



HAL
open science

Role of thermal and mechanical effects on drug release from thermosensitive nanocarriers

Anthony Novell, Jean-Michel Escoffre, Chantal Al-Sabbagh, Christophoros Mannaris, Elias Fattal, Nicolas Tsapis, Michalakis Averkiou, Ayache Bouakaz

► **To cite this version:**

Anthony Novell, Jean-Michel Escoffre, Chantal Al-Sabbagh, Christophoros Mannaris, Elias Fattal, et al.. Role of thermal and mechanical effects on drug release from thermosensitive nanocarriers. 2012 IEEE International Ultrasonics Symposium, Oct 2012, Dresden (GERMANY), Germany. pp.1873-1876, 10.1109/ultsym.2012.0470 . hal-04280329

HAL Id: hal-04280329

<https://hal.science/hal-04280329>

Submitted on 10 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Role of thermal and mechanical effects on drug release from thermosensitive nanocarriers

Anthony Novell¹, Jean-Michel Escoffre¹, Chantal Al-Sabbagh², Christophoros Mannaris³, Elias Fattal²,
Nicolas Tsapis², Michalakis Averkiou³ and Ayache Bouakaz¹

1. UMR Inserm U930, Université François Rabelais, Tours, France
2. UMR CNRS 8612, Faculté de pharmacie, Châtenay-Malabry, France
3. Department of mechanical and manufacturing engineering, University of Cyprus, Nicosia, Cyprus

anthony.novell@univ-tours.fr

Abstract— The combination of focused ultrasound (FUS) with thermosensitive liposomes (TSL) is a promising method for drug delivery since it allows a localized release upon moderate heating with ultrasound. Besides thermal effects, FUS also induces mechanical stresses on drug nanocarriers. We propose in this study to examine the influence of both effects (thermal and mechanical) on drug release. For this, an *in-vitro* setup allowing liposomal drug delivery using FUS was first evaluated. Calcein was used as a model drug. FUS experiments were performed in water at 37°C using a 1 MHz transducer focused at 48 mm, at 1 kHz PRF and 40% duty cycle. The driving pressure and the insonation duration were varied from 1 to 2 MPa and from 0 to 30 min, respectively. Thermal heating using a water-bath was also performed with temperatures from 37 to 49°C. For TSL, the release reaches a plateau above 42°C (45%) after 10 min heating while no release is observed for non-thermosensitive liposomes (NTSL). Using FUS, a rapid calcein release is observed for pressures from 1 to 1.5 MPa (from 0% to 49%) for TSL. Above 1.5 MPa, the release increases slightly (59% at 2 MPa). For NTSL, a weak calcein release is measured for acoustic pressures higher than 1.5 MPa. This release is attributed to the mechanical stress generated by FUS which is sufficient to destabilize the liposomal membrane. Mechanical stress alone can enhance the calcein release by up to 17% for pressures higher than 1.75 MPa.

Keywords—FUS, thermosensitive liposomes, drug delivery, mechanical stress

I. INTRODUCTION

In the conventional anticancer therapy, the therapeutic effectiveness of anticancer drugs such as doxorubicin or 5-fluorouracil is limited by the undesired high drug accumulation in healthy tissues compared to its uptake in the tumor tissues. To overcome these major problems, anticancer drugs have been encapsulated inside PEGylated lipid or polymer nanovehicles preventing their recognition by the reticuloendothelial system [1]. Among these nanocarriers, the liposomes have been investigated for potential therapeutic applications in controlled and targeted *in-vivo* drug delivery. These vehicles are spherical closed, and lipid bilayer structures that encapsulate aqueous solution or gas [2]. The liposomal formulation offers attractive features for therapeutic applications: (i) its ability to protect and encapsulate

hydrophilic or hydrophobic compounds (*e.g.* nucleic acids, anticancer drugs), (ii) its biocompatibility, (iii) its low immunogenicity, (iv) its nanometer size and its PEGylation allowing them to circulate furtively and stably in the bloodstream, (v) its possibility of targeting with ligands or antibodies allowing them to induce its accumulation in the desired tissue area. For targeted drug delivery, the encapsulated drug inside the liposomes should be released at a controlled rate upon an appropriate stimulus in the target tissue. Chemical and physical methods including pH value, temperature, electric field and ultrasound for triggering drug release from these nanocarriers have been investigated. Among these different stimuli, ultrasound has a unique advantage since it can propagate into deep tissues. The mechanisms by which ultrasound triggers drug delivery from responsive liposomal formulation depend on the chemical composition of the liposomes and ultrasound conditions. Indeed, previous studies reported that the exposure of conventional liposomes (*i.e.* non-thermosensitive liposomes (NTSL)) to high intensity focused ultrasound (HIFU) induced the compound release from these liposomes through the membrane rupture of large liposomes (> 100 nm) and the generation of membrane pore-like defects of small liposomes (<100 nm) [3, 4]. These membrane openings are induced by acoustic cavitation resulting from HIFU. Novel liposomal formulations have emerged that allow the temperature-triggered release of drugs [5]. Temperature-sensitive liposomes (TSL) are able to lead to a more rapid release of the encapsulated drugs through transient nanopores formed at their phase transition temperature. Ideally, the drug should remain stably encapsulated in the TSL at body temperature (37°C), whereas a rapid and quantitative drug release should occur at mild hyperthermia (39-43°C). The temperature increment required for such drug release can be triggered by FUS under specific ultrasound conditions. The thermal effect of ultrasound is caused by absorption of the ultrasound energy. As the ultrasound waves are absorbed, their energy is converted into heat in the focused area.

If a thermal effect is clearly involved in the drug release from TSL, the impact of mechanical effect has not been investigated. In this context, we investigated the physical

processes involved in the calcein release from NTL and TSL under FUS insonation.

II. MATERIAL AND METHODS

A. Liposomal formulations

Calcein (40 mM final concentration) was used as a model drug and was encapsulated in liposomes (12 mM final concentration of lipids). Liposomal solutions were designed with different concentrations of DPPC and cholesterol to modify their thermosensitive property. DSPE conjugated with Polyethylene Glycol-2000 was added to get furtive liposomes. For NTL, the formulation was DPPC: 65%, Cholesterol: 30%, and DSPE-PEG₂₀₀₀: 5%. For TSL, the concentration of DPPC and cholesterol was adjusted to 90% and 5%, respectively to obtain a phase transition temperature close to 42°C. Liposome formulation was completed with 5% of DSPE-PEG₂₀₀₀.

B. In-vitro setup

Temperature elevation into an *in-vitro* setup was investigated as a function of the acoustic pressure (from 0 to 2 MPa) and the insonation time (from 0 to 30 min). An arbitrary function generator (33220A, Agilent, Palo Alto, CA) was used to design the excitation signal. Tone burst centered at 1 MHz with duty cycle (DC) of 40% was repeated with a fixed frequency of 1 kHz. This signal was then amplified using a power amplifier (ADECE, Artannes sur Indre, France) and transmitted to a 1 MHz homemade single-element transducer focused at 48 mm with a diameter of 47 mm. HIFU experiments were performed in a water-bath maintained at 37°C. A cuvette system was positioned at the transducer focal distance [6]. This system consisted of a sample holder, containing 25 µL of liposomal solution diluted in 500 µL of PBS, placed into a glycerol-filled plastic cuvette. In this double compartment setup, glycerol is heated by FUS. The heat transfer from the glycerol solution induces a temperature elevation of the nanoparticle solution through diffusion. This setup, using a secondary heating, mimics the *in-vivo* conditions since PBS as water and blood are non-absorptive media. Over the sides of the two containers placed in front of the transducer, acoustical windows were made of TPX foil having a thickness of 20 µm to avoid ultrasound attenuation and reflection. Temperature measurements were performed using a fine-wire thermocouple positioned inside the sample holder for different acoustic parameters (*e.g.* acoustic pressure, exposure time).

C. Spectrofluorometry

The calcein release from liposomes was determined by measuring the fluorescence intensity ($\lambda_{Ex} = 485 \text{ nm} / \lambda_{Em} = 520 \text{ nm}$) using a spectrofluorometer. Subsequently, the solution was heated using water-bath or FUS. Two control assays were selected: 1) Nanoparticles solution after addition of 10 µL of Triton X-100 at 10% v/v (Sigma Aldrich, Saint Louis, MO), which induced a complete calcein release by dissolving the liposomes (F_t positive control) and 2)

Nanoparticles alone (F_0 negative control). The percentage of calcein leakage was calculated according to:

$$\% \text{ calcein release} = \frac{F_x - F_0}{F_t - F_0} \times 100$$

where, F_x is the fluorescence of calcein after heating, F_0 is the initial fluorescence at 37 °C, and F_t is the fully dequenched calcein fluorescence after addition of Triton X-100. Data are presented as mean \pm standard error of the mean (SEM) of 3 independent measurements.

III. RESULTS AND DISCUSSION

A. Validation of in-vitro setup

Temperature increment induced by FUS was first measured in the sample holder containing the solution of PBS and liposomes. The temperature increase as a function of the acoustic pressure is displayed in Fig. 1. Using the following acoustic conditions: frequency 1 MHz, DC 40%, PRF 1 kHz, exposure time 5 min, a pressure higher than 1.25 MPa is necessary to induce a temperature increase above 42°C. The maximal temperature increment is 11.8°C at 2 MPa. Similar measurements were performed where the glycerol- was substituted by water. This condition is equivalent to primary heating as no sufficient ultrasonic absorption occurs in water. In this case, the maximal temperature increase is 39.6°C (data not shown). This demonstrates that the secondary heating setup using the glycerol-filled cuvette is necessary and can be used for thermosensitive activation of the TSL.

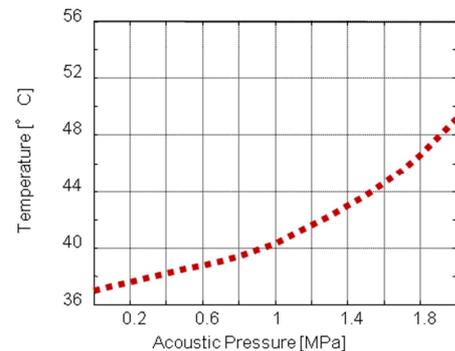


Fig. 1. Temperature elevation inside the nanoparticles holder as a function of the applied acoustic pressure (1 MHz, DC 40%, PRF 1 kHz, 5 min).

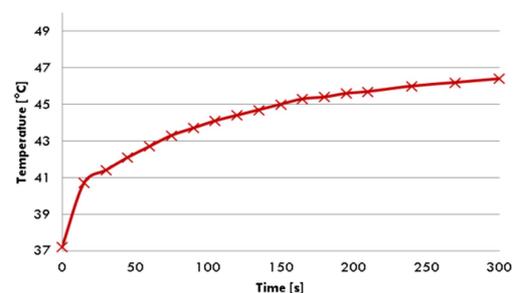


Fig. 2. Kinetics of temperature elevation at 1 MHz, 1.75 MPa, 40% DC; 1 kHz.

The kinetics of temperature elevation after insonation as a function of acoustic pressure is shown in Fig. 2. The curve is

shown for acoustic parameters of 1 MHz, 1.75 MPa, 40% DC, PRF 1 kHz. A quick increase of temperature occurs in the two first minutes of insonation (from 37.2°C to 44.4°C). An insonation of 5 minutes is necessary to achieve a quasi-stabilized temperature.

B. Calcein release measurements

Water-bath heating measurements were performed to evaluate the role of thermal effect alone on calcein leakage. The water-bath temperature was varied from 37 to 49°C in order to reproduce the same maximal temperature increment obtained by FUS (48.8°C at 2 MPa). The results using the water-bath are displayed in the top panel of Fig. 3 after 10 min heating. For TSL, the calcein release increases linearly between 37 and 42 °C and reaches a plateau for temperatures above 42°C. One can notice that calcein release induced by thermal effect never exceeds 45% for this liposomal formulation. Even after heating, most of the calcein is still trapped inside the liposomes. As expected, no temperature-dependent calcein leakage is observed for NTSL.

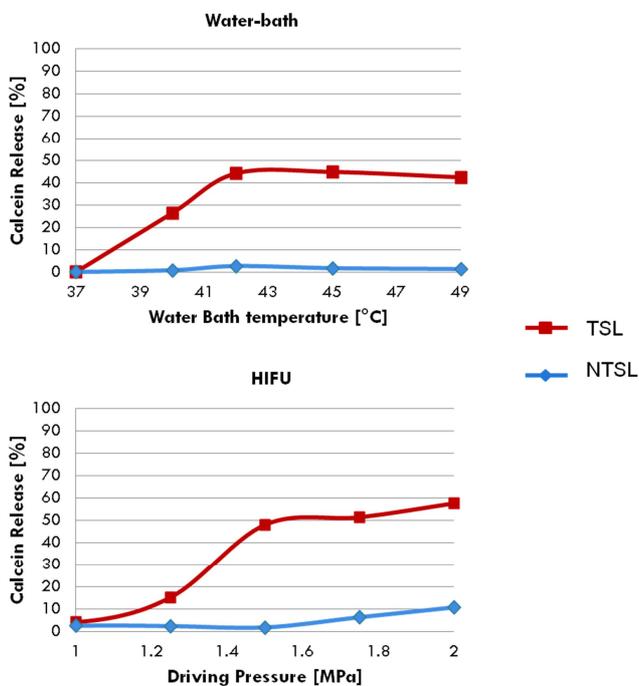


Fig. 3. Calcein release as a function of the water-bath temperature after 10 min heating (top panel) and as a function of applied acoustic pressure (bottom panel) during 10 min insonation.

Results for FUS experiments after 10 min insonation are shown in the bottom panel of Fig. 3. For TSL, a weak calcein release is observed for pressures from 1 to 1.25 MPa (< 18%). In these conditions, the temperature inside the liposome holder remains below 42°C (Fig. 1). At 1.5 MPa, the calcein release is close to the maximal release obtained after water-bath heating. Above this pressure, the release increases slightly and reaches 59% at 2 MPa. For NTSL, no calcein release is

measured from 1 to 1.5 MPa whereas it increases to 7% and 12% at 1.75 MPa and 2 MPa, respectively.

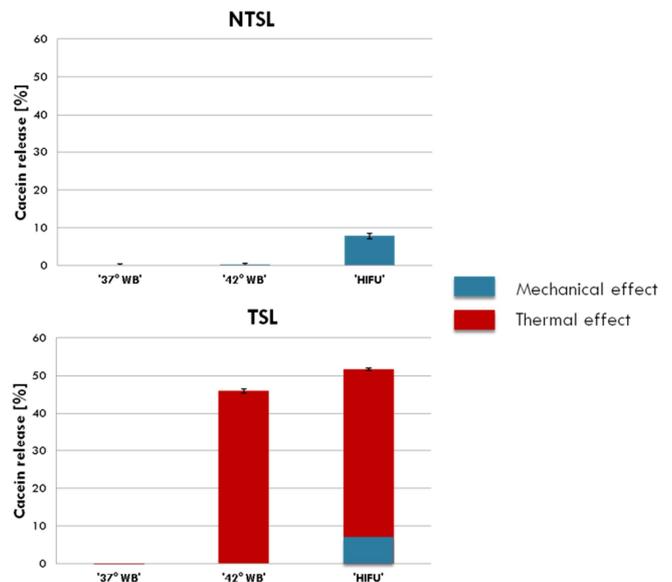


Fig. 4. Role of thermal and mechanical effects on calcein release for NTSL (top panel) and TSL (bottom panel) after 10 min water-bath and FUS heating

Since the NTSL nanocarriers are not sensitive to thermal effects, this increase can be attributed to the mechanical stress generated by FUS, which is sufficient to deteriorate the liposomal membrane and induce a weak calcein release. In fact, PEG intrusion in the liposome formulation renders these nanocarriers sensitive to the ultrasonic excitation [7]. For high acoustic pressures (>1.5 MPa), the mechanical stress also explains the higher release observed for TSL compared to water-bath heating. For example, for water-bath (49°C) and HIFU (2 MPa) experiments inducing the same temperature increment, a higher calcein leakage up to 17% is obtained with FUS.

Thermal effect can be separated from mechanical effect as shown in Fig. 4. Indeed, the mechanical effect can be isolated by a direct comparison between water-bath (thermal only) and FUS (thermal + mechanical) or by analyzing the results from NTSL (mechanical only). The same rate of calcein release due to mechanical effect alone (*e.g.* 8% at 1.75 MPa) is obtained for both NTSL and TSL.

IV. CONCLUSION

Drug release induced by mechanical stress was isolated from that resulting from thermal effects. The mechanical stress induced by FUS can enhance the calcein release by up to 17% for acoustic pressures higher than 1.75 MPa. Optical observations of thermal and mechanical effects on liposomes are currently in progress. The impact of different acoustical conditions on the number and size of liposomes will be investigated.

ACKNOWLEDGMENT

The project was partly funded by ANR-10-TECS-07-01 and Sonodrugs (NMP4-LA-2008-213706). The authors would like to acknowledge Jean-Yves Tartu and Jean-Marc Grégoire for their technical assistance.

REFERENCES

- [1] Y. Yan, G. K. Such, A. P. Johnston, J. P. Best, and F. Caruso, "Engineering particles for therapeutic delivery: prospects and challenges," *ACS Nano*, vol. 6, pp. 3663-9, May 22 2012.
- [2] A. D. Bangham, D. H. Heard, R. Flemans, and G. V. Seaman, "An apparatus for microelectrophoresis of small particles," *Nature*, vol. 182, pp. 642-4, Sep 6 1958.
- [3] D. Chen and J. Wu, "An in vitro feasibility study of controlled drug release from encapsulated nanometer liposomes using high intensity focused ultrasound," *Ultrasonics*, vol. 50, pp. 744-9, Aug 2010.
- [4] J. Wu, D. Chen, J. Pepe, B. E. Himberg, and M. Rieu, "Application of liposomes to sonoporation," *Ultrasound Med Biol*, vol. 32, pp. 429-37, Mar 2006.
- [5] H. Grull and S. Langereis, "Hyperthermia-triggered drug delivery from temperature-sensitive liposomes using MRI-guided high intensity focused ultrasound," *J Control Release*, vol. 161, pp. 317-27, Jul 20 2012.
- [6] C. Mannaris, E. Efthymiou, J. M. Escoffre, A. Bouakaz, M. E. Meyre, M. Germain, and M. Averkiou, "Ultrasound-induced temperature elevation for in-vitro controlled release of temperature-sensitive liposomes " *J Acoust Soc Am*, vol. 130, p. 2502, 2011.
- [7] H. Y. Lin and J. L. Thomas, "PEG-Lipids and oligo(ethylene glycol) surfactants enhance the ultrasonic permeabilizability of liposomes," *Langmuir*, vol. 19, pp. 1098-1105, 2003.