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Research paper

A novel approach to identify non-palpable breast lesions combining fluorescent liposomes and magnetic resonance-guided high intensity focused ultrasound-triggered release

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ABSTRACT

The combination of fluorescein-containing liposomes (FCL) and magnetic resonance-guided high intensity focused ultrasound (MR-HIFU)-triggered release is a promising approach for lesion demarcation and more efficient removal of non-palpable breast lesions. Exposure of FCL to ablation temperatures (60 °C) using MR-HIFU would result in palpable, stained tumors, which are more easy to identify during surgical resection. In this study, proof-of-concept concerning fluorescent FCL for MR-HIFU-triggered release and tumor demarcation of non-palpable breast lesions is presented. *Ex vivo* experiments in human blood and porcine muscle tissue showed increased label release from the liposomes, clear fluorescence enhancement and diffusion of the released compound after heating to 60 °C. Next, fluorescein release of FCL was observed after MR-HIFU-mediated mild hyperthermia (42 °C) and ablation temperature (60 °C) for a short period (30 s), which is in line with the clinically relevant MR-HIFU treatment parameters. These results indicate the potential of the FCL as a tool to improve tumor demarcation in patients by MR-HIFU-triggered release. Therefore, this method may offer a new tool for efficient surgical resection of non-palpable breast tumor lesions by enabling proper discrimination between tumor tissue and adjacent healthy tissue.

1. Introduction

One of the main difficulties in surgical breast tumor resection is to distinguish non-palpable breast lesions from healthy tissue. Smith-Bindman et al. stated after a screening mammography study in the UK and the US that 10–55% of the detected suspicious non-palpable lesions were malignant [1]. These patients must undergo intensive treatments like lumpectomy or mastectomy to ascertain 100% removal of the malignancies. Axillary lymph node dissection and loss of breast tissue severely affects the quality of life of the patients. Hence, it is important to improve lesion demarcation to reduce the removal of healthy tissue. Consequently, there is a need for a method that enables proper discrimination between tumor tissue and adjacent healthy tissue to ensure efficient complete tumor removal.

Nowadays, the use of magnetic resonance imaging (MRI) as a non-invasive diagnostic modality for characterization of suspicious breast lesions has become an additional diagnostic tool next to conventional techniques like mammography and ultrasound [2]. A recent clinically available non-invasive technique, that combines MRI with thermal ablation, is magnetic resonance-guided high intensity focused ultrasound (MR-HIFU) [3]. MR-HIFU is able to heat tissue with pinpoint accuracy thereby ablating malignant cells. Temperatures to be reached for this purpose are 60 °C and higher. Currently, clinical trials are performed on MR-HIFU treatment of uterine fibroids and prostate cancer [4,5]. Extensive preclinical research has been performed to expand the possibilities of this technique to breast tumor treatment [6,7]. Unfortunately, 100% tumor ablation cannot be guaranteed [6]. Especially for tumors with undefined margins and scattered multiple foci, MR-HIFU-mediated tumor ablation is not suitable [8]. Therefore, surgical resection remains important in the clinical setting.

A recent development in anti-cancer therapy is the combination of MR-HIFU and drug-containing nanoparticles like thermosensitive liposomes, which release their content upon application of a

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heat-trigger around 42 °C [9–12]. Interestingly, the combination of MR-HIFU-mediated ablation and thermosensitive liposomes has not been explored as yet. Although treatment of the lesions with MR-HIFU alone will result in coagulated tissue, which becomes palpable and consequently more easy to identify, visual detection of the malignant areas during surgery will still be difficult. So, it would be beneficial to enable visual discrimination of the lesions from healthy tissue. Margin marking of the lesions with a dye would help to facilitate more efficient lesion resection. An appropriate fluorescent dye for this purpose is fluorescein, since it is already used as a diagnostic tool in combination with ultraviolet (UV) light in a clinical setting and can therefore be safely used in patients [13–15]. Moreover, fluorescein is a self-quenching dye, which means that no fluorescence is detected at high intraliposomal concentration [16]. This has the advantage that the dye can only be detected with UV light after it is being released from the liposomes enabling proper discrimination of the MR-HIFU-treated malignant tissue from the non-treated healthy tissue.

The concept of this novel approach to improve efficiency in lesion resection after the MR-HIFU-mediated ablation procedure starts with intravenous administration of fluorescein-containing liposomes (FCL). To ensure a sufficient liposome accumulation at the tumor tissue, it is essential to include cholesterol and poly(ethylene glycol) (PEG) into the liposome formulation for high stability and long circulation, respectively, while the inclusion of a thermosensitive phospholipid such as 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) enables MR-HIFU-mediated fluorescein release from the liposomes. By virtue of the enhanced permeability and retention (EPR) effect, liposomes with a size of around 100 nm are able to extravasate from the blood vessels into the tumor tissue [17–21]. Subsequently, the high ablation temperature as applied by MR-HIFU will trigger the FCL to release their dye, which will diffuse throughout the surrounding tumor tissue, resulting in tumor demarcation.

In this study, FCL, which are able to release their content after exposure to MR-HIFU-mediated ablation temperatures, are developed, and proof-of-concept is provided for the proposed approach to improve lumpectomy based on a combination of FCL and MR-HIFU.

2. Materials and methods

2.1. Materials

All chemicals and lipids were commercially available and used as obtained. 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-ethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (PEG_{2000}-DSPE) were obtained from Lipoid GmbH (Ludwigshafen, Germany). Cholesterol (>99%), glutathione (reduced, >99%); Triton X-100 and silicon dioxide (SiO_2, ~99%, 0.5–10 μm) were supplied by Sigma Aldrich (Steinheim, Germany). Sodium fluorescein (100 mg/mL) was obtained from Serb Laboratories (Paris, France). HMPAO kit was purchased from Ceretec (Amersham, Arlington Heights, IL). ^{99m}Tc-pertechnetate was obtained from a molybdenum-99/technetium-99m generator (Mallinckrodt, Petten, The Netherlands). *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (NBD-PE) was purchased from Invitrogen (Carlsbad, CA). Agarose MP was obtained from Roche Applied Science (Mannheim, Germany).

2.2. Liposome preparation

The FCL were prepared with the conventional thin-film hydration technique as described previously [20] and consisted of DPPC, cholesterol and PEG_{2000}-DSPE in a molar ratio of 1.85:1:0.15. The lipid mixture (100 mM) was dissolved in ethanol (10 mL) and evaporated to dryness by rotary evaporation under vacuum (Rotavapor R-210, BUCHI Laboratory Equipment, Zurich, Switzerland). The resulting lipid film was further dried under N_2 to ensure that all ethanol had evaporated. The lipid film was hydrated with 10 mL fluorescein (100 mg/mL). For control experiments, ^{99m}Tc-HMPAO- and NBD-PE-containing liposomes were prepared using the same technique. For the ^{99m}Tc-HMPAO-containing liposomes, the lipid film was hydrated with 10 mL glutathione (15.4 mg/mL). NBD-PE was incorporated with the lipid bilayer to obtain a liposome composition of DPPC, cholesterol, PEG_{2000}-DSPE and NBD-PE in a molar ratio of 1.85:1:0.15:0.03 (NBD-PE 1% of total lipid concentration) at 15 μmol/mL. The lipid film was hydrated with 10 mL HEPES-buffered saline (HBS, 20 mM HEPES and 135 mM NaCl, pH 7.4). The resulting lipid dispersions were sized with sequential extrusion using a Lipex Extruder (Northern Lipids Inc., Vancouver, Canada) and polycarbonate membrane filters (Poretics Corporation, Livermore, CA) with a pore diameter of 600, 200, 100 nm and 50 nm to obtain liposomes with an average diameter of around 100 nm.

2.3. Liposome characterization

The average hydrodynamic size and polydispersity index (PDI) of the liposomes were determined with dynamic light scattering (DLS) using a Malvern ALV CGS-3 system (Malvern Instruments Ltd., Worcestershire, United Kingdom). The PDI value can range from 0 for a monodisperse to 1 for a heterodisperse formulation. Intensity correlation functions were measured using a wavelength of 632.8 nm at a scattering angle of 90°.

Non-encapsulated fluorescein or glutathione was removed by dialysis in HBS using Slide-A-Lyzer cassettes with a molecular weight cut-off of 10 kDa (Pierce, Rockford, IL) during 48 h with four times a change of buffer. Dye concentration of FCL was determined by spectrophotometric measurement (UV Mini 1240, Shimadzu Scientific Instruments, Columbia, USA, $\lambda = 490$ nm) after lysis of the liposomes using 2% Triton X-100 in HBS.

The total phospholipid concentration of the liposomes after extrusion was determined with the phosphate assay as described by Rouser et al. [22]. Preformed glutathione-containing liposomes were labeled with ^{99m}Tc-HMPAO as described previously by Phillips et al. [23]. Briefly, 740 MBq $^{99m}TcO_4^-$ was added to a lyophilized HMPAO kit. Incubation of 0.5 mL liposomes with 1 mL $^{99m}TcO_4^-$ HMPAO results in a concentration of 14.8 MBq/μmol lipid. Labeling efficiency of ^{99m}Tc-HMPAO-containing liposomes was determined in the liposome fraction after removal of the non-encapsulated ^{99m}Tc-HMPAO using a PD-10 desalting column (Amersham Biosciences, Uppsala, Sweden) with HBS as eluent. The radioactivity of the liposomes was measured with a VDC-404 dose calibrator (Veenstra Instruments, Joure, The Netherlands). Differential scanning calorimetry (DSC) measurements were performed in a capillary cell microcalorimeter instrument (MicroCal VP-DSC, Northampton, MA) to determine the phase transition melting temperature (T_m) of the liposomes. The experiment was performed at temperatures ranging from 20 to 70 °C at a heating rate of 1 °C min^{-1} after an equilibration period of 15 min at 20 °C.

2.4. Liposome stability

To investigate liposomal stability, the liposomes were stored at 4 °C for 6 months. Afterwards, three samples of each liposome batch were taken to measure size and PDI. Besides, the three freshly prepared liposome compositions were heated to 60 °C for 180 min to investigate the size distribution and PDI of the liposomes after an extended heating period.

2.5. In vitro FCL release studies

For quantitative dye release analysis, the FCL and NBD-PE-containing control liposomes were exposed to different temperatures. Therefore, agarose phantoms (0.5%) were prepared with deionized water in 50 mL BD Falcon™ tubes. A small cavity was created with a 100 μL pipette tip in the middle of the agarose phantom before the onset of gelation. The remaining cavity after the tip was removed was filled with liposomes containing a total dye concentration of 130 μM. The agarose phantoms were heated to 20, 37, 40, 43, 47 and 60 °C for 30 s using a thermostat-controlled water bath. To study the effect of room and body temperature, the agarose phantoms were exposed to 20 and 37 °C for an extended period of 180 min. After heat exposure, the agarose phantoms were stored at 4 °C and dye release was measured with a Photon Imager (Biospace Lab, Paris, France) 24 h after heat exposure to allow the released dye to diffuse throughout the agarose phantoms. Quantitative data (number of fluorescent counts) were collected and analyzed using M3Vision software (Biospace Lab, Paris, France). FCL and NBD-PE-containing liposomes lysed with 2% Triton X-100 were used as positive control. Agarose phantoms (0.5%) without liposomes were used as negative control.

2.6. Ex vivo liposome release studies

To determine the feasibility of FCL application in a clinical setting, FCL (800 μM) were mixed with human blood (1 mL:1 mL) and exposed to 20, 37 and 60 °C for 30 s for visual detection of the released dye after heat-triggering. Photographs of the samples were taken under normal light conditions and UV light (350 nm).

Additionally, ^{99m}Tc release from liposomes was investigated in porcine muscle tissue using planar nuclear imaging with a dual-head gamma camera (Vertex-MCD, ADAC, Milpitas, California) before and after heat exposure of the tissue. Two pieces of porcine musculus semitendinosus were obtained from a pig that was previously used as a laboratory animal. The tissue was stored at 7 °C and used within 24 h after the pig was euthanized. The muscle tissues were injected with 1 mL of ^{99m}Tc-HMPAO liposomes of which one was heated to 60 °C using hot air flow and one was kept at room temperature (20 °C), as monitored by a locally placed thermometer. Diffusion of ^{99m}Tc throughout the tissue was allowed for 2 h at room temperature. The acquisition time was 90 s with a count rate of approximately 10,000 cps. The images were acquired in a 256 × 256 matrix with a pixel size of 2.32 mm × 2.32 mm and a 20% window centered around the 140 keV photopeak using a low energy collimator. Posterior and anterior images were fused in one image.

2.7. MR-HIFU-triggered release study

Agarose phantoms were prepared in polypropylene beakers with 0.5% agarose, 2% SiO_2 and 800 mL deionized water. SiO_2 is added for a higher absorption coefficient of the agarose gel, resulting in better absorption of the ultrasound beam. A small cavity was created in the middle of the agarose phantom with a 2-mL pipette tip, which was inserted in the middle of the agarose before the onset of gelation. After the agarose was cooled down, the pipette tip was removed and the remaining cavity was filled with a mixture of liposomes containing a dye concentration of 800 μM and agarose gel, which was cooled down to 37 °C to prevent premature release from liposomes. After cooling down, more agarose gel (37 °C) was put on top until the cavity was completely covered to prevent scattering of the ultrasound beam during MR-HIFU exposure.

The agarose phantoms containing FCL or the control NBD-PE-containing liposomes were heated with the Philips Sonalleve 3T MR-HIFU system (Philips Healthcare, Helsinki, Finland). This system was used to heat a volume of approximately 1.2 × 1.2 × 5 cm^3 using a 256-element phased-array ultrasound transducer with volumetric heating capabilities and feedback control [24]. The ultrasound field was continuous wave with a frequency of 1.2 MHz. Planning of the target area and temperature mapping during treatment was performed by a 3T Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands). The temperature measurements were performed according to the Proton Chemical Shift (PRF) method [25]. MR phase images of a gradient echo sequence were used to calculate temperature maps. When the spatial temperature in the field-of-view of the first acquired phase image is known (e.g. in equilibrium at room temperature), the temperature distribution in the second acquisition can be considered as an absolute temperature map. The temperature maps were used as input for the binary feedback loop of the HIFU transducer. The binary feedback loop modulated in real-time the transducer power resulting in a precise control of the temperature in the field-of-view. The MR gradient echo sequence is a dynamic multi-slice sequence with the following parameters: TR/TE = 28/20 ms; flip angle = 19°; matrix = 160 × 99; field-of-view = 400 × 250 mm; voxel size = 2.5 × 2.5 × 7 mm; acquisition time per dynamic scan = 2.5 s. The MR slices were arranged as described by Kohler et al. [26]. After a short preheating period (around 30–120 s), the desired temperature of 42 and 60 °C was reached and maintained for 30 s. The temperature accuracy was 0.1 °C based on the MR signal intensity [27].

After 24 h of storage at 4 °C, the agarose phantoms were sliced along the midsagittal plane. Dye release from the focal spot was measured with the Photon Imager and analyzed using M3Vision software as described previously. The total dye release after MR-HIFU treatment was compared with FCL or NBD-PE-containing liposomes lysed with 2% Triton X-100 were used as positive control. Liposome-embedded agarose phantoms, which were not treated with MR-HIFU, were used as negative control.

3. Results

3.1. Liposome preparation and characterization

FCL were prepared to determine their implementation as a diagnostic tool in lesion demarcation after MR-HIFU-mediated ablation (Fig. 1). Liposomes containing the lipophilic label NBD-PE were prepared as control liposomes and ^{99m}Tc-HMPAO-containing liposomes were used for *ex vivo* studies (Table 1). Average diameter of the liposomes was around 110 nm and the PDI was

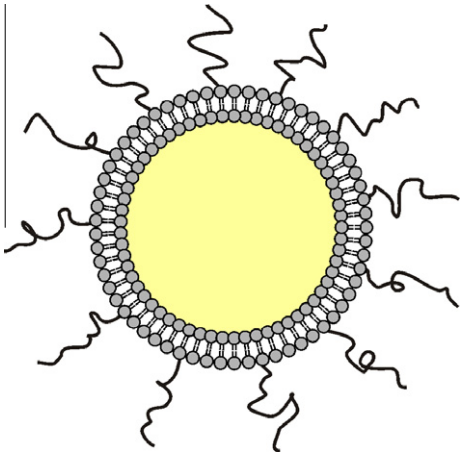

Fig. 1. Schematic representation of the FCL showing a PEG-coated lipid bilayer surrounding an aqueous compartment containing fluorescein.

Table 1
Liposome characteristics.

Liposome type	Label	Intraliposomal location	Dye concentration
FCL	Fluorescein	Internal aqueous phase	7.1 mg/ml
NBD-PE	NBD	Lipid bilayer	131 μg/ml
^{99m}Tc-HMPAO	^{99m}Tc	Internal aqueous phase	134 MBq/ml

low in every liposome dispersion (all $\leqslant 0.05$), which indicates a narrow size distribution. Storage of liposomes for 6 months at 4 °C and heating of liposomes to 60 °C for 180 min did not influence the size distribution of the liposomes, indicating that the liposomes are stable regarding changes in particle size distribution (Fig. 2). DSC measurements confirmed the T_m of the FCL to be at 42 °C, which corresponds with the T_m of DPPC.

3.2. In vitro liposome release studies

The degree of dye release from FCL exposed to 20, 37, 40, 43, 47 and 60 °C for 30 s is presented in Fig. 3a. Exposure to 43 °C resulted in 38% release, while 68% release was observed after exposure to 60 °C. Exposure to 20 and 37 °C, even after an extended exposure period of 180 min, did not result in any detectable release. NBD-PE-containing liposomes exposed to 37 and 60 °C for 180 min did not show detectable release of the fluorescent label from liposomes into the agarose phantoms (Fig. 3c), while NBD-PE liberated using 2% Triton X-100 from the liposomal bilayer was able to diffuse throughout the agarose gels. This indicates that the liposomal formulation remains stable after heating. In order to exclude any direct effects of temperature elevation on fluorescence levels of fluorescein, an experiment was performed on fluorescein solubilized in demineralized water. The samples were heated for 15 min from 20 °C up to 60 °C, and emission spectra were measured. No detectable change in the emission spectra was observed at different temperatures (data not shown), hence the increase in fluorescence can exclusively be ascribed to release from the FCL.

In Fig. 4, photographs from FCL in human blood exposed to different temperatures for 30 s, taken under normal light conditions (Fig. 4a–c) and 350 nm UV light (Fig. 4d–f), are shown. No visual differences between the samples were observed under normal light conditions. However, under UV light conditions, fluorescence was clearly enhanced after exposure of the FCL to 60 °C (Fig. 4f).

3.3. Ex vivo release studies

Distribution of fluorescein throughout tissue cannot be visualized directly in large tissue samples due to the insufficient tissue penetration depth. Therefore, *ex vivo* porcine muscle tissue was injected with liposomes labeled with the water-soluble radioisotope ^{99m}Tc (^{99m}Tc-HMPAO-containing liposomes) to evaluate the release properties of the liposome formulation in tissue. Planar nuclear images of the tissue before and after heat exposure (60 °C) are shown in Fig. 5. Fig. 5b indicates the diffusion of ^{99m}Tc in tissue after heat-triggering, which supports the feasibility of MR-HIFU-triggered release from liposomes in tissue. In control tissue, which was not exposed to heat, ^{99m}Tc did not show such enhanced diffusion from the injection spot throughout the tissue (Fig. 5c and d).

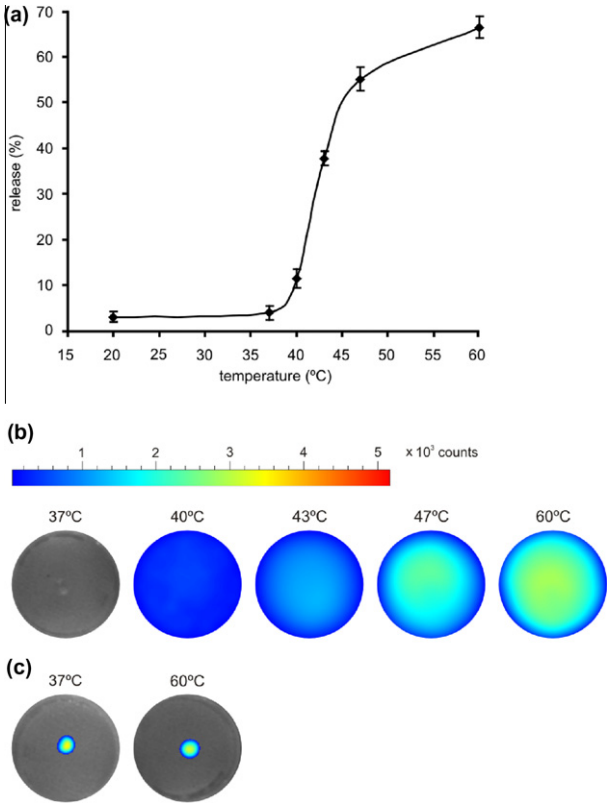

Fig. 3. Percentage of fluorescein release from liposomes after 30 s exposure to different temperatures (mean ± SD, $n = 3$) (a). Fluorescence measurements in FCL-containing phantoms exposed to different temperatures for 30 s (b). Fluorescence of NBD-PE-containing liposomes in agarose phantoms after heat exposure to 37 and 60 °C for 30 s (c).

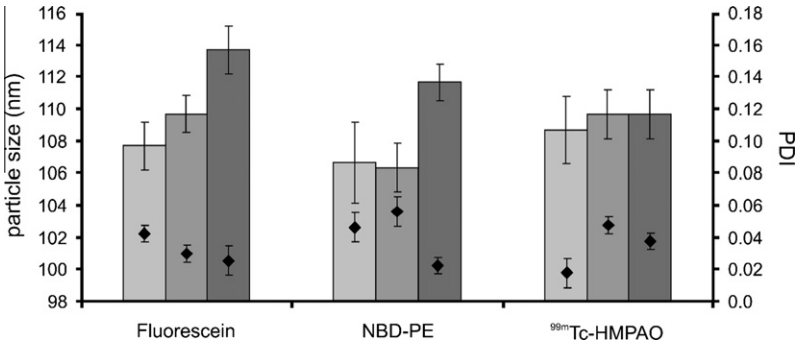

Fig. 2. Liposome size (nm) and PDI (mean ± SD, $n = 3$). Diameter of freshly prepared liposomes (□). Liposome diameter after 180 min of exposure to 60 °C (■). Diameter of liposomes 6 months after storage at 4 °C (■). PDI ± SD is indicated by (◆).

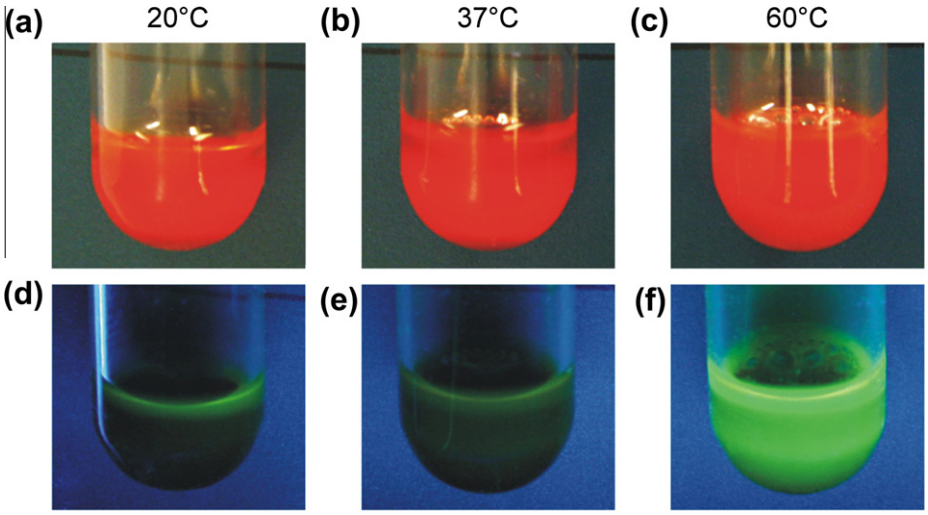

Fig. 4. Photographs from FCL dispersed in human blood. Samples are exposed to 20, 37 and 60 °C for 30 s. Photographs taken under normal light conditions (a–c). Photographs taken under 350 nm UV light emission (d–f). Exposure to 20 and 37 °C (d and e). After exposure to 60 °C, fluorescence was clearly enhanced (f).

3.4. MR-HIFU-triggered release study

The liposome spot inside the agarose phantoms was treated with MR-HIFU, as is depicted in Fig. 6a. This region of interest (ROI) was heated to 42 °C (T_m of DPPC) and 60 °C (ablation temperature) for 30 s with MR-HIFU. In Fig. 6b and c, the heated liposome spot in the agarose phantom is shown with MRI temperature mapping to demonstrate the accuracy of the MR-HIFU procedure. MR-HIFU-induced heating to 42 °C resulted in 28% release, whereas 68% release was observed after heating to 60 °C. These results correspond with the heat exposure results that were obtained with a water bath (Fig. 3). MR-HIFU treatment of NBD-PE-containing liposomes did not lead to fluorophore distribution throughout the agarose phantoms, which is also in agreement with the *in vitro* water bath results (see above).

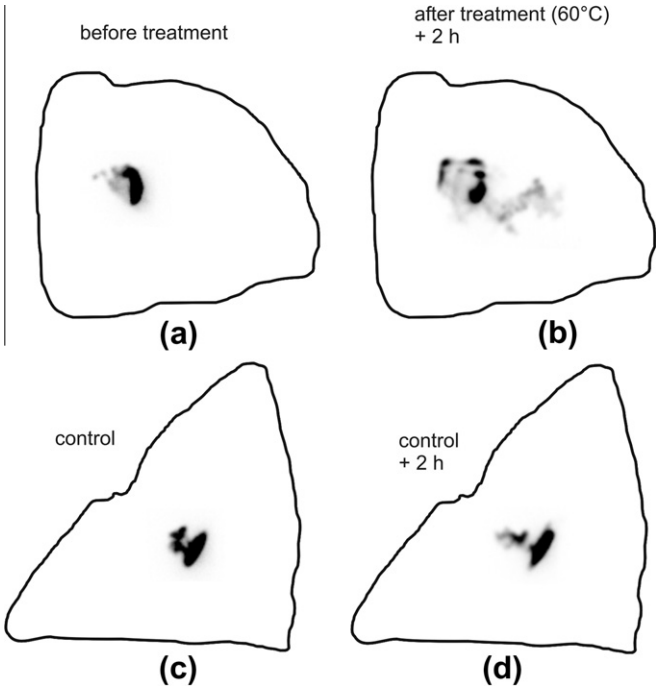

Fig. 5. Planar nuclear images of porcine muscle tissue injected with ^{99m}Tc-HMPAO-containing liposomes. Distribution of ^{99m}Tc in the tissue before and after heat exposure to 60 °C (a and b). Diffusion of ^{99m}Tc throughout the tissue was observed after heat-triggering. In control tissue, such enhanced distribution was not observed (c and d).

4. Discussion

This study was performed to obtain proof-of-concept for the applicability of stable, long-circulating FCL as a tool for tumor demarcation of non-palpable breast lesions after MR-HIFU-mediated ablation. The liposomal size distribution of around 110 nm enables passive tumor targeting facilitated by the EPR effect [17]. A 6-month storage period at 4 °C showed that the FCL do not leak their fluorescent label and are stable regarding changes in particle size distribution. A pharmaceutical acceptable shelf-life is important for usage in a clinical setting and underlines the clinical applicability of the FCL formulation.

Temperature-induced fluorescein release from liposomes was investigated by exposure of liposome-embedded agarose phantoms to different temperatures. Exposure of these phantoms to 20 and 37 °C did not result in detectable release. Fluorescein release was limited at 40 °C (about 10%) after short exposure times (30 s) while it increased to 68% after 30 s exposure to 60 °C (Fig. 3), illustrating the sensitivity of the liposome formulation for temperature changes above 37 °C. No fluorophore distribution was observed after heating the NBD-PE-containing liposomes to 60 °C, demonstrating the high stability of the liposome formulation. Exposure of FCL dispersed in human blood to 20, 37 and 60 °C for 30 s showed a clear enhancement of fluorescence at 60 °C compared to body temperature (37 °C). The liposomal fluorescein concentration used in these experiments is sufficient for staining in a clinical setting. Due to self-quenching inside the liposomes, fluorescence is only observed when fluorescein is released from the liposomes. This attractive property contributes to efficient lesion demarcation since fluorescence will only be detected around the heated area, which excludes false positive staining of healthy tissue.

Although animal-derived breast tissue or *ex vivo* human mammary tissue may resemble breast tissue in a clinical setting, these tissues, however, do not resemble tumor tissue itself with respect to the tissue consistency. Tumor tissue is often characterized as dense tissue, which is highly vascularized at the tumor periphery. For this reason, muscle tissue was determined to be a suitable alternative for tumor tissue in this setup for *ex vivo* diffusion

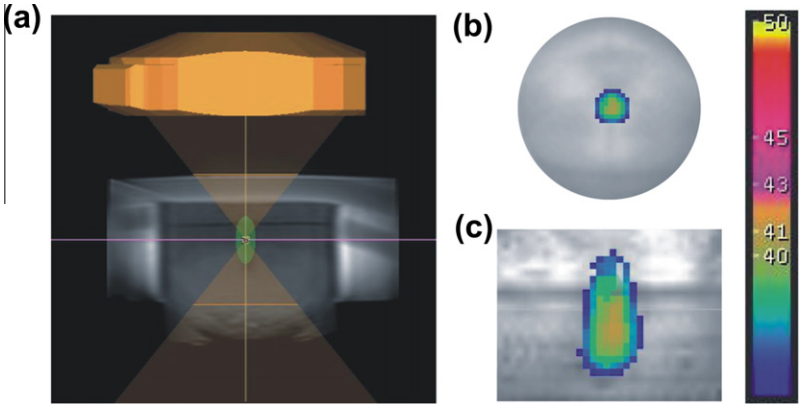

Fig. 6. Schematic drawing of the treatment planning positioned on the MR image of the agarose phantom during MR-HIFU treatment; the intended treatment cell is shown in green (a). Transverse plane of the agarose phantom during the 42 °C treatment, the temperature distribution can be deducted from the colour bar (b). Midsagittal plane of the agarose phantom during 42 °C treatment, indicating the heated area and reflecting the treatment cell as prepared during the treatment planning (c).

experiments after heat-triggered liposome release of ^{99m}Tc. *Ex vivo* experiments with porcine muscle tissue injected with ^{99m}Tc-HMPAO showed enhanced distribution of ^{99m}Tc after heating to 60 °C, indicating release and diffusion of ^{99m}Tc. This result additionally confirms the potential applicability of the liposome formulation for lesion demarcation in tissue.

To demonstrate the occurrence of temperature-induced release after MR-HIFU-triggering, FCL-embedded agarose phantoms were exposed to MR-HIFU ablation for 30 s. Short ablation temperatures are critical when using MR-HIFU in the body. The short exposure period is in accordance with clinically relevant MR-HIFU parameters [24,26]. Long heating periods may cause peripheral absorption of heat in non-targeted tissue, yielding the risk of unpredictable thermal lesions [6]. Short exposure of FCL-embedded agarose phantoms to MR-HIFU at 42 and 60 °C resulted in dye release kinetics in line with the *in vitro* results obtained with a water bath.

5. Conclusion

In conclusion, herein proof-of-concept for a novel approach for lesion demarcation using the combination of long-circulating fluorescent liposomes and MR-HIFU-mediated ablation is presented. FCL, which release their fluorescent content after 30 s exposure to MR-HIFU, which is in line with the clinically relevant MR-HIFU treatment parameters, were successfully prepared. After release, the treated fluorescent lesion can be detected with UV light. This method may offer a new tool for efficient surgical resection of non-palpable breast tumor lesions by enabling proper discrimination between tumor tissue and adjacent healthy tissue.

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