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Effect of High-Frequency Ultrasound on Targeted Liposomes

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Delivering highly toxic drugs inside a safe carrier to tumors while achieving a controlled and effective drug release at the targeted sites represents an attractive approach to enhance drug efficiency while reducing its undesirable side effects. Functionalization of highly biocompatible nanocarriers such as liposomes functionalized with targeting moieties enhances their ability to target specific cancer cells overexpressing the targeted receptors. Furthermore, upon their accumulation at the target site, High-frequency ultrasound (HFUS) can be used to stimulate a controlled release of the loaded drugs. Here, the US-mediated drug release from calcein-loaded non-pegylated, pegylated as well as targeted-pegylated liposomes modified with human serum albumin (HSA) and transferrin (Tf) was investigated. HFUS at two different frequencies (1 MHz and 3 MHz) was found to trigger calcein release, with higher release rates recorded at the lower frequency (1 MHz) compared to the higher frequency (3 MHz) despite a higher power density. Pegylation was found to enhance liposomal sensitivity to HFUS significantly. In addition, targeted pegylated liposomes were more susceptible to HFUS than non-targeted pegylated (control) liposomes. These findings show that pegylation and targeting moieties directly influence liposomal sensitivity to HFUS. Therefore, combining targeted-pegylated liposomes with HFUS represents a promising controlled and effective drug delivery system.

KEYWORDS: *Targeted Drug Delivery, High-Frequency Ultrasound, Active Targeting, Transferrin, Human Serum Albumin.*

INTRODUCTION

Antineoplastic drugs consist of powerful chemicals designed to destroy fast-growing cells. Tumor cells can divide/multiply much faster than the majority of the other cells in the body. Those cytolethal drugs are widely used to treat a wide range of metastatic cancer and can sometimes be combined with other forms of treatment such as surgery and radiotherapy. However, conventional chemotherapeutic drugs are non-selective and attack all the cells with a high proliferation rate [1]. This leads to destroying both cancerous and healthy cells with fast growth rates. Therefore, conventional chemotherapeutics cause systematic toxicity resulting in many unpleasant side effects ranging from mild to severe. Research efforts have been directed towards enhancing these toxic drugs' specificity and targeting ability to maximize their potency while reducing their side effects [2].

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Several nano-sized drug delivery particles that can encapsulate these toxic drugs have been developed to provide a safe drug delivery vehicle to solid tumors. Liposomes are one of the most successful nanocarriers used to date due to their versatile structure, biocompatibility, non-toxicity, non-immunogenicity, and biodegradability [3, 4].

Liposomes are artificial spherical vesicles prepared from naturally derived phospholipids. They are composed of lipid bilayers (one or more) surrounding a hydrophilic aqueous core. Their amphiphilic nature allows them to enclose hydrophilic as well as hydrophobic compounds [5, 6]. Furthermore, liposomes enhance the bioavailability and provide a longer circulation time for the encapsulated water-insoluble drugs by protecting them from degradation. This will allow overcoming the issue of multidrug resistance and improve the therapeutic efficiency of the encapsulated drugs while reducing their unwanted side effects [7]. However, conventional liposomes can be recognized by the body's immune system (reticuloendothelial system), resulting in their clearance from the body. Liposomes can be camouflaged to escape the RES by decorating their surfaces with stealth-imparting polymers, e.g.,

Polyethylene glycol or PEG to form "stealth liposomes" [8]. The success of PEGylation as a stealth technology resulted in the development of several clinically approved liposomal formulations used for cancer treatment, such as Doxil®, Caelyx®, and Myocet® [9].

As seen in Figure 1, normal tissues have blood vessels with intact walls that are impenetrable to liposomes or other nano-sized carriers. Tumor vasculature, on the other hand, has a disordered and leaky structure accompanied by an impaired lymphatic drainage system. This permits the extravasation of the liposomes through the deformed vasculature and their accumulation inside solid tumors, a unique phenomenon that is referred to as the "enhanced permeability and retention effect" or the EPR effect, which allows the passive targeting of tumors using drug-loaded nanocarriers [2, 5, 10]. Conventional liposomes, as well as stealth ones, rely on passive targeting to deliver anticancer drugs to tumor sites. Passive targeting is an excellent drug delivery pathway but suffers from several limitations. This is due to the variations in the size of the pores of the tumor vessels depending on solid tumors' type and stage. These variations can even exist within the same tumor. Therefore, homogeneous targeting of the tumor tissues is not always feasible. Moreover, the elevated interstitial fluid pressure in solid tumors can inhibit the distribution of liposomes within the tumor tissues, which, in turn, may lead to multiple-drug resistance (MDR) [10, 11].

Active targeting improves the specific delivery of drugs to targeted sites. Liposomes' surfaces can be modified for the purpose of active targeting using site-specific targeting ligands that recognize and bind to specific cellular receptors overexpressed on cancer cells, such as antibodies, proteins, vitamins, and peptides [10, 12–14].

Once inside the tumor tissue, a sufficient release of the drug is essential to ensure maximum anticancer efficacy. Stimuli-responsive liposomal systems are promising drug delivery tools that provide a controlled drug release and sufficient drug concentrations at the diseased sites [15, 16]. Stimuli-responsive liposomes can maintain their intact structure while circulating inside the body, accumulate inside tumors and release their loaded drugs when subjected to certain stimuli. Different internal and external triggers are excellent candidates to be employed as triggers to stimulate the release of drugs trapped inside the liposomes. A number of distinctive characteristics of tumor tissues created the unique physiological properties associated with tumors' microenvironment. These characteristics are employed as internal triggers of drug release from the liposomes, such as lower pH levels, higher temperature, reduced environment, and enzymatic levels. External triggers, on the other hand, include ultrasound, magnetic

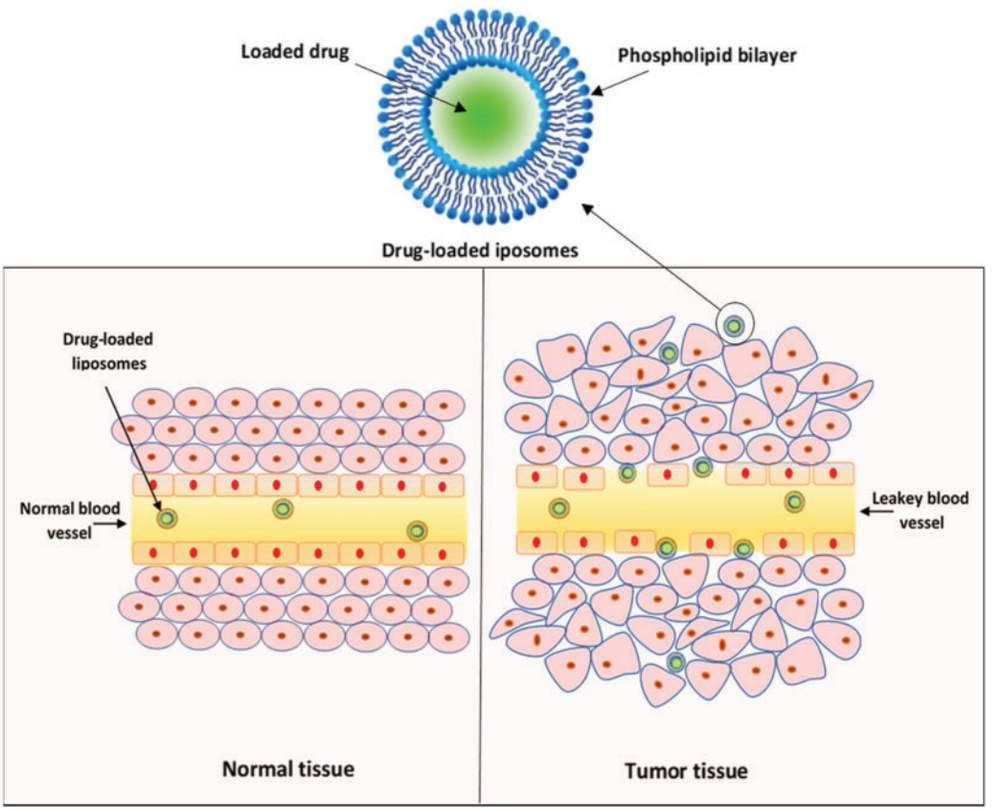


Figure 1. **An illustration showing the extravagation of drug-loaded liposomes through the deformed and leaky tumor vasculature.**

fields, electricity, and light [14, 15, 17]. Figure 2 shows several internal and external triggers used to target tumors inside the body.

Out of the aforementioned external triggers, ultrasound (US) is of particular interest due to its high efficiency and excellent safety profile. US can achieve deep penetration of tissues while being noninvasive. US can also be spatially and temporally controlled to ensure a highly efficient drug release. Together with stimulating liposomes to release drugs, applying US on tumor tissues increases the permeability of the blood vessels surrounding tumors and the membranes surrounding cancer cells [18, 19]. US-mediated drug release is achieved through the action of both the thermal and mechanical effects produced by the US waves as they propagate through the tissues. The energy produced from the US is absorbed, resulting in increasing the medium's temperature, which is associated with the frequency and exposure time. Predominantly, ultrasound waves produce a mechanical effect of the media known as "acoustic cavitation." Cavitation is the creation, growth, oscillation, and possibly, the collapse of gas cavities "bubbles" as a result of the compression of rarefaction regions in the medium created by the ultrasonic waves. Acoustic cavitation can either be "stable cavitation" or "inertial cavitation." During stable cavitation, a steady-state bubble is formed, oscillating within a specific equilibrium size with a small amplitude. Inertial cavitation, on the other hand, forms bubbles that oscillate within a large amplitude resulting in their fast growth and the subsequent violent collapse [20, 21]. The vicinity of these cavitation spots can elevate the surrounding temperature to 5×10^3 K and exert high pressures of up to 1×10^3atm. Furthermore, as the bubbles collapse, shock waves are formed and when hitting another bubble, a high-speed micro-jet is generated in the same direction as the propagating shockwave. This will result in the generation of stress or pressure on the nearby tissues [32–34]. A threshold of the acoustic pressure needs to be achieved for inertial cavitation to occur, the "inertial cavitation threshold," which is directly affected by the size of the bubble and the frequency used [18, 19, 22]. We have previously investigated the effect of low-frequency ultrasound (LFUS) as a stimulus to release calcein entrapped inside targeted liposomes [23–26]. Here, we are studying the effect of applying high-frequency ultrasound (HFUS) on calcein release from non-pegylated, pegylated and targeted liposomes with two types of proteins, albumin (HSA) and transferrin (Tf), conjugated to the pegylated liposomes. HSA is the most prominent constituent of plasma (MW = 66.5 kDa). HSA conjugation to the liposomes enhances their colloidal stability while blocking their recognition by the antibodies [27]. Tf, on the other hand, is a glycoprotein present in the serum (MW = 80 kDa). Many types of cancer cells are known to overexpress Tf receptors, such as ovarian cancer, colon cancer, and glioblastoma cell lines [28–30]. Thus, targeting transferrin receptors presents an appealing method for delivering chemotherapeutics to tumors [31].

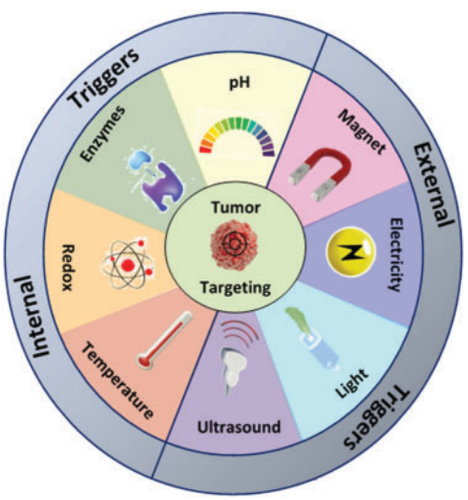


Figure 2. Different internal and external stimuli utilized as drug release triggers from the liposomes.

EXPERIMENTAL DETAILS

Materials

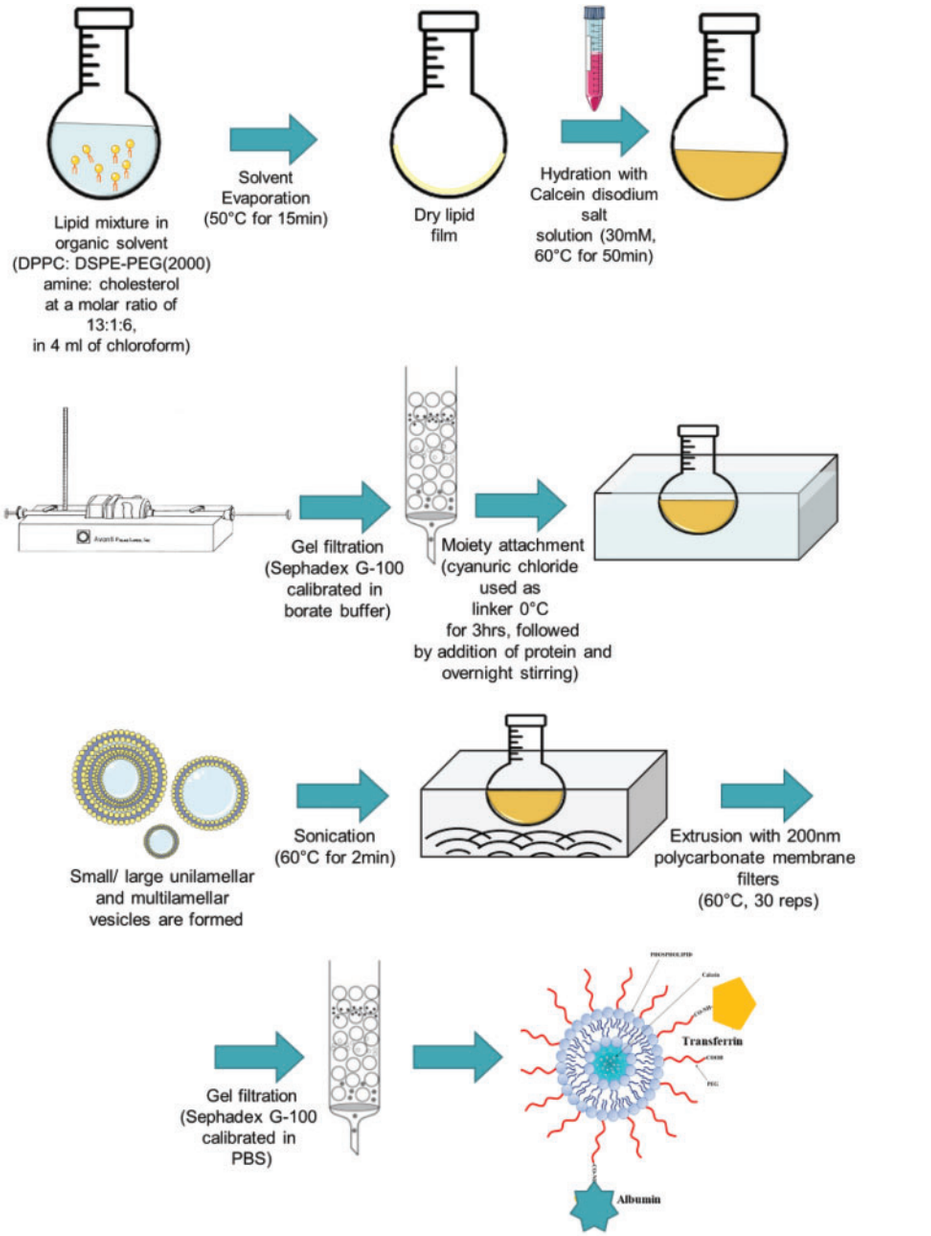
1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG(200)-NH_2) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) were obtained from Avanti Polar Lipids Inc. (Alabaster, Alabama, USA, provided by Labco LLC. Dubai, UAE). All the other chemicals used in this study were purchased from Sigma-Aldrich Chemie GmbH (by Labco LLC. Dubai, UAE).

Liposomes Preparation

Non-targeted liposomes used here were either pegylated (stealth) or non-pegylated, pegylated liposomes were synthesized according to the thin-film hydration method [32]. The detailed procedure used in preparing, conjugating, and characterizing the liposomes is provided in our previous works [24, 31, 33, 34]. In addition, Figures 3 and 4 below summarize the synthesis and conjugation process, respectively.

Characterization

The size of the prepared liposomes and the polydispersity percentage was measured using dynamic light scattering (DLS). The Stewart assay was used for the calorimetric determination of the phospholipids' content in the liposomes [35]. In addition, the bicinchoninic acid (BCA) assay was performed to confirm conjugation and quantify protein content. Figure 5 summarizes the protocols followed to perform the characterization tests.



*35 kHz sonicating bath (Elma D-78224, Melrose Park, Illinois, IL, USA); Avanti polar mini-extruder (Avanti Polar Lipids, Inc., Alabaster, Alabama, AL, USA)

Figure 3. **Summary of liposomes preparation protocol.**

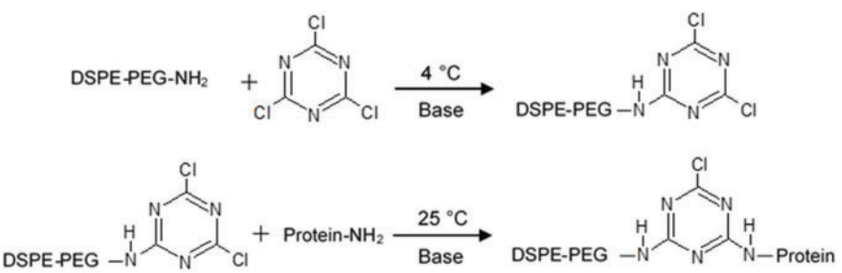


Figure 4. **Conjugation of protein molecules (Tf and HSA) to liposomes using cyanuric chloride as a coupling agent.**

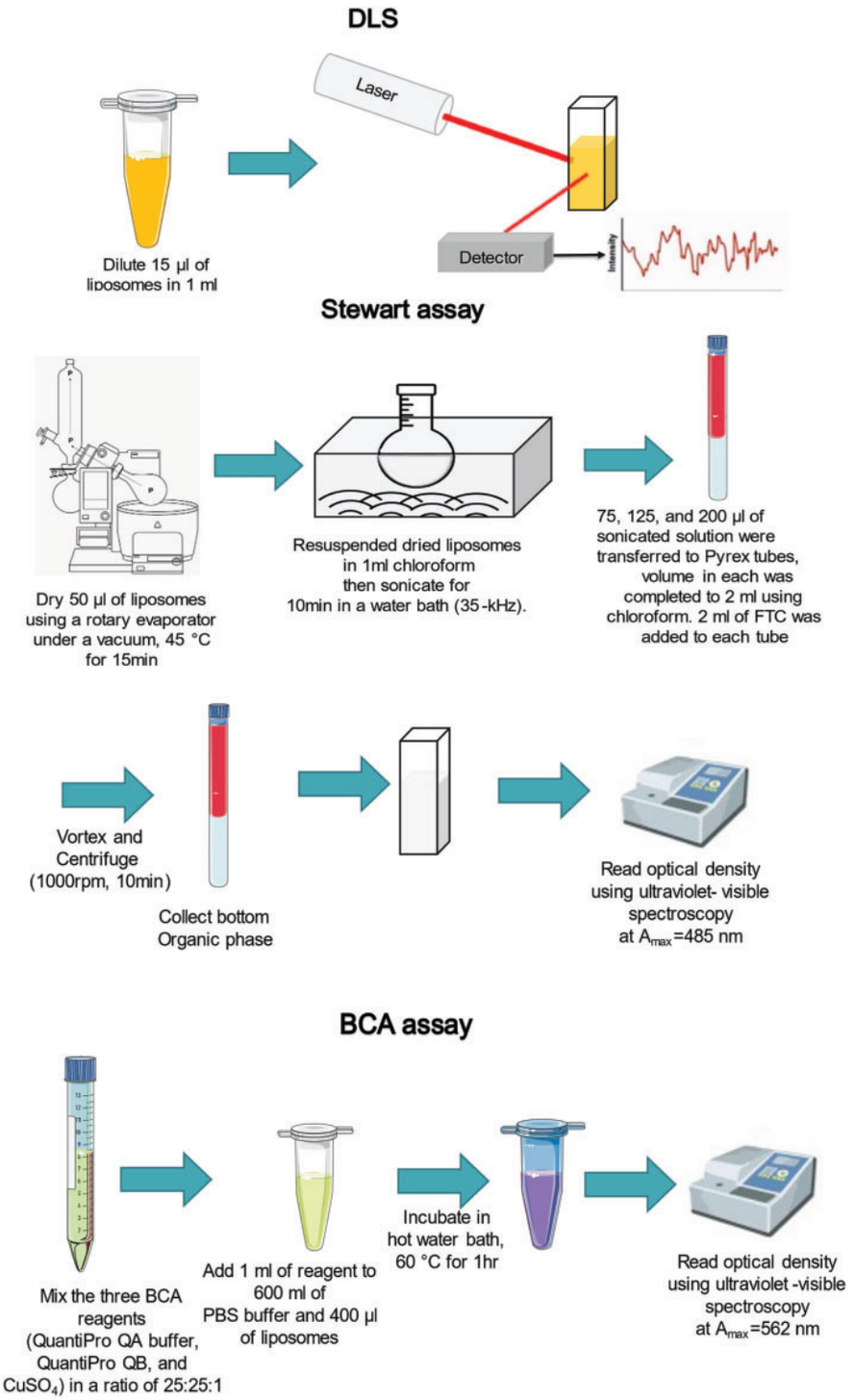


Figure 5. **Summary of characterization tests performed in this study.**

High-Frequency Ultrasound (HFUS) Release

Calcein release triggered with HFUS was performed using two frequencies 1 MHz (power density of 22.59 W/cm^2) and 3 MHz (power density of 158 W/cm^2). The fluorescence of the released calcein was determined using the QuantaMaster QM 30 phosphorescence spectrofluorimeter (Photon Technology International, Edison NJ, USA); the excitation and emission wavelengths used for calcein were 495 nm and 515 nm, respectively. Ultrasound waves were delivered from an ultrasonic probe placed inside a

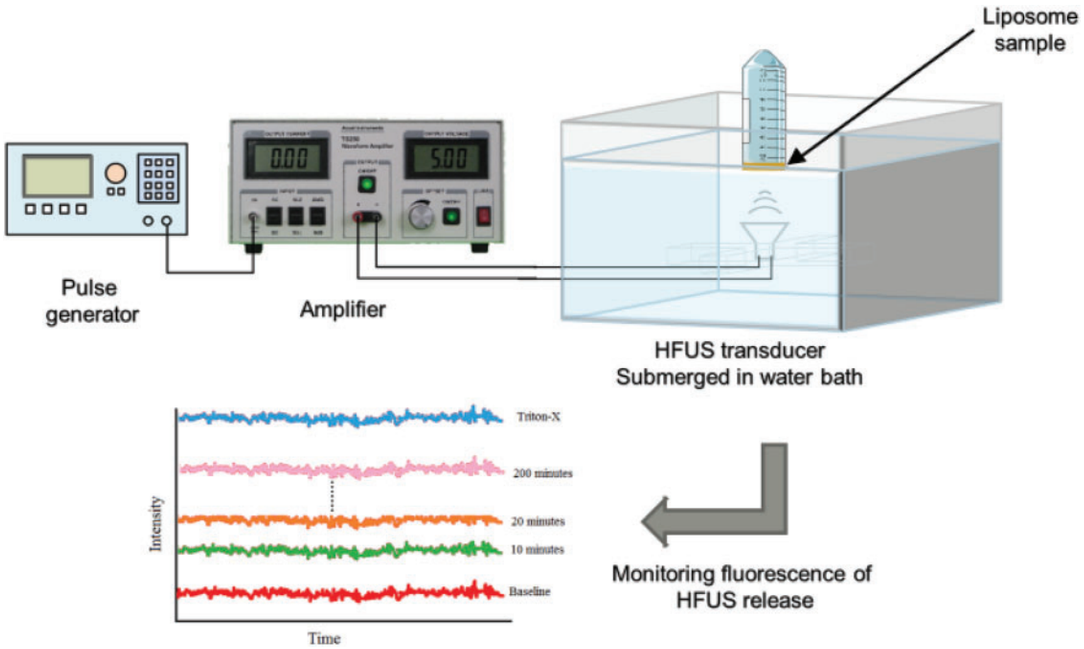


Figure 6. **HFUS apparatus used to trigger calcein release from the liposomes.**

water bath and connected to an AC amplifier (High Voltage Amplifier WMA-300, Falco Systems, Amsterdam, Netherlands). Figure 6 shows the arrangement of the used HFUS apparatus. Each sample was prepared, in a cuvette, by diluting 75 μl of liposomes with 3 ml of PBS buffer. The initial fluorescence intensity (baseline) was first determined using the spectrofluorometer. The sample was sonicated for 10 minutes using a 50 ml tube covered with a Parafilm and was then transferred into a cuvette and the fluorescence intensity of the sample was recorded. The process was repeated until a total sonication time of 200 min was reached. Finally, the sample was mixed with Triton X-100 (1%) to ensure an immediate and total release of the calcein remaining inside the liposomes corresponding to the final calcein release. The cumulative fraction released (CFR) was calculated using the recorded fluorescent intensities obtained experimentally, as shown in the following equation:

$$\mathrm{CFR} = \frac{I_t - I_o}{I_\infty - I_o} \quad (1)$$

Where I_0 represents the baseline intensity, I_t represents the intensity at time t, and I_∞ represents the highest fluorescence intensity value obtained.

Statistical Analysis

All the results reported here are the average $\pm$ standard deviation (SD). Two-tailed t-tests were used to determine the statistical significance of the results; p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Characterization

The average size (hydrodynamic radius) of the non-pegylated, pegylated (control) and pegylated-targeted liposomes, as well as their %polydispersity (%Pd), are presented in Table I. The formed liposomes are below 200 nm in diameter, with an acceptable percentage of polydispersity of less than 17%.

The results of the t-test showed that pegylation did not affect liposomes' size ($p = 0.326$). In addition, no significant difference was found between the sizes of pegylated liposomes following their conjugation to HSA and Tf molecules showing p-values of $p = 0.287$ and $p = 0.0775$, respectively.

All types of liposomes (non-pegylated, pegylated, TF-liposomes, and HSA-liposomes) showed similar stability, releasing less than 1% of the encapsulated calcein following their incubation in FBS for 4 hours at 37 °C. All the used liposomes had similar phospholipids concentration (0.727 ± 0.003 μg/mL for control liposomes, 1.63 ± 0.01 μg/mL for Tf-liposomes ($p = 0.0163$), and 1.14 ± 0.32 μg/ml ($p = 0.0163$) for HSA-liposomes. In contrast, the

Table I. **Size and polydispersity (%Pd) measurements of the different liposomal formulations.**

Liposome Type	Average radius (nm)	%Pd
Non-pegylated liposomes	84 ± 0.89	15 ± 4.21
Pegylated liposomes (control)	83.67 ± 3.32	16.00 ± 0.88
Tf-liposomes	90.13 ± 1.99	18.30 ± 3.26
HSA-liposomes	87.25 ± 2.44	13.43 ± 1.39

BCA assay showed that the apparent protein concentrations obtained from the absorbance values for the targeted liposomes (Tf-liposomes and HSA-liposomes) were higher than those obtained for the control liposomes with no protein conjugation. Figure 7 shows the values of the measured protein concentrations, as well as an illustration of the different types of liposomes used in this study. Qualitative microscopic images of the targeted liposomes (Tf-liposomes) obtained by transmission electron microscopy (TEM) confirmed that the produced liposomes were uniform in size (Fig. 8).

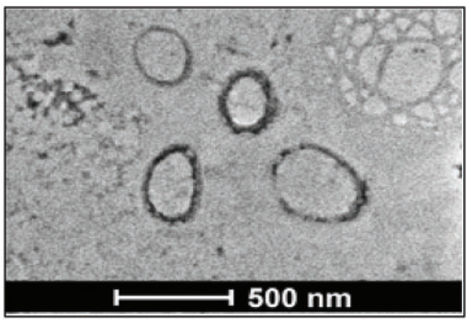


Figure 8. **TEM image of Tf-liposomes.**

HFUS Offline Release

HFUS applied at two different frequencies, 1 MHz (power density = 22.59 W/cm^2) and 3 MHz (power density = 158 W/cm^2), were investigated here. We first studied the effect of pegylation on the HFUS-triggered calcein release from the liposomes. As seen in Figure 8, non-pegylated liposomes were not sensitive to HFUS applied at the higher frequency (3 MHz, 158 W/cm^2), releasing only 2.87% of their encapsulated calcein following 200 min of total sonication time. At the lower frequency of 1 MHz (22.59 W/cm^2), also showed a slow but slightly higher calcein release following 200 min of total sonication time (6.39%). These results suggest that non-pegylated liposomes are generally not sensitive to HFUS applied at the tested frequencies.

The effect of applying HFUS on pegylated liposomes on triggering calcein release was strongly dependent on the frequency used. Upon exposure to HFUS at 1 MHz, more calcein was released as sonication time increased, with 38.03% of the calcein released after 200 min of total sonication time. However, sonicating with 3 MHz HFUS resulted in a much lower drug release with only 14.64% following 200 min of total sonication time, showing similar release kinetics to non-pegylated liposomes triggered with HFUS at 1 MHz (Fig. 9). These results show that pegylation directly affects their sensitivity to HFUS depending on the frequency used.

To investigate the effect of conjugating targeting molecule to the surface of pegylated liposomes on

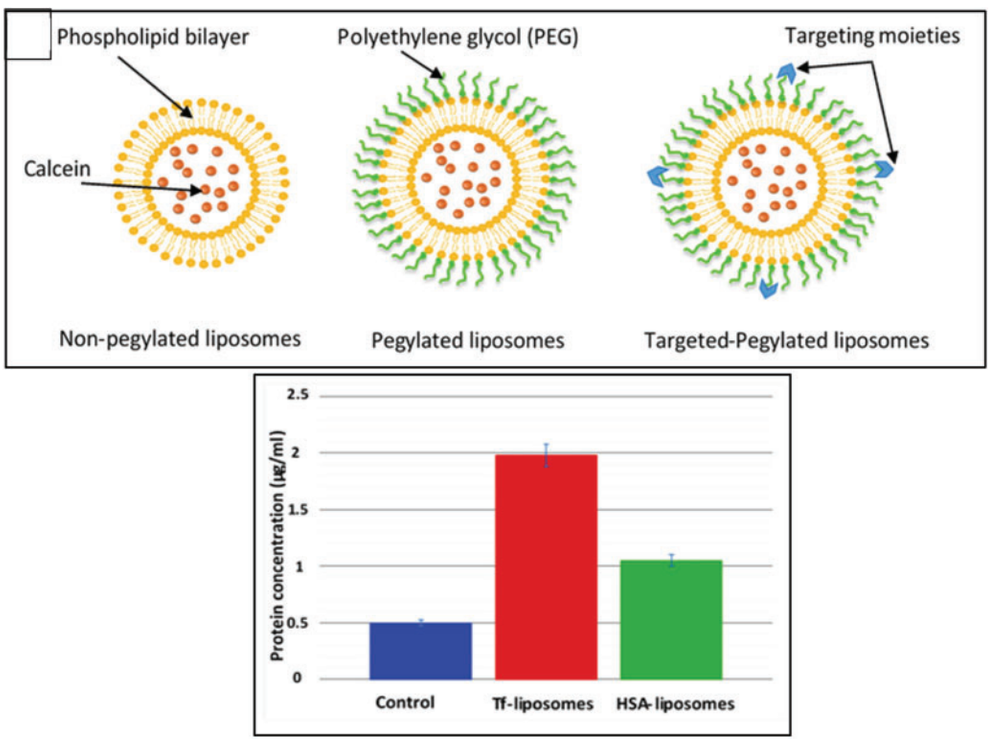


Figure 7. **An illustration of the different types of liposomes used in this study (A) and protein concentrations of the control and targeted liposomes using the BCA assay.**

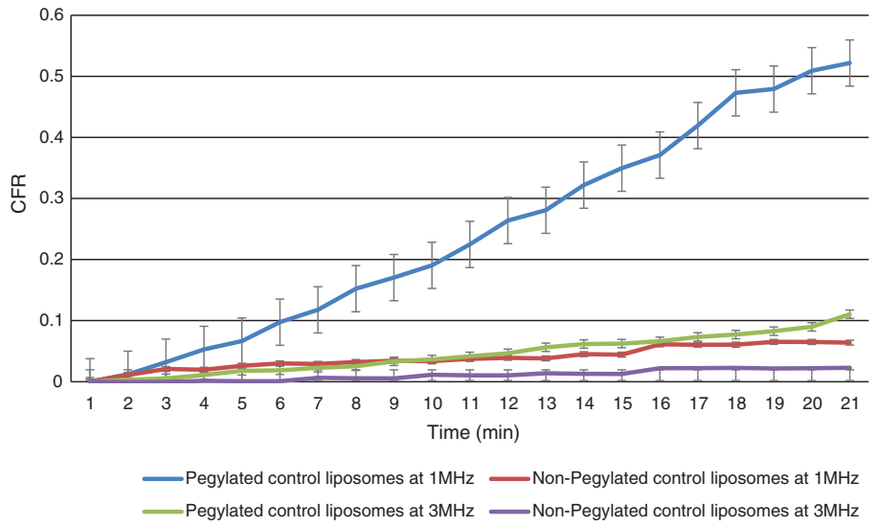


Figure 9. Normalized release profiles for non-pegylated as well as pegylated liposomes at 1 MHz and 3 MHz. Results are average of 3 liposomes batches (3 replicates each).

HFUS-mediated calcein release, a comparison between pegylated liposomes with no targeting moiety conjugated to their surfaces (control liposomes) and targeted-pegylated liposomes (Tf-liposomes and HSA-liposomes) was carried out (refer Fig. 10). Generally, calcein release increased with increasing HFUS exposure time at both frequencies for both types of targeted liposomes. At 3 MHz, both the Tf-liposomes and HSA-liposomes showed a significantly lower calcein release following 200 min of sonication (54.76% for Tf-liposomes and 49.65% for HSA-liposomes) compared to that achieved at 1 MHz (40.40% for Tf-liposomes and 42.14% for HSA-liposomes) ($p < 0.05$).

Generally, Tf-liposomes and HSA-liposomes showed fraction release of higher values in comparison to those obtained for the control at both frequencies. The final percentage of release achieved following 200 min of sonication at 1 MHz and 3 MHz for control and targeted liposomes is summarized in Table II.

At 1 MHz, Tf-liposomes and HSA-liposomes showed a significant increase in release ($p = 0.005618$ and 0.03717, respectively) compared to the control liposomes. At 3 MHz, Tf-liposomes and HSA-liposomes also showed a higher calcein release ($p = 2.17 \times 10^{-6}$ and 2.27×10^{-6}, respectively) compared to the release obtained from the control liposomes. Comparing the performance of the moieties, no statistical difference was observed when comparing calcein release values at 1 MHz and 3 MHz ($p = 0.199$ and 1.22×10^{-6}, respectively).

Encapsulating highly toxic antineoplastic agents in liposomal systems represents a safer approach to treating cancer by minimizing its adverse side effects. Decorating the surfaces of the nano-size liposomes with conjugated moieties to target cancer cells increases their selectivity, which in turn can improve their overall efficacy at delivering drugs to the targeted cancer cells. Once at their targeted site, different triggering mechanisms can be applied to facilitate the opening of the liposomes and the subsequent controlled drug release within tumors. We investigated the effect of applying HFUS in triggering calcein release from non-pegylated, pegylated, or targeted-pegylated liposomes. HFUS has shown promising potential in numerous clinical and research applications due to its high safety profile, noninvasive approach, low cost, and reproducibility. HFUS is usually used in clinical settings with frequencies ranging from 1 to 12 MHz, with the lower frequencies achieving much deeper penetration into the body [36, 37]. Furthermore, HFUS can be focused on a tiny, targeted area. Therefore, a drug delivery system composed of targeted liposomes triggered with HFUS has excellent potential for the safe delivery and controlled release of chemotherapeutic drugs inside tumors, including those embedded deeper within the body.

Ultrasound waves produce either thermal or mechanical effects. The liposomes used in this study are designed to be sensitive to elevation in temperature above a specific temperature and the mechanical effects of the ultrasound (cavitation). DPPC is the main phospholipid forming the lipid bilayer of our liposomes ($T_c = 41.3$ °C). Below this temperature, the lipid bilayer will be in the solid (gel) order and will turn into the liquid-disordered phase once heated to this temperature or above, resulting in calcein release. The sensitivity of our liposomes to the mechanical force produced by the cavitation effect depends on the packing parameter of the phospholipids used to prepare the liposomes. We have used different liposomal formulations to understand the effect of pegylation and conjugation of protein molecules on the phospholipids and the subsequent sensitivity to the cavitation effects.

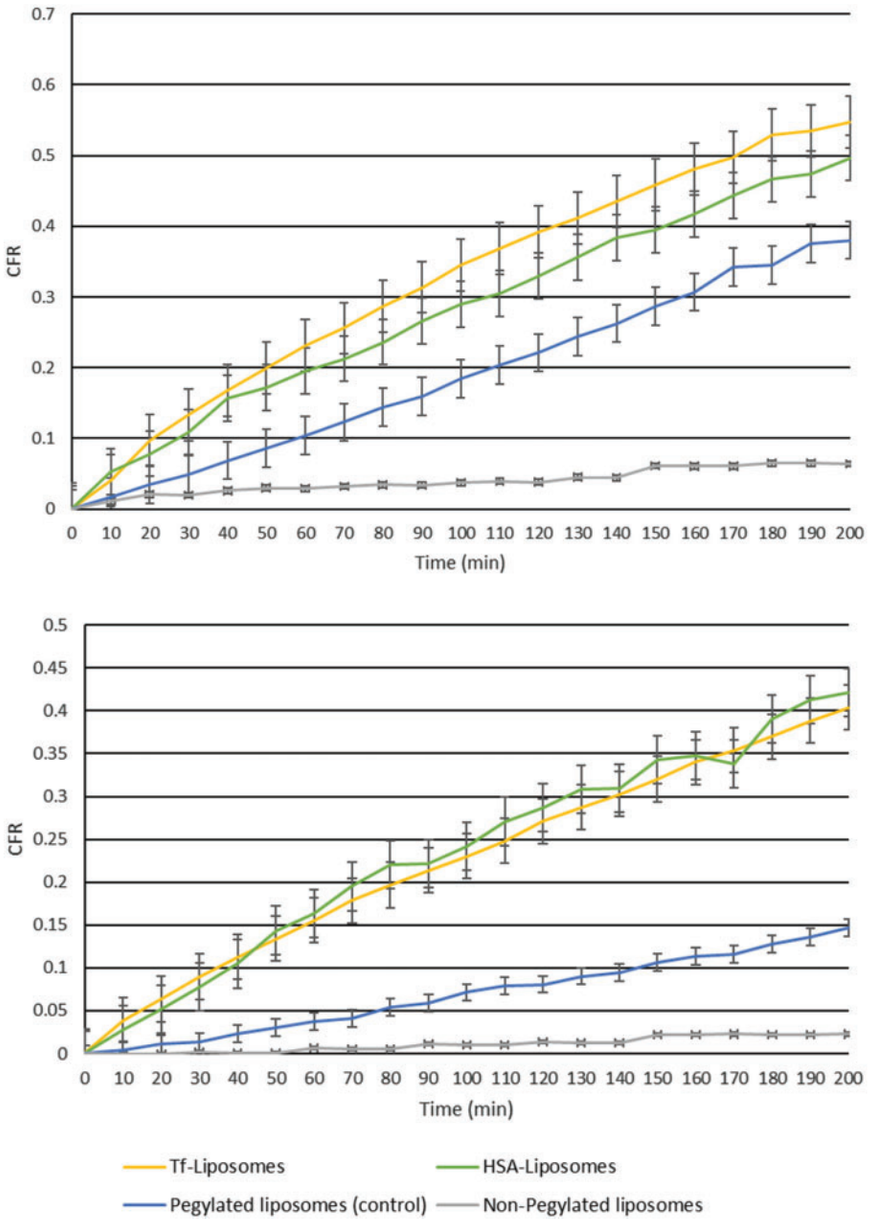


Figure 10. **Normalized release profiles for all tested liposomes at 1 MHz (top), and 3 MHz (bottom). Results are average of 3 liposomes batches (3 replicates each).**

When compared to HFUS (1 and 3 MHz), LFUS (20 kHz) was found to show higher efficiency in triggering drug release from the liposomes [38]. This is because the occurrence of cavitation is mainly dependent on the intensity of the applied ultrasound [36, 39, 40]. Generally, the lower the frequency, the lower the value of the intensity required to provoke transient cavitation [41]. Thus, transient cavitation occurs more frequently at low frequencies. Subsequently, the induction of drug release from liposomes exposed to LFUS is anticipated to be higher than those exposed to HFUS due to increased cavitation events. This is because LFUS waves produce more cavitation nuclei in liquids while reducing the threshold of cavitation. Furthermore, LFUS provides ample time for nucleation as the formed bubbles are given a longer time to expand and grow slowly during negative pressure. [22, 42]. This was supported by an earlier study reported by our research group [24]; we have used LFUS (20 kHz) to trigger drug release from liposomes similar to those tested here. The power densities used were 12, 7, and 6 mW/cm^2. The released calcein from all the tested formulations was significantly higher than the values reported here using the HFUS.

Table II. Summary of percent final release for control and targeted liposomes at 1 MHz and 3 MHz.

Liposomes	% Final release at 1 MHz	% Final release at 3 MHz
Non-pegylated liposomes	6.39	2.87
Pegylated liposomes (control)	38.03	14.69
HSA-liposomes	49.65	42.14
Tf-liposomes	54.76	40.40

Two different frequencies were used in this study: 1 MHz (power density = 22.59 W/cm^2) and 3 MHz, (power density = 158 W/cm^2). Overall, we have found that HFUS applied at the lower frequency/lower intensity (1 MHz) triggered higher calcein release, from all liposomal formulations, compared to the higher frequency/higher intensity (3 MHz). The Mechanical Index (MI) was introduced to indicate the possibility of transient cavitation in a medium exposed to US. MI will be mathematically defined as follows [39]:

$$MI = \frac{P_{neg}}{\sqrt{f}} \quad (2)$$

$$P_{neg} = \sqrt{2IZ} \quad (3)$$

P_{neg} represents the highest negative pressure, f represents the frequency, I is the intensity of US, and Z represents the acoustic impedance of water. Generally, an MI values of more than 0.7 indicates an increased probability of the occurrence of cavitation.

The mechanical indices were calculated using Eq. (2). The Z value used to determine P_{neg} was that of water (1.48 MPasec/m). MI was calculated to be around 0.79 and 1.249, corresponding to 1 MHz (22.59 W/cm^2) and 3 MHz (158 W/cm^2), respectively. Generally, cavitation does not exist below an MI of 0.3–0.4 [43–45], with biological effects being reported at an MI higher than 0.7. The calculated MI for the tested frequencies indicates that the observed HFUS-triggered release of the encapsulated calcein is mainly mediated by collapse (inertial) cavitation. Also, the recorded increase in calcein release with the lower power density (22.59 W/cm^2) compared to the higher power density (158 W/cm^2) is because a higher frequency (3 MHz) was used at the higher power density. This agrees with the established fact that the attenuation of the propagating ultrasonic waves increases with the increase in the frequency used [46].

Our results showed that pegylation, as well as the presence of protein targeting moieties, have a direct effect on determining liposomal sensitivity to HFUS. Generally, pegylation was found to enhance liposomal sensitivity to HFUS. Non-pegylated liposomes released significantly less calcein compared to the pegylated liposomes when 1 MHz LFUS was used. During the 10 min sonication intervals, the temperature increased from 22.1 °C to 33 °C, which is lower than the T_c of DPPC, indicating that temperature may have played a role in calcein release from the liposomes. However, the limited release reported with the non-pegylated liposomes despite the same increase in temperature indicates that the reported temperature increase is not the main drive behind the reported release. The effect of cavitation is expected to be the main driving force for the reported calcein release from the pegylated liposomes through disturbing the membrane of these liposomes by creating transient pores which form as ultrasound waves are applied and reseal when stopped. Evjen et al. [49] reported that DPPC liposomes formed transient pores when exposed to ultrasound. This will result in enhancing the permeability of the liposomes, and more drugs will be released. Furthermore, other studies had shown that the presence of PEG on the outer membrane of the liposomes enhanced their permeability when LFUS was applied [25, 42, 50]. The packing parameter of the pegylated phospholipid used here (DSPE-PEG) is 0.5, which is lower than that of DPPC (0.74). Thus, exposing the liposomes to ultrasound waves makes DSPE-PEG more prone to ejection from the liposomes to create micellar structures [42, 46, 51–53]. We also showed that the conjugation of protein molecules TF and HSA, with molecular weights of 80 kDa and 66.5 kDa, respectively, to PEG boosted their sonosensitivity to HFUS compared to PEG alone. This could be due to the added weight of protein molecules combined with the already weak packing parameter of DSPE-PEG. This suggests that using targeted liposomes not only enhances their localized drug delivery but enhances their sensitivity to ultrasound triggering using HFUS.

This study showed that HFUS could be used with liposomes as a drug release triggering mechanism. However, compared to previous findings, HFUS is less efficient than LFUS as a triggering mechanism. However, liposomes' sensitivity to HFUS can be enhanced by pegylation and the incorporation of targeting moieties such as TF and HSA. This study is a proof of concept; future studies using animal models are needed to fully understand the potential of combining HFUS with liposomes to treat solid tumors.

CONCLUSIONS

This study investigated the effect of HFUS on drug release from liposomes (non-pegylated, pegylated, and pegylated-targeted liposomes). All the tested formulations showed similar stability and size. Pegylation of the liposomes was found to increase their sensitivity to HFUS with higher drug release recorded at the lower frequency (1 MHz) compared to the higher frequency of 3 MHz. Furthermore, pegylated liposomes with Tf or HSA conjugation (Tf-liposomes and HSA-liposomes) showed higher sonosensitivity to HFUS compared to the pegylated liposomes. This proposes that incorporating HFUS is likely to enhance the efficiency of drug delivery using targeted liposomes. Further studies are needed in order to explore this method in more detail.

Conflicts of Interest

The authors declare no conflict of interest.

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