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Liposomal nanoparticles enhance contrast of fluorescence, speckle-contrast imaging, and ultrasound measurements in phantoms and murine model

Olga A. Stelmashchuk[a], Andrey Vinokurov[a], Mikhail Apanaykin[b], Igor Kozlov[c], Andrian Mamoshin[a], A. Borsukov[d], Andrey V. Dunaev [c]

[a]The Cell Physiology and Pathology Laboratory, Orel State University, Orel, Russia 302026
[b]Orel State University named after I.S. Turgenev, Komsomolskaya 95, Orel, Russia, 302026
[c]Research and Development Center of Biomedical Photonics, Orel State University named after I.S. Turgenev, 95 Komsomolskaya St., Orel, Russia 302026
[d]Smolensk State Medical University, Problem Scientific and Research Laboratory "Diagnostic Researches and Minimally invasive Technologies", Krupskaya 40, Smolensk, Russia, 214019

ABSTRACT

Liposomal particles are used as an instrument for drug delivery and as fluorescent labels. Due to its properties, liposomal particles can be used as a dual-purpose contrast agent both in fluorescence imaging, laser speckle contrast imaging and ultrasound diagnostics. Liposomes obtained by one purpose but subsequently contained a fluorescent label or air. This work aimed to prepare liposomal particles and to study possibilities of its application for the dual-use in fluorescence spectroscopy and ultrasound diagnostics. In this work, we used fluorescence spectroscopy to investigate the efficiency of propagation in the circulatory system of fluorescent-labelled liposome nanocapsules loaded with eosin-Y (disodium salt of 2,4,5,7 tetrabromofluorescein These results demonstrate that liposomal particles can be used in fluorescence spectroscopy, in ultrasound diagnostics and in LSCI. It may find the application in the field of drug delivery control and high-throughput screening during drug development.

1. KEYWORDS: LIPOSOMAL PARTICLES, LASER SPECKLE CONTRAST IMAGING, FLUORESCENCE SPECTROSCOPY, BLOOD MICROCIRCULATION, OPTICAL MEASUREMENTS IN VIVO, ULTRASONIC CONTRAST

1. INTRODUCTION

Liposomal particles have found application as a means of delivery of drug agents, contrast agents or fluorescent labels. Paramagnetic liposome particles, due to their ability to withstand the high payload of a Gd-containing lipid, can serve as a highly effective contrast agent for magnetic resonance imaging. [1]. The use of non-ionic iodinated vascular contrast agent encapsulated in polyethene glycol-stabilized (PEGylated) liposomes can be used to increase the contrast of the vascular bed using coherent tomography (CT). [2]. The acoustic and drug release properties of developed echogenic liposomes are discussed for use as agents for simultaneous imaging in ultrasound diagnostics and drug/gene delivery. [3,4]. Although microbubbles are currently the only FDA-approved ultrasound contrast media, liposomes and perfluorocarbon nanoparticles also can demonstrate their effectiveness as targeted contrast agents for fluorescence imaging.. Microbubble liposomal particles have proven to be useful in assessing the blood vessel velocity of microvessels in cardiology and radiology. Their predominant concentration in the vasculature, within the cell phagocytes, or the reticuloendothelial or lymphatic system allows passive targeting [5,6].

One of the promising methods for using liposomal particles is the contrasting of blood flow during laser speckle contrast imaging (LSCI). LSCI is a convenient and simple method for visualizing blood flow dynamics in real-time. LSCI for physiological research is relatively simple and not expensive to create[7]. It can quantify blood flow changes with excellent spatial and temporal resolution. LSCI is based on the principle that light scattered in the opposite direction from tissue illuminated by coherent laser light forms a random interference pattern on the detector, the so-called speckle pattern. The

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movement of particles inside the tissue causes oscillations in this speckle pattern, which leads to blurring of speckle images upon receipt with an exposure time equal to or greater than the speckle fluctuation time scale. This erosion may be associated with blood flow if fluctuations are caused by the movement of red blood cells (red blood cells)[8].
Measurements are limited to surface tissues without resolution in depth. The creation of a contrast drug for LSCI with fluorescence and ultrasound control of particle distribution has an essential role in preclinical studies. It will also use in the clinical practice of diseases of the circulatory system, including dermatological, neurosurgical and endoscopic studies [9,10].
This work aimed to obtain particles for multi-use in LSCI, fluorescence spectroscopy and ultrasound diagnostics.

2. MATERIAL AND METHODS

In this work, using the method of fluorescence spectroscopy, ultrasound diagnostics and laser speckle imaging, we investigated the effectiveness of using liposomes to increase the signal level in these methods. Liposomes obtained by one purpose but subsequently contained a fluorescent label or air. Liposomes were prepared using a modified version of the reverse-phase evaporation method. Phospholipids, cholesterol and PEG were dissolved in chloroform. The organic solvent was removed in a rotary evaporator under vacuum with the temperature of water bath 40 °C and rotor speed 100 rpm. Evaporation was stopped after thin lipid film formation. Ultrasound sonification was used for the hydration of lipid film. The duration of ultrasound treatment was chosen large enough for the complete film flushing and forming of liposomes with the diameter less than 500 nm. Fluorescent dye eosin-Y (the disodium salt of 2,4,5,7 tetrabromofluorescein) was encapsulated inside the liposomes. Passive inclusion was performed at the stage of the lipid film hydration. In case of empty liposomes lipid film was hydrated with the sterile saline. Unrelated eosin was removed from the liposome suspension using dialysis against saline. After obtaining of air-filled liposomes in the process of washing off the lipid film, air passed through a physiological solution through a thin needle using a compressor.
Experimental studies were performed on clinically healthy mice of outbred stock CD-1 at the age of 5 months [11](n = 6 in the group). Before the transfer of the animals to the clean zone of the vivarium and the beginning of the experiment, the animals were kept in quarantine for 14 days. When placed in quarantine, the veterinarian conducted a primary assessment of the animals' condition with the introduction of the results of the examination in the relevant act and examined animals daily. Immediately before the transfer to a clean zone and the formation of experimental groups, the veterinarian conducted a clinical examination of the animals. In the study, animals were selected with no signs of health related abnormalities, so that the average body weight did not differ statistically between the groups. Each animal was assigned an individual number. The basic rules of maintenance and care corresponded to the standards set out in the sanitary rules for the arrangement, equipment and maintenance of experimental biological clinics (and the position-guidance "Laboratory animals"[12].
Fluorescence spectroscopy was used to investigate the efficiency of propagation in the circulatory system of fluorescent-labelled eosin-Y (the disodium salt of 2,4,5,7 tetrabromofluorescein) liposome capsules. Maximum of intrinsic fluorescence of tested fluorescent dye (eosin-Y) lies in the region of 520±5 nm. Fluorescence channel with a fibreprobe series of multifunctional laser non-invasive diagnostic system "LAKK-M" ("LAZMA" Ltd, Russia) was used as the measuring equipment. A fluorescence channel with a fibre-optics probe in series with the multifunctional laser-based non- for fluorescence spectroscopy (ex = 450 nm)[13] on the Figure 1 has been used in this study.

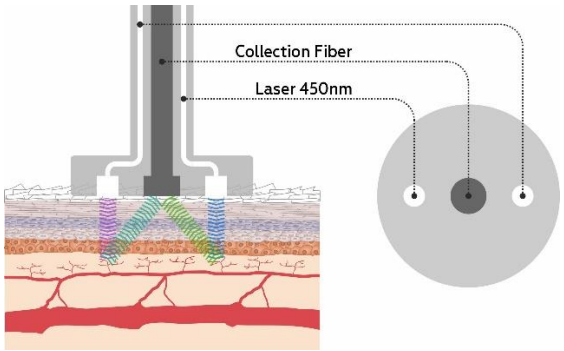


Figure 1.The study of fluorescence spectra in mice using "LAKK-M" system.

The following LSCI-based experimental setup illustrated on the Figure 2 has been used in this study: 10 mW laser source working at 635 nm (Edmund Optics Inc, USA) illuminated the object. Further, CMOS- camera DCC 3260M (Thorlabs, USA) with 1936×1216 pixels and 5.86 μm pixel size was used in combination with 34 mm Mitutoyo Plan Apochromat Objective MY5X-802 (Thorlabs, USA) for raw speckle image acquisition. The obtained images were processed by a custom-developed algorithm in the offline regime using a MATLAB r2017b software environment.
The phantom consisted of a capillary tube (diameter 1 mm). Suspension of Intralipid 20% andliposomes in water was pumped through the capillary tube by a syringe. Changes in the speed of movement of Intralipid/liposomes in a test tube were recorded by a camera, then frame sequences were processed[14].

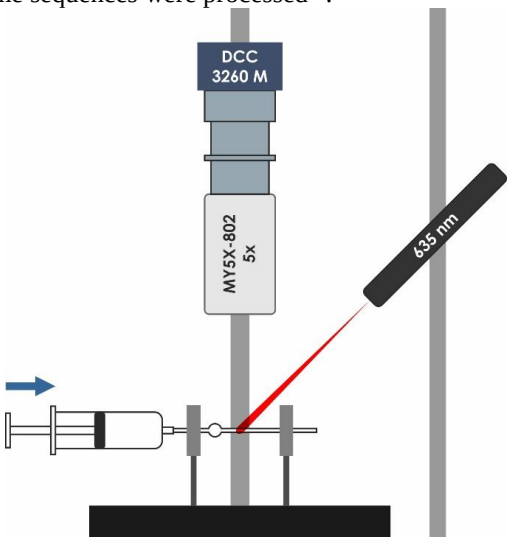


Figure 2. The setup with capillary tube and syringe.

For ultrasound examination, an apparatus was used by the ultrasound apparatus Esaote MyLab 50, with a frequency of 1-8 MHz. During the study, experiments carried out on phantom units to verify the increase in contrast properties of the synthesized particles. The phantom setup is shown in Figure 3.

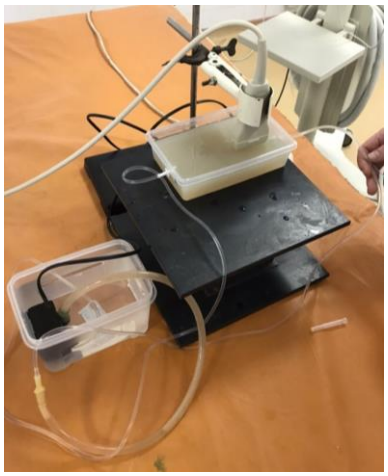


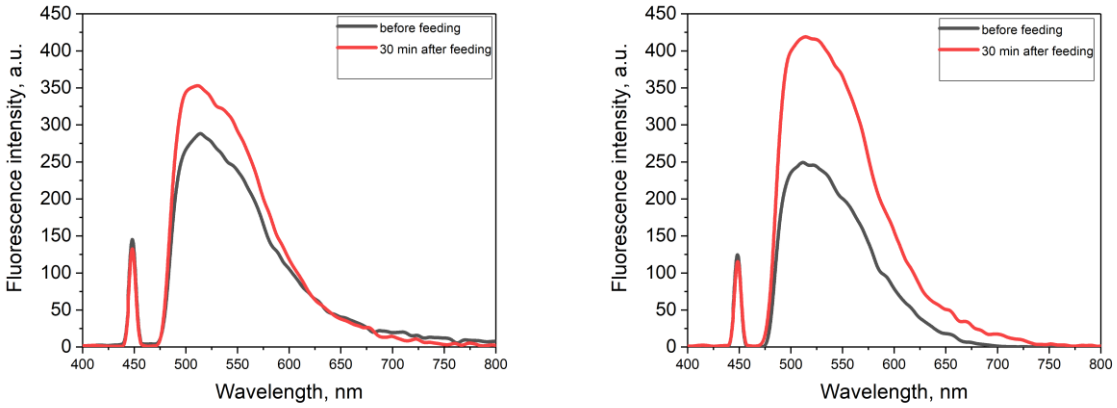
Figure 3. Phantom unit for ultrasound diagnostics.

The study conducted on a phantom setup with elastic tubes placed in agar-agar, through which an isotonic solution passed at a speed of 0 to 20 cm/s.

3. EXPERIMENTAL RESULTS AND DISCUSSION

Using the method of fluorescence spectroscopy, we were able to assess the degree of penetration of the particles into the bloodstream upon oral administration. Not invasive measurements performed on the proximal part of the mouse's tail. Due to its anatomical structure, the tail is well suited for fluorescence measurements of blood flow in upper layers of the dermis. The rear has a well developed subcutaneous arterial and venous network with a large number of anastomoses between the vessels. The degree of Eosin labelled liposomes penetration through the intestine can be inferred by recording changes of fluorescence in the blood flow. To reduce photobleaching affect the exposure of optical radiation on a tissue did not exceed 2 seconds per measurement.

Twelve mice of outbred stock CD-1 were divided into two groups: treated with eosin-Y (n = 6) and control dye without liposomes (n = 6). Maximum fluorescence intensity, 140% of the initial level, was recorded 30 min after oral administration. In the group of animals which received fluorescent dye without liposome membrane fluorescence, the increase did not exceed 110% of the initial level. Presented in Figure 4.

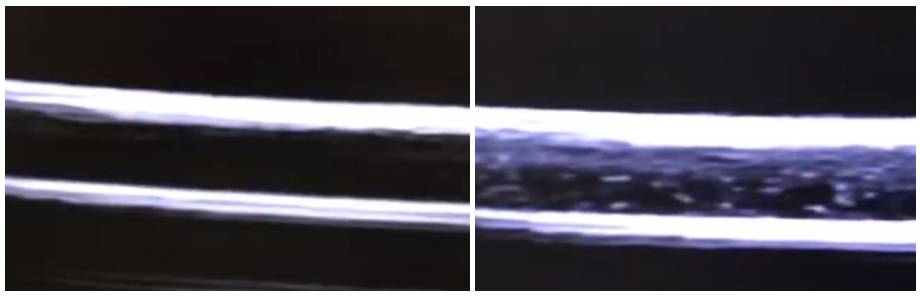


(a) (b)

Figure 4. Fluorescence spectra of the: a) group received fluorescent dye; b) group received liposome with incorporated fluorescent dye.

Experimental results show that the received multilayer liposome particles may increase the effectiveness of the transported material entering the bloodstream via the gastrointestinal tract after oral administration[12].

For studies of increasing echogenicity, liposome particles used phantom setup. It had an imitation of vascular-thin-walled latex tubes in the agitated agar solution[15]. The tube system had a cyclic structure with a pump to maintain the speed of the solution. The introduction of liposomes in the model system made it possible to evaluate the quality of the proposed particles from the standpoint of increasing echogenicity in the lumen of the tube. Figure 5 presents a snapshot of a sonographic image.



(a) (b)

Figure 5. Phantom unit sonographic image: a) saline; b) saline after administration liposome with air.

The circulation time in a closed system at a speed of 20 cm / s was 11 minutes until an ultrasonic wave utterly destroyed the particles. The contrast level increased by 57% of the initial concentration in control. The particle circulation time was 11 minutes until the ultrasonic wave utterly destroyed the particles. The increase in signal is due to the physical features of gas-filled particles. Modern pulse ultrasound devices use the principle of summation of signals. Pulses from tissues are linear, and from gas-containing particles - nonlinear. Particles begin to pulsate with a specific resonant frequency and give an answer that is different from the tissue. Based on these properties, modern contrast preparations for ultrasound diagnostics have created [16,17].

To test the ability of liposomal particles filled with air to increase the contrast of vessels for laser speckle imaging spectroscopy, a setup made as described in 10, the diagram and general view of which shown in Fig. 2. The installation consists of a capillary tube (inner diameter 1.6 mm), through which an 8% (by volume) solution of intralipid 20% (Fresenius Kaby, USA), intralipid with liposomal particles and intralipid infused using an electric pump calibrated by current / speed with liposomes with air. The selected 8% concentration of intralipid approximately corresponds to the optical scattering properties of blood at wavelengths of 635 and 785 nm. A camera recorded changes in the speed of motion of scattering particles in a capillary tube, data post-processing performed in MATLAB r2017b. To test the LSCV system, intralipid solutions with liposomes passed through a capillary tube with linear velocities of 0, 0.5, 1, 1.5, and 2 mm / s, which covers the in vivo range of blood velocities in capillaries and arterioles, as well as the expected increase in speeds by 2–3 times at the time of reperfusion. The measurements performed twice: when the capillary tube illuminated with 635 nm wavelength of laser radiation. Thus, the selected speed of the scattering liquid in the capillary exceeded the average rate of the capillary blood flow, the camera recording frequency for this part of the experiment was 40 frames/s 10.

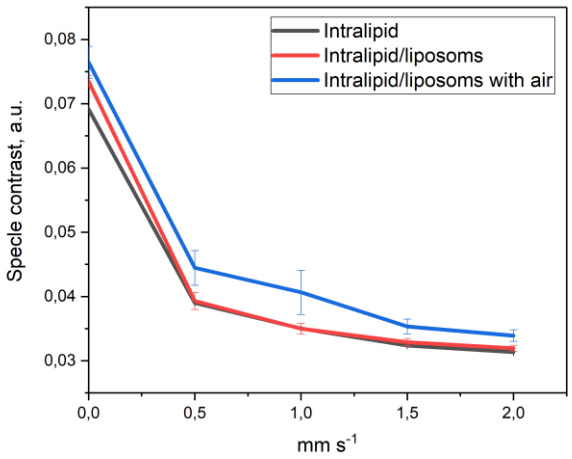


Figure 6. Phantom unit specle contrast.

Analyzing the data obtained, we can conclude that the proposed gas-filled liposomal particles can increase the level of contrast. It is believed that the optical properties of moving fluid and surrounding tissue can influence LSCI values. Several values can affect local contrast values in laser speckle contrast imaging (LSCI), regardless of relative motion. Khaksari found that the influence of the particle scattering coefficient on the increase in speckle contrast is greater than the impact of the absorption coefficient.[18]. Filling bioavailable particles with gas allows you to change the dispersion coefficient and thereby increase the level of speckle contrast parameter.

4. CONCLUSION

These results demonstrate that liposomal particles can be used in fluorescence spectroscopy, in ultrasound diagnostics and in LSCI. The combination of these properties will make it possible to obtain more informative data in studies of blood flow, perfusion level and the state of parenchymal organs with the possibility of targeted drug delivery and simultaneous fluorescence imaging. Using a single dose of particles will provide a wide range of information and offer fewer invasive procedures. The results can be used in the field of drug control and in the process of high-throughput screening during drug testing

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