Review

Journal of Biomedical Nanotechnology
Vol. 19, 1–14, 2023
www.aspbs.com/jbn

AMERICAN SCIENTIFIC PUBLISHERS
Copyright © 2023 American Scientific Publishers
All rights reserved
Printed in the United States of America

Liposomal Encapsulation of Chemotherapeutics Agents Combined with the Use of Ultrasound in Cancer Treatment

Zeyad Almajed[1], Nour M. Al Sawaftah[2], Hana Sulieman[3], and Ghaleb A. Husseini[1, 2, 4*]

[1] *Biomedical Engineering Graduate Program, College of Engineering, American University of Sharjah, Sharjah, P.O. Box 26666, United Arab Emirates*
[2] *Materials Science and Engineering Program, College of Arts and Sciences, American University of Sharjah, Sharjah, P.O. Box 26666, United Arab Emirates*
[3] *Department of Mathematics and Statistics, College of Arts and Science, American University of Sharjah, Sharjah, P.O. Box 26666, United Arab Emirates*
[4] *Chemical and Biological Engineering Department, College of Engineering, American University of Sharjah, Sharjah, P.O. Box 26666, United Arab Emirates*

Ultrasound (US) has numerous uses in the medical field, including imaging, tumor ablation, and lithotripsy; another interesting application of US in cancer therapy is as an external trigger in targeted drug delivery. Cancer-targeted drug delivery involves delivering chemotherapeutic drugs to tumor sites with a high degree of precision, which would minimize the adverse side effects experienced by patients. Several nanocarriers have been studied as possible nanocarriers; however, liposomes stood out from the rest because of their non-immunogenicity, amphiphilic nature, ease of functionalization, and stimuli-responsiveness. This review addresses the role of US in the synthesis of liposomes, its ability to induce localized and controlled drug release from liposomes, as well as the integration of US-induced release and US-imaging using liposomes as contrast agents utilizing thermal and/or mechanical effects.

KEYWORDS: *Ultrasound, Cavitation, Liposome, Targeted Delivery, Contrast Agent, Theranostic.*

CONTENTS

*Author to whom correspondence should be addressed.
Email: ghusseini@aus.edu
Received: 19 January 2023
Accepted: 17 February 2023

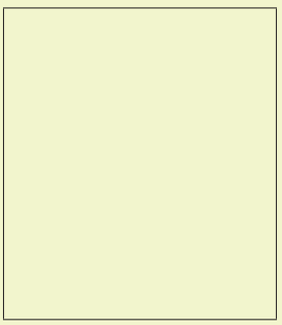
INTRODUCTION

Ultrasound (US) waves are high-frequency sound waves (higher than the audible range >20 kHz) [1]. The first medical application of US dates back to 1942 when Karl Dussik used US to locate brain tumors [2–4]. The applications of US in the medical field have been growing ever since. US waves can be generated using piezoelectric crystal, magnetostrictive crystals, or via a whistle generator (refer to Table I) [5–7].

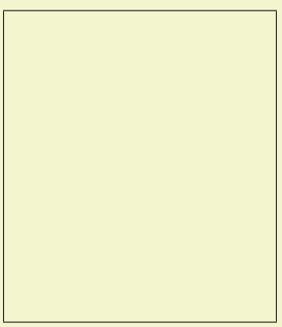
US interacts with biological matter in different ways, namely, reflection, refraction, scattering, and attenuation. Reflection is the change in the direction of a wave at an interface between two different media, while refraction is the deflection of a wave from its original direction as it passes between tissues with different acoustic properties. Scattering occurs when the width of the boundary is smaller than the wavelength of the US wave. Finally,

attenuation is when the US wave loses some of its energy as heat [1, 8, 9].

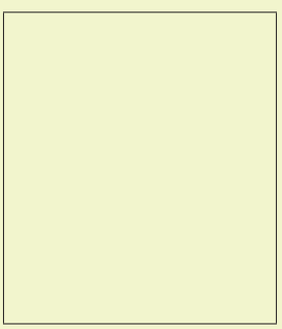
In the medical field, US has both diagnostic and therapeutic applications. Diagnostic applications usually employ low-intensity US to obtain information about the different tissues and organs in the body. In contrast, therapeutic applications use high-intensity US to manipulate matter and induce biological effects [1, 6, 7, 10, 11]. The therapeutic applications of US include lithotripsy, focused US surgery, and high-intensity focused US ablation of cysts and tumors. The biological effects associated with US can be divided into thermal and mechanical effects. Thermal effects involve an increase in the medium's temperature when irradiated with US waves. As mentioned earlier, when an US wave passes through a material, it loses some of its energy to the surroundings



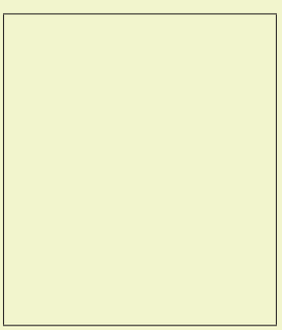
Zeyad Almajed



Nour M. Al Sawaftah

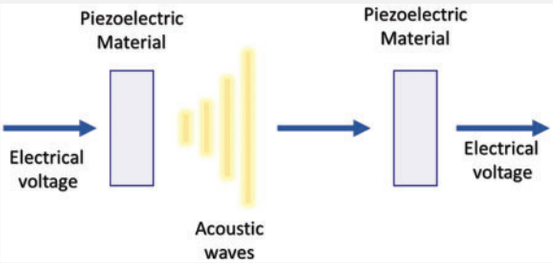
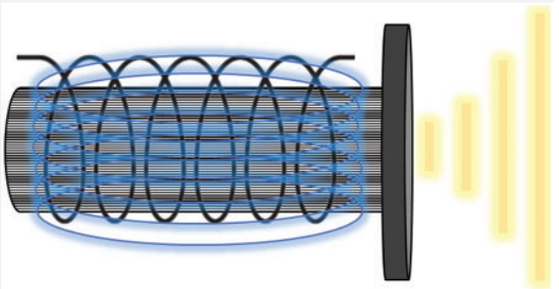
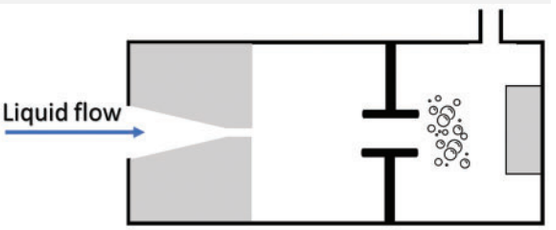


Hana Sulieman



Ghaleb A. Husseini

Table I. Types of US sources.

US source	Schematic
Piezoelectric crystal	
Magnetostrictive crystal	
Liquid whistle	

as heat, i.e., is attenuated. Attenuation is measured in relative units based on the intensity of the sound energy along the propagation path [1, 8, 9, 12]. The thermal effects of US depend on the intensity or frequency of the US wave, the absorption coefficient of the material, as well as the exposure time [13–16].

On the other hand, the mechanical effects of US manifest as acoustic cavitation. Acoustic cavitation is a process in which pre-existing bubbles or nuclei in a fluid grow, oscillate, and eventually collapse due to pressure changes induced by US irradiation. Based on the fate of the oscillating bubbles, acoustic cavitation can be classified into stable and transient (inertial) cavitation (refer to Fig. 1). In stable cavitation, the bubble oscillates about an equilibrium radius, and these oscillations emit pressure to the surrounding fluid, which generates flow around the bubble, termed microstreaming. In contrast, transient cavitation involves the rapid growth of the bubble to two- or three times its limiting size and then its violent collapse, producing shock waves, free radicals, and fluid jetting. The vicinity of these transient cavitation spots has been characterized by high temperatures (~5000 K) and high pressure (~1000 atm) [1, 15, 17, 18].

CANCER AND TARGETED DRUG DELIVERY

Cancer is a disease in which abnormal cells in the body do not undergo apoptosis (programmed cell death) and continue to grow uncontrollably and may spread to other parts of the body (metastasize). If the growing mass remains localized, the tumor is referred to as benign; however, if the cancer metastasizes, it is referred to as malignant. Another characteristic of cancer cells is that they have the ability to influence healthy cells in their vicinity and induce the formation of blood vessels, a phenomenon known as neo-angiogenesis, to support and supply the growing mass with oxygen and nutrients. This new vasculature, along with some cells, molecules, acidic pH levels, and blood vessels, comprise the tumor microenvironment. Currently, there are several treatment options for cancer ~~treatment~~, such as surgery, radiotherapy, chemotherapy, immunotherapy, and targeted therapy [19–23]. The side effects attributed to chemotherapy occur due to the systemic uptake of these toxic chemical drugs, causing adverse reactions in the body and systemic cytotoxicity.

One of the primary motivations behind developing nanocarriers for cancer therapy is the mitigation of

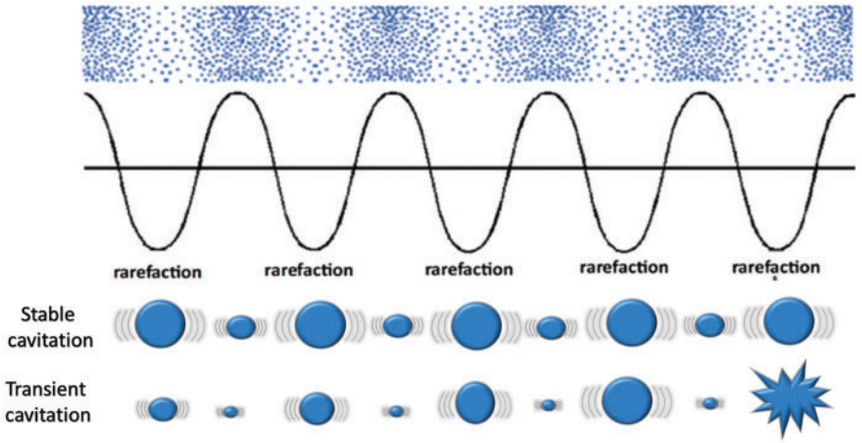


Figure 1. **Types of acoustic cavitation.**

chemotherapy side effects. Thus, a drug delivery system in which a chemotherapy dose is precisely delivered to the tumor sites instead of the whole system is highly desirable. In addition to the delivery to specific sites, a slow, controlled release of a highly cytotoxic drug is also desirable as it reduces system shock and alleviates side effects [24, 25]. Presently, several nanocarriers have been employed in drug delivery vessels for cancer therapy, including [26–29]:

1. Polymeric nanoparticles: are particles within the size range of 1 to 2500 nm and can be loaded with compounds entrapped within or surface-adsorbed onto the polymeric core.
2. Dendrimers: are nano-scale symmetrical molecules (often polymers) in which a small atom or group of atoms is surrounded by symmetric branches known as dendrons. Dendrimers have well-defined structures, are highly compatible with biological systems, and their three-dimensional structure can form a variety of active drug conjugates.
3. Hydrogels: are a group of 3-dimensional cross-linked networks of polymers. They can be built in many shapes, such as slabs, particles, and films. They have hydrophilic structures and are capable of holding large amounts of water.
4. Carbon nanotubes (CNTs): are carbon structures with desirable properties such as high surface-to-volume ratios, enhanced conductivity, and strength, biocompatibility, ease of functionalization, and optical responsiveness. They have been used as novel drug and gene delivery carriers. Many different cell types effectively take up CNTs.
5. Metal-organic frameworks (MOFs): are a class of hybrid porous materials constructed from metallic clusters connected by organic linkers. MOFs have excellent properties for drug delivery, such as flexible composition, well-defined pore size, tunable size, high agent loading, and, depending on the choice of materials, are highly biocompatibility.
6. Liposomes, which are the focus of this review, are spherically shaped microscopic vesicles that consist of one or more phospholipid bilayer membranes. They are widely used as drug delivery nanocarriers and have several formulations that have been FDA-approved. Table II summarizes the advantages and disadvantages of different organic and inorganic nanocarriers [30–37].

Liposomes

Liposomes are widely used nanocarriers in drug delivery [38, 39]. They are spherically shaped synthetic nanoparticles made from phospholipid bilayers, with diameter ranges between 20 and 1000 nm. Each lipid layer consists of ~~a~~ hydrophilic (polar) ~~head~~ and ~~a~~ hydrophobic (nonpolar) ~~tail~~. Liposomes are biocompatible since their lipid bilayers membranes are similar to cell membranes. Hydrophilic drugs can be contained within the core of the liposome, while the region within the bilayer can entrap hydrophobic drugs. Liposomes are classified according to their sizes which can range from 20 nm to 1,000 nm, as well as by the number of bilayers. Liposomes with a single bilayer are called unilamellar vesicles (ULVs), while ones with multiple layers nested inside each other are referred to as multilamellar vesicles (MLVs). Vesicle size affects the circulation half-life of liposomes and the concentration of drugs entrapped. Unilamellar liposomes with diameters between 50 and 150 nm are the most suitable for drug delivery applications because this size range allows the liposomes to penetrate deep into the tissues, and, in the case of cancer therapy, they can accumulate in the fenestrations between cancer cells [38–40].

Table III ~~Presents~~ a summary of some FDA and EMA-approved (up to 2017) liposomal drugs [41].

Liposomes can be surface functionalized to acquire stealth properties through PEGylation (covalent and/or non-covalent attachment of polyethylene glycol) and to promote receptor-mediated endocytosis (i.e., active targeting) via targeting ligands such as antibodies, peptides,

Table II. Advantages and disadvantages of different organic and inorganic nanocarriers.

Type of nanoparticle	Nanoparticle	Advantages	Disadvantages
Organic	Liposomes	-Biocompatible -Increased circulation time -Amphiphilic -Functional modification -Protect drug from environmental conditions -Low toxicity	-May trigger an immune response -Poor stability (depending on formulation)
	Polymeric micelles	-Biodegradable -Self-assembling -Biocompatible -Functional modification -Versatility in chemical composition -Increase solubility of lipophilic drugs -Protect drugs from environmental conditions	-Occasional cytotoxicity -Degradation of the carrier -Low drug-loading capacity -Stability issues
	Dendrimers	-Uniformity in size, shape, and branch length -Increased surface area -Increased loading -Multiple functional groups for targeted drug delivery	-Complex synthesis route -Not veiy suitable for hydrophilic drugs -High synthesis cost
	Solid lipid nanopaiticles	-Good solubility and bioavailability -Low toxicity	-Low drug loading capacity -Risk of gelation -Drug expulsion due to lipid polymorphism
	Nanoeimilsions	-Stable -Amphiphilic	-Toxicity
Inorganic	Gold nanopaiticles	-Increased surface area -Increased loading	-Potential toxicity
	Magnetic nanopaiticles	-Uniformity in size -Potential in imaging, theranostic systems	-Potential toxicity
	Metal organic frameworks (MOFs)	-Large porosity -Large surface area -Open metal sites for interactions -Easy to fiinctionalize	-Low thermal stability -Low chemical stability -Biocompatibility issues -Premature release of drug
	Carbon nanotubes	-Multiple functions -Chemical modification -Water-soluble -Biocompatible -Efficient loading	-Potential toxicity
	Quantum dots	-Fluorescent properties for imaging and drug tracking	-Potential toxicity

Table III. A list of FDA and EMA approved liposomal formulations.

Trade name	Year approved	Active ingredient	Indication
Visudyne	2000	Verteporfin	Photodynamic therapy for age-related muscular degeneration
AmBisome	1990	Amphotericin B	Fungal infections
Abelcet	1995	Amphotericin B	Fungal infections
DepoDur	2004	Morphine	Extended-release morphine
Octocog alfa	2009	Factor VIII	Hemophilia A
Definity	2001	Octofluoro-propane	Ultrasound contrast agent
Doxil/caelyx	1995	Doxorubicin	Antineoplastic
Myocet	2001	Doxorubicin	Metastatic breast cancer
DepoCyte	1999	Cytarabine	Lymphomatous meningitis
Daunoxome	1996	Daunirubicin	Antineoplastic
Mepact	2009	Mifamurtide	Osteosarcoma
Marqibo® (Onco TCS)	2012	Vincristine	Acute lymphoblastic leukemia

proteins, carbohydrates, and various other molecules [42]. Liposomes can also be designed to be sensitive to internal and/or external triggers, such as temperature, pH, redox levels, enzymatic levels, US, electric and magnetic fields. Recently, liposomal-based US-guided drug delivery has immerged as a promising approach to treating certain types of cancer because the technology is noninvasive, readily available, and permits the spatially confined delivery and tracking of drugs to targeted areas with a high degree of precision, thus minimizing the adverse effects on healthy tissues [43].

ULTRASOUND-GUIDED DRUG DELIVERY

Using Ultrasound to Form Liposomes

Several methods can be used to synthesize liposomes, including the thin-film hydration method, ethanol/ether injection methods, the emulsification method, detergent dialysis, etc. [44]. The majority of the aforementioned techniques rely on the self-assembly of phospholipids when exposed to aqueous media. The formed liposomes, in this case, tend to be multilamellar and with a wide size distribution. Post-processing by extrusion or sonication is often required to reduce the sizes and break MLVs into ~~(~~ULVs~~)~~ [44, 45]. The preparation of sonicated ~~SUV~~ typically involves sonicating MLVs using either sonication baths or probes [46, 47]. Bath sonicators are favored over probe sonicators because they do not come into contact with the sample, which in the case of probe sonicators may lead to metal particles being released into the sample and the need to be removed by centrifugation (refer to Fig. 2) [12, 46, 48–50]. One of the earliest studies on the use of US to size liposomes was conducted by Papahadjopoulos and Miller [51, 52], in which they showed that exposing phospholipid suspensions to low-frequency ultrasound (LFUS) led to the formation of ~~small unilamellar vesicles (~~SUVs~~)~~. These studies were followed by the work of Huang [53], who carefully studied these SUVs using molecular sieve chromatography on large pore aerosol gels [1]. Husseini et al. [54] investigated the effect of acoustic cavitation on liposomal size. Their findings showed that stable, not transient, cavitation causes a reduction in liposomal size. This was established when the authors saw a change in the diameter of liposomes even when collapse cavitation was inhibited by lowering ultrasound intensity or increasing hydrostatic pressure. Microfluidics is emerging as a promising technique to synthesize liposomes. The liquid flow in microfluidic channels (micron size range) can be controlled to establish laminar flow conditions suitable for liposome formation. Huang et al. [45] combined microfluidic technologies and US to produce liposomes. The microfluid channels were used to control the flow rates of phospholipids and solvent, while a sonicator bath was used for size reduction. The results showed that liposome size decreased as the buffer-to-solvent fraction increased. The size decreased even further in response to sonication (increasing the flow rate ratio from 8 to 12 with sonication decreased the liposome size from ~150 nm to ~50 nm).

Using Ultrasound to Trigger Release from Liposomes

Another advantage of liposomes as drug delivery systems is that they can be designed to release their payload in response to an internal (i.e., pH, redox, enzymatic level) or external trigger (temperature, US, electric field, magnetic field). This review focuses on US-induced release from liposomes [55]. US-responsive nanocarriers are designed to respond to the thermal effects, the mechanical effects of US, or a combination of both. Drug release through US-induced hyperthermia occurs when US is focused on a particular region, causing a rise in local temperature; this elevated temperature is usually higher than the transition temperatures of the phospholipids composing the liposomes disrupting the orderly packing of the lipid bilayer and releasing the drug [1, 15]. As mentioned earlier, the oscillation or bursting of cavitation bubbles causes microstreaming, shock waves, or micro-jets, which also disrupt the liposomal bilayer and induce drug release [1, 15, 43, 56].

In a study conducted by Kim et al. [57], US-sensitive liposomes encapsulating the chemotherapeutic agent Doxorubicin (Dox) were synthesized using ethanol injection and achieved a loading efficiency of $97.1 \pm 1.44\%$. Under continuous US irradiation, the Dox release reached 60%. *In vivo* studies were conducted using breast cancer (MDA-MB-231) xenografted mice, and the combination of US and liposomes suppressed tumor growth 56% more than unsonicated liposomes and 98% more than the control group (refer to Fig. 3). In another study, ~~Matos~~ et al. [58] encapsulated the cytotoxin mistletoe lectin-1 (ML1) in US-responsive liposomes and studied ML1 release in response to high-intensity focused US (HIFU). The release experiments results showed an 80% release of ML1 when the liposomes were sonicated at a frequency of 1.3 MHz. *In vitro* experiments showed that the cytotoxicity of the liposomal formulation was enhanced when combined with HIFU in murine colon carcinoma (CT26) cells (IC_{50} 400 ng/ml; free ML1 IC_{50} 345 ng/ml) was compared to non-triggered USL loaded with ML1. Olsman et al. [59] investigated the effect of FUS in combination with microbubbles on the delivery and therapeutic efficacy of MMP enzyme-sensitive-Dox-loaded liposomes *in vivo*. The highest tumor uptake was seen when mice were treated with FUS at a MI of 0.8 and ~~microbubbles~~; moreover, compared to the control group (treated with saline), the group treated with liposomes and FUS, showed a 58% reduction in tumor growth. Husseini et al. [60–71] have also done extensive work with regard to both low-intensity and high-intensity US-mediated liposome drug release.

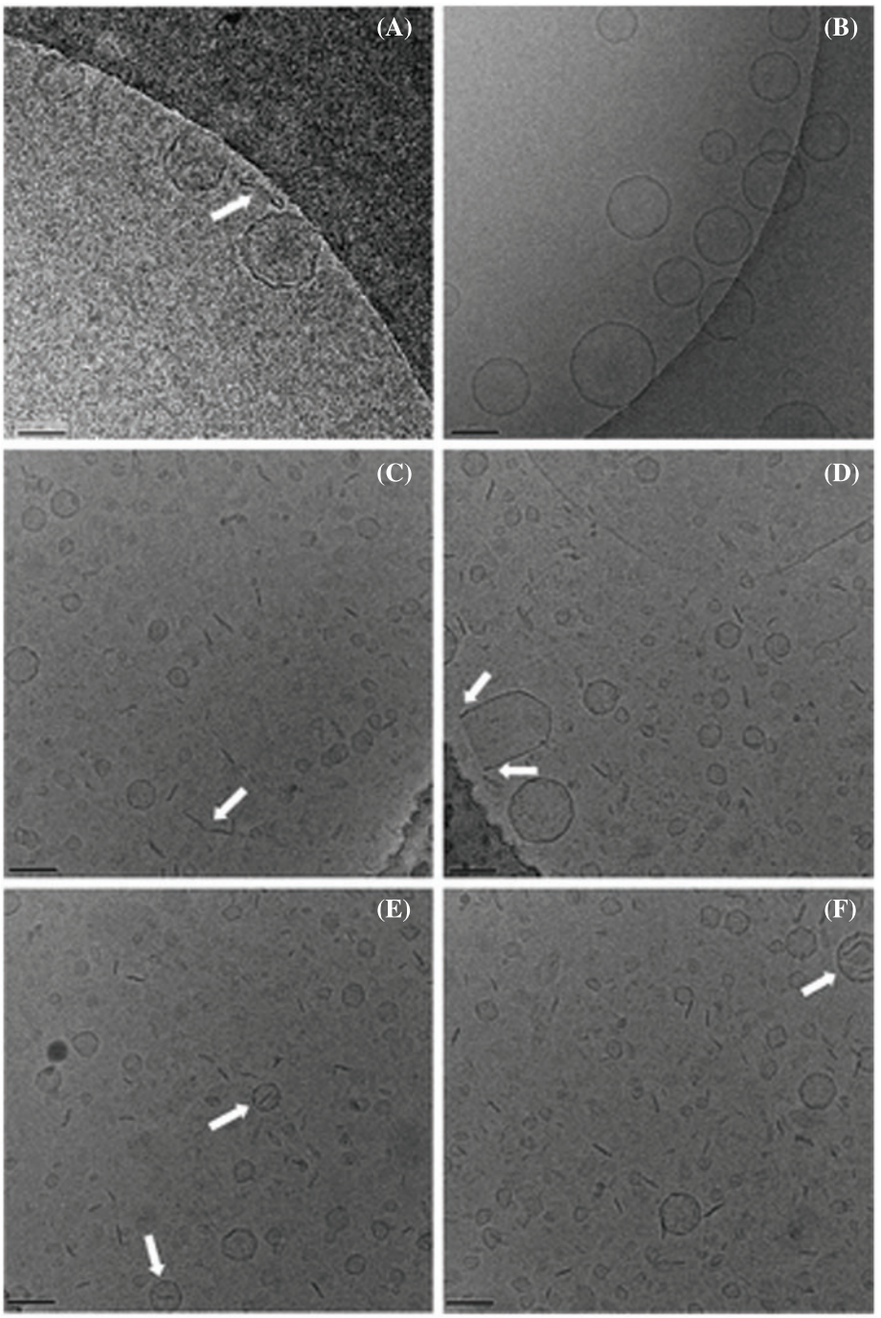


Figure 2. **Cryo-TEM images of (A) 0.1 mM DPPC MLV after 20 min AFU (B) 1 mM DPPC MLV passed through polycarbonate filters of 200 nm pore; (C)–(F) 20 mM DPPC/LysoPC/DSPE-PEG-2000 (87:9:4, molar ratio) MLV after 20 min AFU. Reprinted with permission from [46], Tejera-Garcia, R., Ranjan, S., Zamotin, V., Sood, R. and Kinnunen, P.K.J., 2011. Making unilamellar liposomes using focused ultrasound. *Langmuir*, *27*(16), pp.10088–10097. Copyright@American Chemical Society.**

Using Ultrasound-Triggered Liposomes to Enhance US Cancer Imaging

A pre-requisite for an effective cancer therapy nanocarrier is the ability to target and accumulate in specific body locations in order to increase drug concentration at the target site and reduce systemic toxicity. When devising treatment plans, being able to visualize the nanocarrier accumulation and therapeutic release at the target site would help physicians overcome issues related to penetration depth, the limitations of therapeutic strategies in tumors, as well as monitoring post-treatment changes at the tumor site [72, 73]. A wide range of imaging modalities has been studied for potential image-guided nanocarrier delivery applications, including optical

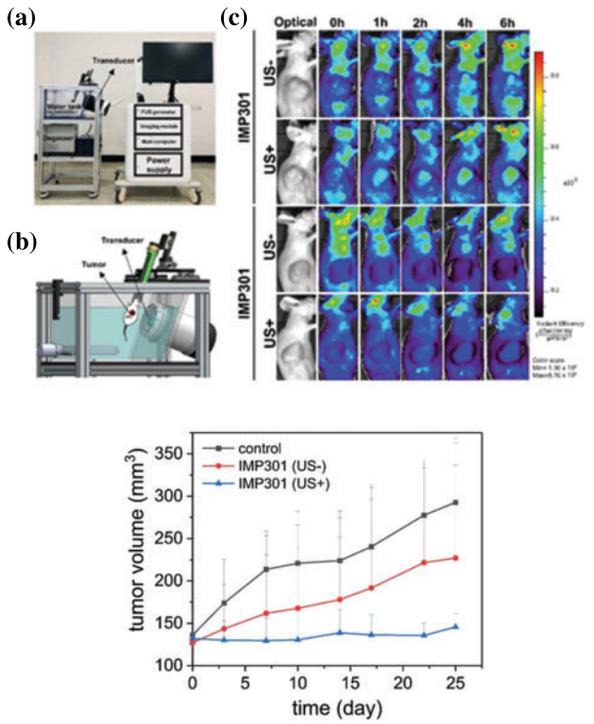


Figure 3. ***In vivo*** **Dox release from IMP301 and DOXIL under FUS irradiation (a) IMD-10R system set up. (b) Schematic illustration of exposing mice to FUS (c) The fluorescence intensity of Dox after intravenous injection for the DOXIL, IMP301 without FUS, and with FUS treatment. (d) Tumor growth in MDA-MB-231 tumor-bearing mice treated with saline (control; black line), IMP301 (IMP301 US-; red line), and IMP301 with FUS irradiation (IMP301 US+; blue line). Reprinted with permission from [57], Kim, Y.S., Ko, M.J., Moon, H., Sim, W., Cho, A.S., Gil, G. and Kim, H.R., 2022. Ultrasound-responsive liposomes for targeted drug delivery combined with focused ultrasound.** ***Pharmaceutics****,* ***14*****(7). Copyright@MDPI.**

imaging, X-rays, computed tomography, magnetic resonance, and US. US cancer imaging is widely used in clinical settings due to its non-invasiveness, low cost, high tissue-penetrating ability, and ease of controllability [74]. A key component to successful US imaging is the contrast agent; microbubbles (MBs) were the first US-imaging contrast agents discovered and have been extensively used ever since, especially in echocardiography. The first-generation MBs consisted of free air-gas ~~microbubbles (~~MBs~~)~~ produced by hand agitation of saline; however, these contrast agents suffered from short lifetimes because of the solubility of air in water. This led to the development of second-generation contrast agents MBs of perfluorocarbons, nitrogen gas, or sulfur hexafluoride stabilized by phospholipid, or polymer vesicles ranging in size from 1 to 8 μm (e.g., Definity, SonoVue/Lumason®, Sonazoid®). Upon US application, these gas-filled vesicles oscillate/cavitate (compression under positive pressure and expansion in the negative pressure phase of the US wave) reflecting the incident US waves which are then captured by the US-transducer and converted to an image. The compressibility of these MBs is higher than that of biological tissues, meaning that they are better at reflecting US waves which enhances the contrast of the region of interest (i.e., echo reflection or echo enhancement) [73, 75, 76]. However, the main limitation to the use of MBs is their relatively large size (10 μm) which restricts their efficient penetration into the solid tumor microenvironment (endothelial gaps size range between 380 and 780 nm). In addition, MBs have limited drug loading capacity, and short circulation time, and may cause irreversible damage to off-target tissues [75–77]. Chandan and Banerjee [78] were able to synthesize submicron-sized (756 ± 180.0 nm) US-responsive, phosphatidylserine (PS)-based paclitaxel-liposomes-nanobubble conjugates (PSPLBC). To exert a PSPLBCs exhibited anticancer effects and enabled US-contrast enhancement. *In vitro* experiments showed a 10-fold increase in cellular internalization compared to a control sample, as well as significant tumor growth inhibition *in vivo* ($98.3 \pm 0.8\%$ tumor growth inhibition). The *in vitro* contrast enhancement potential of PSPLBCs was evaluated using a clinical 5–7 MHz phased array convex US-probe. The US-images showed similar bright contrast for both the free nanobubbles and PSPLBCs. Furthermore, an extended gradual decrease in contrast intensity duration was observed; this meant that the PSPLBCs achieved a longer contrast duration which increased the time available for investigation (refer to Fig. 4). The following year, Prabhakar and Banerjee [73] were able to synthesize even smaller-sized (528.7 ± 31.7 nm) nanobubble–paclitaxel liposome complexes for US imaging and US-responsive drug delivery in cancer cells. The *in vitro* cellular uptake was increased by 2.5-fold after sonication compared to the liposomes alone. Moreover, the nanobubbles-liposomes complexes showed better echogenic stability than SonoVue® MBs.

Recently, echogenic liposomes have been investigated as contrast agents for US imaging. Echogenic liposomes are submicron-sized liposomal particles encapsulating a gas or a gas-generating molecule in their central core. The gases used typically include air, nitrogen, perfluorocarbons (PFCs), or sulfur hexafluoride. Kim and Lee [79] loaded liposomes with Melanin, perfluorohexane (PFH), and 5-fluorouracil (5-FU) (melanin@PFH@5-FU-liposomes). The synthesized liposomes could generate bubbles upon near-infrared (NIR) irradiation, which significantly improved drug release and US imaging. Lin et al. [72] developed 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (AIPH)-loaded liposomes (Lip-AIPH) that can generate gas bubbles and reactive oxygen species (ROS), simultaneously upon exposure to US. The enhanced US imaging contrast of the LipAIPHs was assessed by confocal microscopy. Following US irradiation, bright gas bubbles were observed, which was attributed to the formation of gas bubbles. When compared to sonicated PBS, and control liposomes, LipAIPH,

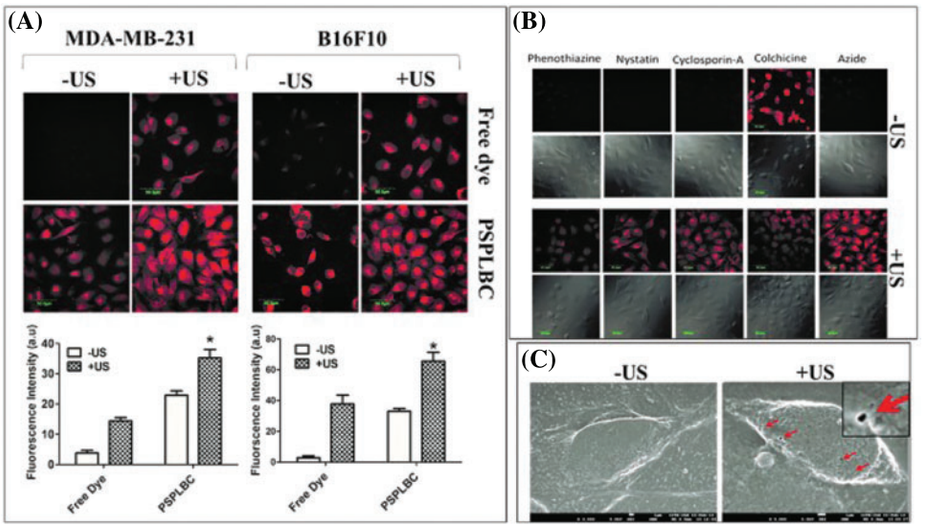


Figure 4. ***In vitro*** **cellular internalization studies. (A) Confocal laser scanning microscopy (CLSM) images of MDA-MB-231 and B16F10 cells for the free rhodamine-6G dye, free dye + US, rhodamine-6G loaded PSPLBC, and rhodamine-6G loaded PSPLBC + US treatment groups. (B) CLSM images show the mechanism of PSPLBC uptake by pretreated cells, both with and without application of US. (C) Cryo-FEG-SEM images of the cell without and immediately after PSPLBC + US treatment. Reprinted with permission from [78], Chandan, R. and Banerjee, R., 2018. Pro-apoptotic liposomes-nanobubble conjugate synergistic with paclitaxel: A platform for ultrasound responsive image-guided drug delivery.** ***Scientific Reports****,* ***8****(1). Copyright@Springer Nature.**

exhibited a 4.2-fold increase in echo intensity. Moreover, *in vivo* experiments showed that the survival rate of breast cancer (MCF-7) tumor-bearing mice treated with Lip-AIPH and US was prolonged over the monitoring duration of 40 days. Fernandes and Kolios [80] synthesized perfluorohexane-BODIPY-labeled nanoemulsions (PFH-NEs) for theranostic applications. The synthesized PFH-Nes were incubated with breast cancer (MCF-7) cells and US signals were measured after 4, 24, and 48 hours of incubation. US signals from the cells treated with PFH-NEs were two times greater compared to cells without any PFH bubbles; moreover, the signals were relatively constant with time (average signals of $11.33 \times 10^3 \pm 0.53$, $14.16 \times 10^3 \pm 0.63$, and $12.05 \times 10^3 \pm 1.60$ after 4, 24 and 48 hours of incubation). Park et al. [81] investigated echogenic liposomes as a nanocarrier for siRNA. The results showed that around 10% of siRNA used in the experiment was successfully protected by echogenic liposomes. In addition, the release of siRNA from the liposomes was successfully triggered using 1 W/cm^2 US sonication at a frequency of 1 MHz. Moreover, US images were obtained in order to verify the echogenic response and stability of the synthesized echogenic liposome compared to those generated with commercial ~~microbubbles~~ (Definity® and SonoVue®). The images were collected during a 10-min period to evaluate the lifetimes of individual ~~microbubbles~~ in a degassed water condition. According to Figure 5, the synthesized echogenic liposome had the lowest brightness in the US image; however, all three ~~microbubbles~~ showed decreasing US signals. This observation was attributed to the bubble density difference, as Definity® has approximately 60 times more ~~microbubbles~~ than SonoVue® according to manufacturer descriptions. Hence, higher concentrations of echogenic liposomes would increase the initial signal level.

CHALLENGES AND FUTURE PROSPECTS

Combining US with nanomedicine provides a powerful theranostic tool that could be beneficial in the fight against cancer. Despite the promising results of US-mediated liposomal release in cancer therapy, there is still a need for further research into the optimization of US parameters

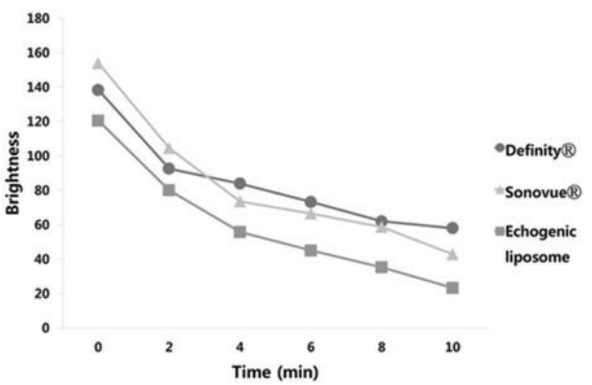


Figure 5. Comparison of contrast US signal with respect to time. Reprinted with permission from [81], Park, D.H., Jung, H.C., Park, J., Bae, S., Shin, U.C., Kim, S.W., Kim, C.W., Lee, Y.H. and Seo, J., 2022. Synthesis of echogenic liposomes for sonoporation. ***Micro & Nano Letters****,* ***17****(11), pp.276–285. Copyright@Elsevier.**

to help this technology transition into clinical settings [12, 82]. Existing imaging techniques, such as US imaging, photoacoustic imaging, and fluorescent imaging, have their own limitations; for instance, many cancers cannot be detected via US imaging; additionally, calcifications that are visible on mammograms cannot be detected by ultrasound scans, preventing the early diagnosis of the breast cancers that begin with calcifications. One way to address these limitations could be by using multimodal imaging, where several complementary imaging techniques are combined to acquire images at different times (asynchronous), then fuse them together or simultaneously acquire images (synchronous) and merge them automatically [83]. Multimodal imaging agents, which permit the combination of two or more imaging modalities by using a single agent, can provide multimodal contrast imaging concurrently with complementary temporal, spatial, and depth resolution for a more accurate and reliable diagnosis [84, 85]. However, the pharmacological profiles, biodistribution, degradation behaviors, and metabolism need further study to enable their translation into clinical applications [75].

Liposomes can be designed in such a way as to render them more echogenic. Liposomes release therapeutic agents at a slower rate than micelles when US is used as a stimulus. Our research group has extensively studied acoustic agents release kinetics from micelles [86–98], liposomes [99–101], and metal-organic frameworks [102–105]. While liposomes are more efficient drug delivery vehicles at releasing the therapeutic content [99] compared to MOFs [103], they are not as echogenic as micelles [86, 87, 91–93]. Micelles are capable of releasing 10% of their content within 2 seconds of applying US) [94–97]; on the other hand, liposomes release between 10–30% of their content within 20 seconds of ultrasonication (and less than 5% within 2 seconds of application) [62, 64–69, 106]. In triggered drug delivery, the shorter the applied time, the lower the side effects and hence the higher the chance of translating the technology into clinics. MOFs, on the other hand, release their contents slowly (it could take up to 30 minutes for US to release 10% of their contents) [102–104]. A new generation of liposomes encapsulates perfluorocarbon nanoemulsions that can easily vaporize in 1–2 seconds, destroying the liposomal structure in the process and releasing the encapsulated contents [61, 107]. These liposomes can release up to 50% of the agents within a few seconds of US at higher power densities.

The mechanism of release differs between the different nanocarriers employed in our lab. In the case of liposomes, cavitation (both stable and transient) is the main culprit [103, 104]. Cavitation is thought to cause the release either by the complete or partial destruction of the liposomal structure [48, 63–69]. The mechanism of micellar destruction follows a similar trend [54, 94]. ~~Microbubbles~~ present in the vicinity of the micelles cavitate, and as they oscillate back and forth (via stable cavitation) and eventually implode (via collapse or transient cavitation), they produce microjets and microstreams that pierce a "hole" (or "holes") in the micellar structure and allow the contents to escape [86, 87, 91–93]. Collapse cavitation is more efficient in causing the release from micelles, as shockwaves, after the MBs implode, can shear the micellar structure and destroy it entirely [54]. Emulsion liposomes (eLiposomes) are destroyed by acoustic droplet vaporization, which occurs during the negative pressure portion of the acoustic wave. The lower pressure allows the liquid emulsion to overcome the Laplace pressure and vaporize [61, 107]. The evaporation of these low-boiling point liquid emulsions is accompanied by a 100-fold increase in volume, enough to destroy the whole nanocarrier quickly, leading to the spilling of the encapsulated agent. Our research on MOFs shows a combination of factors that enhance the acoustic release, including improving diffusion out of the MOF pores and loosening the physisorption of the agent encapsulated inside the frameworks [102–105]. Cavitation is also thought to play a role in the release from MOFs.

Another advantage of micelles is that they reencapsulate their contents once the acoustic field is turned off or once the micelles leave the sonicated region [93, 95, 97–99]. In contrast, once liposomes are destroyed, they do not reform; hence, their contents may interact with healthy cells [69]. As a future direction recommendation, we believe that more research should be directed toward synthesizing liposomes that are more responsive to acoustic power (i.e., sonosensitive) and are capable of reencapsulating their contents outside the sonicated region.

As with other triggering mechanisms in drug delivery, we recommend developing an instrument by which the operator can control the release of therapeutic agents from nanoparticles in time and space. This can be done via a feedback controller that automatically increases or decreases US power density when the concentration of the released agent is above or below the therapeutic window. This way, we can control and mitigate the side effects of these cytotoxic agents, and guarantee a high enough concentration at the tumor site that will hinder multidrug resistance development [100]. Finally, artificial intelligence techniques can be used to optimize the parameters involved in acoustically-triggered drug delivery platforms [88, 96].

CONCLUSION

Reducing the detrimental systemic side effects of chemotherapy is an area that is being heavily investigated, particularly the use of stimuli-responsive nanocarriers to enhance anticancer drug delivery, release, and accumulation at tumor sites. A wide range of nanocarriers and triggering mechanisms have been proposed to address this issue. This review focuses on US-induced

drug release from liposomes. The potential of MB-, nanobubble-conjugated, and gas-filled (echogenic) liposomes as US-contrast agents has also been discussed. This US-based theranostic approach has shown promising results, both *in vitro* and *in vivo*, and with continued research, may become a successful alternative to conventional chemotherapy.

Conflicts of Interest

There are no conflicts to declare.

Acknowledgments: The authors would like to acknowledge the financial support of the American University of Sharjah Faculty Research Grants [FRG20-L-E48, FRG22-C-E08], the Al-Jalila Foundation [AJF 2015555], the Al Qasimi Foundation, the Patient's Friends Committee-Sharjah, the Biosciences and Bioengineering Research Institute [BBRI18-CEN-11], GCC Co-Fund Program [IRF17-003], the Takamul Program [P OC-00028-18], the Technology Innovation Pioneer (TIP), Healthcare Awards, Sheikh Hamdan Award for Medical Sciences [MRG-57-2019–2020], Friends of Cancer Patients (FoCP) and the Dana Gas Endowed Chair for Chemical Engineering. We also would like to acknowledge student funding from the Material Science and Engineering Ph.D. program at AUS.

REFERENCES

1. Schroeder, A., Kost, J. and Barenholz, Y., **2009**. Ultrasound, liposomes, and drug delivery: Principles for using ultrasound to control the release of drugs from liposomes. *Chemistry and Physics of Lipids*, *162*(1–2), pp.1–16.
2. Kane, D., Grassi, W., Sturrock, R. and Balint, P.V., **2004**. A brief history of musculoskeletal ultrasound: "From bats and ships to babies and hips". *Rheumatology*, *43*(7), pp.931–933.
3. Muir, T.G. and Bradley, D.L., **2016**. Underwater acoustics: A brief historical overview through world war II by Thomas G. Muir and David L. Bradley. Acoustics today. *Acoustics Today*, *12*(3),
4. Newman, P.G. and Rozycki, G.S., **1998**. The history of ultrasound. *The Surgical Clinics of North America*, *78*(2), pp.179–195.
5. Clark, J.W., Neuman, M.R., Olson, W.H., Peura, R.A., Primiano, F.P., Seidband, M.P., Webster, J.G. and Wheeler, L.A., **2010**. *Medical Instrumentation Application and Design*, 4th ed., Hoboken, NJ, USA, John Wiley & Sons, Inc.
6. Sahu, P.K., **2018**. Ultrasonics-how ultrasonic waves are generated. pp.5–6,
7. Ultrasound. https://www.nibib.nih.gov/science-education/science-topics/ultrasound.
8. Powles, A.E., Martin, D.J., Wells, I.T. and Goodwin, C.R., **2018**. Physics of ultrasound. *Anaesthesia & Intensive Care Medicine*, *19*(4), pp.202–205.
9. Coltrera, M.D., **2010**. Ultrasound physics in a nutshell. *Otolaryngologic Clinics of North America*, *43*(6), pp.1149–1159.
10. Kondo, T., **2015**. Application of ultrasound in medicine and biotechnology. In: *Sonochemistry and the Acoustic Bubble*, Elsevier, pp.207–230,
11. Ranganayakulu, S.V., Rao, N.R. and Gahane, L., **2016**. Ultrasound applications in medical sciences. *International Journal of Modern Trends in Engineering and Research*, *3*(2), pp.287–293.
12. AlSawaftah, N., Pitt, W.G. and Husseini, G.A., **2021**. Dual-targeting and stimuli-triggered liposomal drug delivery in cancer treatment. *ACS Pharmacology & Translational Science*, *4*(3), pp.1028–1049.
13. Martins, A.M., Ahmed, S.E., Vitor, R.F. and Husseini, G.A., *Ultrasonic Drug Delivery Using Micelles and Liposomes*. http://link.springer.com/10.1007/978-981-287-470-2_29-2,
14. Smith, N.B. and Webb, A., 2010. *Introduction to Medical Imaging*. Cambridge, Cambridge University Press.
15. Frenkel, V., **2008**. Ultrasound mediated delivery of drugs and genes to solid tumors. *Advanced Drug Delivery Reviews*, *60*(10), pp.1193–1208.
16. Azagury, A., Khoury, L., Enden, G. and Kost, J., **2014**. Ultrasound mediated transdermal drug delivery: Mechanisms and applications. *Advanced Drug Delivery Reviews*, *72*, pp.127–143.
17. Baykal-Caglar, E., Hassan-Zadeh, E., Saremi, B. and Huang, J., **2012**. Preparation of giant unilamellar vesicles from damp lipid film for better lipid compositional uniformity. *Biochimica et Biophysica Acta-Biomembranes*, *1818*(11), pp.2598–2604.
18. Bader, K.B. and Holland, C.K., **2013**. Gauging the likelihood of stable cavitation from ultrasound contrast agents. *Physics in Medicine and Biology*, *58*(1), p.127.
19. Golombek, S.K., May, J.N., Theek, B., Appold, L., Drude, N., Kiessling, F. and Lammers, T., **2018**. Tumor targeting via EPR: Strategies to enhance patient responses. *Advanced Drug Delivery Reviews*, *130*, pp.17–38.
20. Scabini, M., Stellari, F., Cappella, P., Rizzitano, S., Texido, G. and Pesenti, E., **2011**. In vivo imaging of early stage apoptosis by measuring real-time caspase-3/7 activation. *Apoptosis*, *16*(2), pp.198–207.
21. Kondo, M., Asai, T., Katanasaka, Y., Sadzuka, Y., Tsukada, H., Ogino, K., Taki, T., Baba, K. and Oku, N., **2004**. Anti-neovascular therapy by liposomal drug targeted to membrane type-1 matrix metalloproteinase. *International Journal of Cancer*, *108*(2), pp.301–306.
22. Steinberg, I., Huland, D.M., Vermesh, O., Frostig, H.E., Tummers, W.S. and Gambhir, S.S., **2019**. Photoacoustic clinical imaging. *Photoacoustics*, *14*, pp.77–98.
23. Lai, C.-C., Chen, S.-Y., Tu, Y.-K., Ding, Y.-W. and Lin, J.-J., **2021**. Effectiveness of low level laser therapy versus cryotherapy in cancer patients with oral mucositis: Systematic review and network meta-analysis. *Critical Reviews in Oncology/Hematology*, *160*, p.103276.
24. Cancer Research UK, Cancer. https://www.cancerresearchuk.org/about-cancer/cancer-in-general/treatment/chemotherapy/when-you-might-have-chemotherapy.
25. Morales-Cruz, M., Delgado, Y., Castillo, B., Figueroa, C.M., Molina, A.M., Torres, A., Milián, M. and Griebenow, K., Smart targeting to improve cancer therapeutics. http://www.ncbi.nlm.nih.gov/pubmed/31802849,
26. Zielińska, A., Carreiró, F., Oliveira, A.M., Neves, A., Pires, B., Venkatesh, D.N., Durazzo, A., Lucarini, M., Eder, P. and Silva, A.M., **2020**. Polymeric nanoparticles: Production, characterization, toxicology and ecotoxicology. *Molecules*, *25*(16), p.3731.
27. Abbasi, E., Aval, S.F., Akbarzadeh, A., Milani, M., Nasrabadi, H.T., Joo, S.W., Hanifehpour, Y., Nejati-Koshki, K. and Pashaei-Asl, R., **2014**. Dendrimers: Synthesis, applications, and properties. *Nanoscale Research Letters*, *9*(1), p.247.
28. Zhang, W., Zhang, Z. and Zhang, Y., **2011**. The application of carbon nanotubes in target drug delivery systems for cancer therapies. *Nanoscale Research Letters*, *6*(1), p.555.
29. Designing hydrogels for controlled drug delivery. DOI: 10.3390/books978-3-03928-357-6,
30. Lamberti, M., Zappavigna, S., Sannolo, N., Porto, S. and Caraglia, M., Advantages and risks of nanotechnologies in cancer patients and occupationally exposed workers,

31. Lee, J.J., Yazan, L.S. and Abdullah, C.A.C., **2017**. A review on current nanomaterials and their drug conjugate for targeted breast cancer treatment. *International Journal of Nanomedicine*, *12*, pp.2373–2384.

32. Kahraman, E., Güngör, S. and Özsoy, Y., Potential enhancement and targeting strategies of polymeric and lipid-based nanocarriers in dermal drug delivery.

33. Saleem, K., Khursheed, Z., Hano, C., Anjum, I. and Anjum, S., **2019**. Applications of nanomaterials in leishmaniasis: A focus on recent advances and challenges. *Nanomaterials (Basel, Switzerland)*, *9*(12), p.1749.

34. Dhakshinamoorthy, A., Alvaro, M., Corma, A. and Garcia, H., **2011**. Delineating similarities and dissimilarities in the use of metal organic frameworks and zeolites as heterogeneous catalysts for organic reactions. *Dalton Transactions*, *40*(24), pp.6344–6360.

35. **2019**. *Nanomaterials for Drug Delivery and Therapy*. Amsterdam.

36. Mitchell, M.J., Billingsley, M.M., Haley, R.M., Wechsler, M.E., Peppas, N.A. and Langer, R., **2020**. Engineering precision nanoparticles for drug delivery. *Nature Reviews Drug Discovery*, *20*(2), pp.101–124.

37. Ghezzi, M., Pescina, S., Padula, C., Santi, P., Del Favero, E., Cantù, L. and Nicoli, S., **2021**. Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. *Journal of Controlled Release*, *332*, pp.312–336.

38. Sharma, V.K. and Agrawal, M.K., **2021**. A historical perspective of liposomes-a bio nanomaterial. *Materials Today: Proceedings*, *45*, pp.2963–2966.

39. Akbarzadeh, A., Rezaei-Sadabady, R., Davaran, S., Joo, S.W., Zarghami, N., Hanifehpour, Y., Samiei, M., Kouhi, M. and Nejati-Koshki, K., **2013**. Liposome: Classification, preparation, and applications. *Nanoscale Research Letters*, *8*(1), p.102.

40. Shaheen, S.M., Shakil Ahmed, F.R., Hossen, M.N., Ahmed, M., Amran, M.S. and Ul-Islam, M.A., **2006**. Liposome as a carrier for advanced drug delivery. *Pakistan Journal of Biological Sciences*, *9*(6), pp.1181–1191.

41. Bajwa, S.Z., Munawar, A. and Khan, W.S., **2017**. Nanotechnology in medicine: Innovation to market. *Pharmaceutical Bioprocessing*, *5*(2), pp.11–15.

42. Immordino, M.L., Dosio, F. and Cattel, L., **2006**. Stealth liposomes: Review of the basic science, rationale, and clinical applications, existing and potential. *International Journal of Nanomedicine*, *1*(3), pp.297–315.

43. Mullick Chowdhury, S., Lee, T. and Willmann, J.K., **2017**. Ultrasound-guided drug delivery in cancer. *Ultrasonography*, *36*(3), pp.171–184.

44. Šturm, L. and Ulrih, N.P., **2021**. Basic methods for preparation of liposomes and studying their interactions with different compounds, with the emphasis on polyphenols. *International Journal of Molecular Sciences*, *22*(12).

45. Huang, X., Caddell, R., Yu, B., Xu, S., Theobald, B., Lee, L.J. and Lee, R.J., **2010**. Ultrasound-enhanced microfluidic synthesis of liposomes. *Anticancer Research*, *30*(2), p.463.

46. Tejera-Garcia, R., Ranjan, S., Zamotin, V., Sood, R. and Kinnunen, P.K.J., **2011**. Making unilamellar liposomes using focused ultrasound. *Langmuir*, *27*(16), pp.10088–10097.

47. Basu, S.C. and Basu, M., **2002**. Liposome methods and protocols. *Liposome Methods and Protocols*.

48. Awad, N.S., Paul, V., Alsawaftah, N.M., Ter Haar, G., Allen, T.M., Pitt, W.G. and Husseini, G.A., Ultrasound-responsive nanocarriers in cancer treatment: A review. DOI: 10.1021/acsptsci.0c00212.

49. Vemuri, S. and Rhodes, C.T., **1995**. Preparation and characterization of liposomes as therapeutic delivery systems: A review. *Pharmaceutica Acta Helvetiae*, *70*(2), pp.95–111.

50. Mendez, R. and Banerjee, S., **2017**. Sonication-based basic protocol for liposome synthesis. *Methods in Molecular Biology (Clifton, N.J.)*, *1609*, pp.255–260.

51. Papahadjopoulos, D. and Miller, N., **1967**. Phospholipid model membranes. I. Structural characteristics of hydrated liquid crystals. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *135*(4), pp.624–638.

52. Papahadjopoulos, D. and Watkins, J.C., **1967**. Phospholipid model membranes. II. Permeability properties of hydrated liquid crystals. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *135*(4), pp.639–652.

53. Huang, C.-H., **1969**. Phosphatidylcholine vesicles. Formation and physical characteristics. *Biochemistry*, *8*(1), pp.344–352.

54. Husseini, G.A., Diaz de la Rosa, M.A., Richardson, E.S., Christensen, D.A. and Pitt, W., **2005**. The role of cavitation in acoustically activated drug delivery. *Journal of Controlled Release*, *107*(2), pp.253–261.

55. Schroeder, A., Avnir, Y., Weisman, S., Najajreh, Y., Gabizon, A., Talmon, Y., Kost, J. and Barenholz, Y., **2007**. Controlling liposomal drug release with low frequency ultrasound: Mechanism and feasibility. *Langmuir*, *23*(7), pp.4019–4025.

56. Karimi, M., Ghasemi, A., Zangabad, P.S., Rahighi, R., Masoud, S., Basri, M., Mirshekari, H., Amiri, M., Pishabad, Z.S., Aslani, A., Ghosh, D., Beyzavi, A., Vaseghi, A., Aref, A.R., Haghani, L., Bahrami, S., Hamblin, M.R., Village, O., Cancer, D. and Hospital, M.G., **2017**. Smart micro/nanoparticles in stimulus-responsive drug/gene delivery systems. *HHS Public Access*, *45*(5), pp.1457–1501.

57. Kim, Y.S., Ko, M.J., Moon, H., Sim, W., Cho, A.S., Gil, G. and Kim, H.R., **2022**. Ultrasound-responsive liposomes for targeted drug delivery combined with focused ultrasound. *Pharmaceutics*, *14*(7).

58. de Matos, M.B.C., Deckers, R., van Elburg, B., Lajoinie, G., de Miranda, B.S., Versluis, M., Schiffelers, R. and Kok, R.J., **2019**. Ultrasound-sensitive liposomes for triggered macromolecular drug delivery: Formulation and in vitro characterization. *Frontiers in Pharmacology*, *10*, p.1463.

59. Olsman, M., Sereti, V., Andreassen, K., Snipstad, S., van Wamel, A., Eliasen, R., Berg, S., Urquhart, A.J., Andresen, T.L. and de Lange Davies, C., **2020**. Ultrasound-mediated delivery enhances therapeutic efficacy of MMP sensitive liposomes. *Journal of Controlled Release*, *325*, pp.121–134.

60. Pitt, W.G., Husseini, G.A., Roeder, B.L., Dickinson, D.J., Warden, D.R., Hartley, J.M. and Jones, P.W., **2011**. Preliminary results of combining low frequency low intensity ultrasound and liposomal drug delivery to treat tumors in rats. *Journal of Nanoscience and Nanotechnology*, *11*(3), pp.1866–1870.

61. Husseini, G.A., Pitt, W.G., Williams, J.B. and Javadi, M., **2015**. Investigating the release mechanism of calcein from eLiposomes at higher temperatures. *Journal of Colloid Science and Biotechnology*, *3*(3), pp.239–244.

62. AlSawaftah, N.M., Husseini, G. and Pitt, W.G., **2022**. The kinetics of calcein release from mixed targeted liposomes using ultrasound. *Journal of Biomedical Nanotechnology*, *18*, pp.1–12.

63. AlSawaftah, N.M., Paul, V., Awad, N.S. and Husseini, G., **2022**. Effect of high-frequency ultrasound on targeted liposomes. *Journal of Biomedical Nanotechnology*, *8*, pp.1–12.

64. AlSawaftah, N.M., Awad, N.S., Paul, V., Kawak, P.S., Al-Sayah, M.H. and Husseini, G.A., **2021**. Transferrin-modified liposomes triggered with ultrasound to treat HeLa cells. *Scientific Reports*, *11*(1), p.11589.

65. Awad, N.S., Paul, V., Al-Sayah, M.H. and Husseini, G.A., **2019**. Ultrasonically controlled albumin-conjugated liposomes for breast cancer therapy. *Artificial Cells, Nanomedicine, and Biotechnology*, *47*(1), pp.705–714.

66. Salkho, N.M., Paul, V., Kawak, P., Vitor, R.F., Martins, A.M., Al Sayah, M. and Husseini, G.A., **2018**. Ultrasonically controlled estrone-modified liposomes for estrogen-positive breast cancer therapy. *Artificial Cells, Nanomedicine, and Biotechnology*, *46*(sup2), pp.462–472.

67. Elamir, A., Ajith, S., Sawaftah, N.A., Abuwatfa, W., Mukhopadhyay, D., Paul, V., Al-Sayah, M.H., Awad, N. and Husseini, G.A., **2021**. Ultrasound-triggered herceptin liposomes for breast cancer therapy. *Scientific Reports*, *11*(1), p.7545.

68. AlSawaftah, N.M., Paul, V., Kosaji, D., Khabbaz, L., Awad, N.S. and Husseini, G.A., **2022**. Ultrasound-sensitive cRGD-modified liposomes as a novel drug delivery system. *Artificial Cells, Nanomedicine, and Biotechnology*, *50*(1), pp.111–120.

69. Awad, N.S., Paul, V., Mahmoud, M.S., Al Sawaftah, N.M., Kawak, P.S., Al Sayah, M.H. and Husseini, G.A., **2020**. Effect of pegylation and targeting moieties on the ultrasound-mediated drug release from liposomes. *ACS Biomaterials Science and Engineering*, *6*(1), pp.48–57.

70. Awad, N.S., Haider, M., Paul, V., AlSawaftah, N.M., Jagal, J., Pasricha, R. and Husseini, G.A., **2021**. Ultrasound-triggered liposomes encapsulating quantum dots as safe fluorescent markers for colorectal cancer. *Pharmaceutics*, *13*(12), p.2073.

71. Ben-Daya, M., Paul, V., Awad, N.S., AlSawaftah, N.M., Al-Sayah, M. and Husseini, G., **2021**. Targeting breast cancer using hyaluronic acid-conjugated liposomes triggered with ultrasound. *Journal of Biomedical Nanotechnology*, *1*(17), pp.90–99.

72. Lin, X., Qiu, Y., Song, L., Chen, S., Chen, X., Huang, G., Song, J., Chen, X. and Yang, H., **2019**. Ultrasound activation of liposomes for enhanced ultrasound imaging and synergistic gas and sonodynamic cancer therapy. *Nanoscale Horizons*, *4*(3), pp.747–756.

73. Prabhakar, A. and Banerjee, R., **2019**. Nanobubble liposome complexes for diagnostic imaging and ultrasound-triggered drug delivery in cancers: A theranostic approach. *ACS Omega*, *4*(13), pp.15567–15580.

74. Farooq, A., Sabah, S., Dhou, S., Alsawaftah, N. and Husseini, G., **2022**. Exogenous contrast agents in photoacoustic imaging: An in vivo review for tumor imaging. *Nanomaterials*, *12*(3), p.393.

75. Frinking, P., Segers, T., Luan, Y. and Tranquart, F., **2020**. Three decades of ultrasound contrast agents: A review of the past, present and future improvements. *Ultrasound in Medicine & Biology*, *46*(4), pp.892–908.

76. ~~Lu, S., Zhao, P., Deng, Y. and Liu, Y., **2022**. Mechanistic insights and therapeutic delivery through micro/nanobubble-assisted ultrasound. *Pharmaceutics*, *14*(3).~~

77. Fateh, S.T., Moradi, L., Kohan, E., Hamblin, M.R. and Dezfuli, A.S., **2021**. Comprehensive review on ultrasound-responsive theranostic nanomaterials: Mechanisms, structures and medical applications. *Beilstein Journal of Nanotechnology*, *12*(1), pp.808–862.

78. ~~Chandan, R. and Banerjee, R., **2018**. Pro-apoptotic liposomes-nanobubble conjugate synergistic with paclitaxel: A platform for ultrasound responsive image-guided drug delivery. *Scientific Reports*, *8*(1).~~

79. Kim, M.A. and Lee, C.M., **2022**. NIR-mediated drug release and tumor theranostics using melanin-loaded liposomes. *Biomaterials Research*, *26*(1), pp.1–13.

80. ~~Fernandes, D.A. and Kolios, M.C., **2018**. Intrinsically absorbing photoacoustic and ultrasound contrast agents for cancer therapy and imaging. *Nanotechnology*, *29*(50).~~

81. Park, D.H., Jung, H.C., Park, J., Bae, S., Shin, U.C., Kim, S.W., Kim, C.W., Lee, Y.H. and Seo, J., **2022**. Synthesis of echogenic liposomes for sonoporation. *Micro & Nano Letters*, *17*(11), pp.276–285.

82. Zhou, L.Q., Li, P., Cui, X.W. and Dietrich, C.F., **2020**. Ultrasound nanotheranostics in fighting cancer: Advances and prospects. *Cancer Letters*, *470*, pp.204–219.

83. Martí-Bonmatí, L., Sopena, R., Bartumeus, P. and Sopena, P., **2010**. Multimodality imaging techniques. *Contrast Media & Molecular Imaging*, *5*(4), pp.180–189.

84. Li, J., Xi, A., Qiao, H. and Liu, Z., **2020**. Ultrasound-mediated diagnostic imaging and advanced treatment with multifunctional micro/nanobubbles. *Cancer Letters*, *475*, pp.92–98.

85. Guo, C., Jin, Y. and Dai, Z., **2014**. Multifunctional ultrasound contrast agents for imaging guided photothermal therapy. *Bioconjugate Chemistry*, *25*(5), pp.840–854.

86. Abusara, A., Abdel-Hafez, M. and Husseini, G., **2018**. Measuring the acoustic release of a chemotherapeutic agent from folate-targeted polymeric micelles. *Journal of Nanoscience and Nanotechnology*, *18*(8), pp.5511–5519.

87. Wadi, A., Abdel-Hafez, M. and Husseini, G.A., **2017**. Identification of the uncertainty structure to estimate the acoustic release of chemotherapeutics from polymeric micelles. *IEEE Transactions on Nanobioscience*, *16*(7), pp.609–617.

88. Husseini, G.A., Mjalli, F.S., Pitt, W.G. and Abdel-Jabbar, N.M., **2009**. Using artificial neural networks and model predictive control to optimize acoustically assisted doxorubicin release from polymeric micelles. *Technology in Cancer Research and Treatment*, *8*(6), pp.479–488.

89. Husseini, G.A., Pitt, W.G., Christensen, D.A. and Dickinson, D.J., **2009**. Degradation kinetics of stabilized pluronic micelles under the action of ultrasound. *Journal of Controlled Release*, *138*(1), pp.45–48.

90. Díaz De La Rosa, M.A., Husseini, G.A. and Pitt, W.G., **2013**. Mathematical modeling of microbubble cavitation at 70 kHz and the importance of the subharmonic in drug delivery from micelles. *Ultrasonics*, *53*(1), pp.97–110.

91. Martins, A.M., Tanbour, R., Elkhodiry, M.A. and Husseini, G.A., **2016**. Ultrasound-induced doxorubicin release from folate-targeted and non-targeted P105 micelles: A modeling study. *European Journal of Nanomedicine*, *8*(1), pp.17–29.

92. Abdel-Hafez, M. and Husseini, G.A., **2015**. Predicting the release of chemotherapeutics from the core of polymeric micelles using ultrasound. *IEEE Transactions on Nanobioscience*, *14*(4), pp.378–384.

93. Husseini, G.A., Kherbeck, L., Pitt, W.G., Hubbell, J.A., Christensen, D.A. and Velluto, D., **2015**. Kinetics of ultrasonic drug delivery from targeted micelles. *Journal of Nanoscience and Nanotechnology*, *15*(3), pp.2099–2104.

94. Diaz De La Rosa, M.A., Husseini, G.A. and Pitt, W.G., **2013**. Comparing microbubble cavitation at 500 kHz and 70 kHz related to micellar drug delivery using ultrasound. *Ultrasonics*, *53*(2), pp.377–386.

95. Husseini, G.A., Velluto, D., Kherbeck, L., Pitt, W.G., Hubbell, J.A. and Christensen, D.A., **2013**. Investigating the acoustic release of doxorubicin from targeted micelles. *Colloids and Surfaces B: Biointerfaces*, *101*, pp.153–155.

96. Husseini, G.A., Abdel-Jabbar, N.M., Mjalli, F.S., Pitt, W.G. and Al-Mousa, A., **2011**. Optimizing the use of ultrasound to deliver chemotherapeutic agents to cancer cells from polymeric micelles. *Journal of the Franklin Institute*, *348*(7), pp.1276–1284.

97. Husseini, G.A., Diaz De La Rosa, M.A., Alaqqad, E.O., Al Mamary, S., Kadimati, Y., Al Baik, A. and Pitt, W.G., **2011**. Kinetics of acoustic release of doxorubicin from stabilized and unstabilized micelles and the effect of temperature. *Journal of the Franklin Institute*, *348*(1), pp.125–133.

98. Husseini, G.A., Stevenson-Abouelnasr, D., Pitt, W.G., Assaleh, K.T., Farahat, L.O. and Fahadi, J., **2010**. Kinetics and thermodynamics of acoustic release of doxorubicin from non-stabilized polymeric micelles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *359*(1–3), pp.18–24.

99. ~~AlMajed, Z., Salkho, N.M., Sulieman, H. and Husseini, G.A., **2022**. Modeling of the in-vitro release kinetics of sonosensitive targeted liposomes. *Biomedicines*, *10*(12).~~

100. Moussa, H.G., Husseini, G.A., Abel-Jabbar, N. and Ahmad, S.E., **2017**. Use of model predictive control and artificial neural networks to optimize the ultrasonic release of a model drug from liposomes. *IEEE Transactions on Nanobioscience*, *16*(3), pp.149–156.

101. Ahmed, S.E., Moussa, H.G., Martins, A.M., Al-Sayah, M.H. and Husseini, G.A., **2017**. Effect of pH, ultrasound frequency and power

density on the release of calcein from stealth liposomes. *European Journal of Nanomedicine*, *8*(1).

102. Ibrahim, M., Sabouni, R., Husseini, G.A., Karami, A., Bai, R.G. and Mukhopadhyay, D., **2020**. Facile ultrasound-triggered release of calcein and doxorubicin from iron-based metal-organic frameworks. *Journal of Biomedical Nanotechnology*, *16*(9), pp.1359–1369.

103. Ahmed, A., Karami, A., Sabouni, R., Husseini, G.A. and Paul, V., **2021**. Ph and ultrasound dual-responsive drug delivery system based on PEG–folate-functionalized iron-based metal–organic framework for targeted doxorubicin delivery. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *626*, p.127062.

104. ~~Karami, A., H.S., A., Sabouni, R., Husseini, G. and Paul, V., **2022**. Combined and single doxorubicin naproxen drug loading and dual-responsive pH ultrasound release from flexible metal-organic framework nanocarriers. *Journal of Biomedical Nanotechnology*, *18*,~~

105. Karami, A., Ahmed, A., Sabouni, R., Husseini, G.A., Sharabati, M.A., AlSawaftah, N. and Paul, V., **2022**. Hybrid liposome/metal-organic framework as a promising dual-responsive nanocarriers for anticancer drug delivery. *Colloids and Surfaces. B, Biointerfaces*, *217*, p.112599.

106. Ben Daya, S.M., Paul, V., Awad, N.S., Al Sawaftah, N.M., Al Sayah, M. H. and Husseini, G.A., **2021**. Targeting breast cancer using hyaluronic acid-conjugated liposomes triggered with ultrasound. *Journal of Biomedical Nanotechnology*, *17*(1), pp.90–99.

107. Lattin, J.R., Pitt, W.G., Belnap, D.M. and Husseini, G.A., **2012**. Ultrasound-induced calcein release from eLiposomes. *Ultrasound in Medicine & Biology*, *38*(12), pp.2163–2173.