liposomes are usually injected several hours or days before sonication, to allow preferential accumulation in the tumor before applying the US. Fig. (**3**) shows how DOX-liposomes; injected into the bloodstream, accumulate at the tumor site, then DOX release is triggered by US waves.

The sensitivity of liposomes to the acoustically triggered release can be enhanced by incorporating gas bubbles; also known as nano-emulsions, into the inner compartment of the liposomes (echogenic liposomes). This often requires the use of PFC in various forms, from decafluorobutane [254] and perfluoropentane [255] to perfluorohexane [256]. The energy of the US wave causes the evaporation and expansion of the gas, hence, the contents of liposomes will be released once the stress is sufficient to stretch the membranes of the carriers beyond their elastic limit [257, 258]. The phase transitions of perfluorocarbon nano-emulsion induced with ultrasound has been studied experimentally and numerically [259, 260]. Recent studies showed that these emulsion Liposomes are stable at physiological temperatures [261,262]. Microbubbles (gaseous spheres) have also been used in the US-enhanced drug release. Microbubbles are much larger than liposomes (1-5 μm diameter) and can be stabilized with a lipid shell. DOX liposomes can then be conjugated to the lipid shell of such microbubbles. When applying US, the microbubbles will oscillate by expanding and compressing in response to the pressure wave.

Table 4. Examples of publications on US-guided DOX delivery using liposomes.

Study	Cancer Type	*In Vivo*/*In Vitro*	US Parameters	Liposomes	Effect
Santos *et al*., 2017 [264]	Human FaDu squamous cell carcinoma cells	*In vivo*	1.2 MHz short 30s bursts at 42°C	Low-temperature sensitive liposomes (LTSL)	Focused US and hyperthermia improved drug delivery.
Dromi *et al*., 2007 [265]	Murine mammary adenocarcinoma cell line	*In vivo/ in vitro*	pulsed-HIFU, intensity (4 W/cm^2; Frequency (1.189 MHz)	Low-temperature sensitive liposomes (LTSL)	Enhanced and more rapid local delivery of DOX to tumors.
Kheirolomoom *et al*., 2013 [266]	Murine breast cancer	*In vivo*	US pulses consisted of 100-cycle bursts at 1.54 MHz center frequency	Low temperature sensitive liposomes (LTSL)+ Cu-DOX forming (CuDOXLTSLs)	Tumor elimination and no systemic toxicity of DOX was detected.
Um *et al*., 2017 [267]	EMT6 mouse mammary tumor cells	*In vivo/ in vitro*	Pulses of US (20 W/cm^2-15 Hz), (10 W/cm^2-15Hz) and 10 μW/cm^2.	Fatty acid-conjugated, elastin-like peptide bearing thermosensitive liposomes	Similar enhancement of DOX release by both thermal and non-thermal acoustic treatment.
Lentacker *et al*., 2010 [268]	Melanoma	*In vivo*	1MHz, 50% duty cycle, US intensityof 2W/cm^2	Liposomes containing microbubbles	Following the exposure to US, DOX-liposomes containing microbubble were more effective in tumor elimination.
Escoffre *et al*., 2013 [269]	Human glioblastoma cells (U-87 MG)	*In vitro*	1 MHz	Liposomes containing microbubbles	US enhanced the uptake of free DOX by glioblastoma cells from DOX-liposomes containing microbubble.
Park *et al*., 2013 [270]	-	*In vivo*	Pulsed US 1MHz	Temperature sensitive liposomes	significant tumor regression following 2 days of treatment.
Afadzi *et al*., 2013 [271]	The HeLa cell line	*In vitro*	Pulses of 300 KHz	Dox-liposomes and microbubbles	Treating the cells with US in the presence of microbubbles increased the cellular uptake of DOX.

Eggen *et al*., 2012 [272]	Prostate cancer	*In vivo*	1 MHz and 300 kHz	Sonosensitive DEPCbased liposomes	Both 1 MHz and 300 kHz exposure caused a significant Increased in Tumor Uptake of DOX.
Aryal *et al*., 2013 [273]	Rat glioma tumor	*In vivo*	Pulse US repetition frequency: 690 Hz	PEGylated liposomes	US enhanced the delivery of liposomal DOX.
Treat *et al*., 2012 [274]	9L gliosarcoma cells	*In vivo*	Pulsed US at 1.7 MHz	PEGylated liposomes	Reduced brain tumour size.
Yu *et al*., 2016 [263]	Squamous cell carcinoma (SCC)	*In vitro*	Pulses, with intensities from 0.0038 to 1.5 W/cm^2 and	Liposomal DOX conjugated to larger microbub-bles	Low-intensity US releases up to 70% of DOX.
Uenoet *al*., 2011 [275]	Murine osteosarcoma cell line LM8	*In vivo/ in vitro*	Pulses. power density 0.5 and 2 W/cm^2; frequency 1 and 2 MHz	Liposomes containing microbubbles	The effect of DOX was markedly enhanced by combining it with BL and US.
Hamano *et al*., 2013 [276]	293T human embryonic kidney carcinoma cells	*In vitro*	Pulses power density 0.25-1 W/cm^2; frequency 2 MHz	DOX-liposomes and bubble liposomes	The combination of DOXliposomes with bubble liposomes and US enhance drug release.

(Table 4) contd….

Study	**Cancer Type**	***In Vivo*/*In Vitro***	**US Parameters**	**Liposomes**	**Effect**
Yang *et al.*, 2012 [277]	GBM8401 glioma cells	*In vivo*	Pulses, power density; 2.9 W/cm^2; frequency 1 MHz	(AP-1)-conjugated liposomes	Enhanced targeted drug delivery in brain tumor therapies.
Geers *et al*., 2011 [278]	Melanoma cells	*In vitro*	Pulses, power density 2 W/cm^2 and frequency 1 MHz	DOX-liposome-loaded (lipid-shelled) microbubbles	US enhances the release from liposomes loaded microbubbles.
Pitt *et al.*, 2011 [279]	Colorectal cancer cells (DHD/K12/TRb)	*In vivo*	20-kHz (temporal average power density = 1 W/cm^2)	DOX-liposomes	US reduced the size of tumors in rats injected with the Doxloaded nanocarriers.

These oscillations can enhance endocytosis and sonoporation [263].

In drug delivery, thermal effects of US are more utilized for delivery than its mechanical effects, as it is employed to create real-time, controllable, focused hyperthermia. The frequency is usually in the MHz range. The pulse mode can be used to control the excess heat, as the interpulse will allow a decrease in temperature. Thermal effects of US enhances the drug release and reduces toxicity profiles of the encapsulated drug [264].

3.4.2. Summary of In Vitro, In Vivo and Clinical Work of US-Guided DOX Delivery Using Liposomes

Table **4** gives examples of research studies that investigated acoustically triggered liposomes loaded with DOX.

CONCLUSION AND FUTURE DIRECTIONS

Since early ages, researchers have tried tirelessly to find ways to defeat cancer; as it continues to be one of the deadliest, wide-spread diseases worldwide.

Chemotherapy is one of the main strategies used against cancer. It aims to deliver chemical antineoplastic agents to the infected locations to promote tumors self-destruction *via* apoptosis.

Doxorubicin is considered one of the most utilized chemotherapeutic drugs, due to its ability to treat a wide range of malignancies. Nevertheless, using this agent induces severe and chronic side-effects, due to the lack of targetability, limitation of tolerated doses and the development of multi-drug resistance.

The enabling field of nanotechnology has shown potentials in overcoming the challenges facing conventional chemotherapy. For instance, loading active anti-tumor substances into a nanocarrier, such as liposomes, results in a reduction in undesired interactions between the drugs and healthy cells, aprotection of the agent from early discovery and elimination from the bloodstream *via* the RES, an increase in drug uptake by cancerous cells, and improved efficacy, bioavailability, accumulation in tumors, biodistribution and overall performance. These qualities can further be enhanced as suitable targeting and triggering techniques are used.

Ultrasound is a safe- non-invasive technique that has been used in medical applications for decades. It can induce a controlled release from a variety of nano-particles whenever the drug has accumulated in the desired locations.

The design of an optimum nano-delivery system necessitates choosing the right design parameters of the carrier, in terms of the size, shape, type, charge, loading capacity and solubility. The ligand should also be chosen so that it will bind only to the receptors overexpressed in the tumor site(s). Finally, the trigger applied should cause drug release from the system in a controllable manner. Current research in the field has shown promise in multiple areas.

Future research might consider sequestering a combination of chemotherapeutic agents in one carrier to increase therapeutic efficiencies of the treatment and reduce the chance of developing multi-drug resistance. It is also expected that in the near future, more focus will be directed towards developing individual therapeutic systems that act upon certain genes or patient's conditions. It is probably early to see results of such endeavors. Yet, it is certain that more nano-therapeutics will find their way to be approved by health authorities and global markets.

AUTHORS' CONTRIBUTION

All authors have read the journal's publication ethics and publication malpractice statement available at the journal's website and hereby confirm that they comply with all its parts applicable to the present scientific work.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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

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