Review: optically-triggered phase-transition droplets for photoacoustic imaging

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Abstract

Optically-triggered phase-transition droplets have been introduced as a promising contrast agent for photoacoustic and ultrasound imaging that not only provide significantly enhanced contrast but also have potential as photoacoustic therapeutic molecular probes incorporated with targeting molecules and therapeutics. For further understanding the dynamics of optical droplet vaporization process, an innovative, methodical analysis by concurrent acoustical and ultrafast optical recordings, comparing with a theoretical model has been employed. In addition, the repeatability of the droplet vaporization-recondensation process, which enables continuous photoacoustic imaging has been studied through the same approach. Further understanding the underlying physics of the optical droplet vaporization and associated dynamics may guide the optimal design of the droplets. Some innovative approaches in preclinical studies have been recently demonstrated, including sono-photoacoustic imaging, dual-modality of photoacoustic and ultrasound imaging, and super-resolution photoacoustic imaging. In this review, current development of optically triggered phase-transition droplets and understanding on the vaporization dynamics, their applications are introduced and future directions are discussed.

Keywords Photoacoustic agent · Multi-mode agent · Droplet · Optical vaporization · Recondensation · Perfluorocarbon

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1 Introduction

Photoacoustic (PA) imaging is a promising biomedical imaging modality that provides optical contrast at relatively deep depth complemented to traditional ultrasound imaging [1]. PA effect refers to the generation of acoustic waves by the excitation of optical radiation. Several mechanisms for generating PA signals have been known, which include electrostriction, thermal expansion, photochemical changes, gas evolution and plasma formation [2, 3]. For its reasonable PA conversion efficiency and safety, PA agents based on thermal expansion have been traditionally and widely utilized in biomedical imaging applications [3–5]. In recent years, optically-triggered phase-transition droplets were introduced as a promising PA contrast agent that produce significantly stronger PA signals through the vaporization process in response to a short pulse laser [5]. If synthesized in nanoscale, these tiny droplets before being activated and vaporized can passively accumulate to the target area in a tumor, due to the enhanced-permeability and retention (EPR) effect [6]. These droplets can also be utilized as a dual-mode contrast agent for combined ultrasound (US) and PA multimodal

imaging, as the laser-activated droplets that become gaseous microbubbles are hyperechoic due to significant mismatch of acoustic impedance at the boundary [5, 7, 8]. In this review, we introduce initiative efforts in developing the optically-triggered phase-transition droplets and understanding the underlying mechanisms of the vaporization and associated dynamics for PA imaging. Their applications and future directions are also discussed.

1.1 Optically-triggered phase-transition droplets for photoacoustic imaging

Laser-activated phase-transition droplets have been suggested as a promising PA contrast agent because of the significantly stronger PA signals mainly due to higher energy conversion efficiency through vaporization compared to thermal expansion. The droplets in general consist of a core of unstable PFC liquid, such as perfluoropentane (PFP, C_5F_{12} , boiling temperature of 29 °C) incorporated with chromophores and very thin outer shell of polymer, lipid, or albumin as shown in Fig. 1a. Therapeutics or targeting moieties may be incorporated for additional functional extension of the droplets. Near-infrared laser with laser fluence around 1.2 J/cm² at focus was utilized as the energy source for activating the droplets. Absorbed optical energy by the chromophores is converted into thermal energy, resulting in local, transient temperature rise and therefore vaporization of the unstable liquid, called the optical droplet vaporization (ODV) that is illustrated in Fig. 1b [9]. This rapid volume expansion during the ODV generates PA signal with large amplitude. Because PFC-family typically absorbs little optical energy in near-

infrared (NIR) region [10], a chromophore with a strong absorption peak at NIR, such as indocyanine green (ICG) or methylene blue, is incorporated as a heating source. NIR light is preferably adopted due to its low background absorption by human tissues, which maximizes the penetration depth. The shell of a droplet stabilizes the unstable liquid core by increasing the boiling temperature due to the Laplace pressure [11, 12]. The Laplace pressure is built depending on the surface tension at the boundary interface and droplet size as described by

$$P_{\text{inside}} - P_{\text{outside}} = \frac{2\sigma}{R}, \quad (1)$$

where P_{inside} and P_{outside} are the pressure inside a droplet and an ambient pressure, respectively, σ is the surface tension at the boundary and R is a droplet radius [11, 12]. By plugging the calculated Laplace pressure into the Antoine equation with the premeasured vapor pressure, vaporization threshold of the droplet can be estimated [13]. The vaporization threshold temperature of the PFP-based droplet with 1, 5, and 9 μm in diameter is estimated to be 73.2, 42.6 and, 37.3 °C, respectively, while the bulk boiling temperature of PFP is 29 °C [12]. Therefore, the droplets formed in small size (< 3 μm) can sustain its liquid phase at quasi-superheated state during circulation when administered, because its increased vaporization threshold temperature is higher than the physiological temperature (37 °C) [12]. Considering the exponentially increasing Laplace pressure for submicron size droplets, a liquid core with a very low bulk boiling temperature can be chosen depending on an appropriate purpose although it maintains in a gaseous state at room temperature. For example,

Fig. 1 **a** Structure of PA theranostic agent using optically triggered droplet. Plasmonic nanoparticles (chromophores) were used as a heating source. **b** Dual phase mechanisms of optically triggered

droplet; Vaporization (Step 1–3) and Thermal expansion (Step 4–6). Figures are reproduced with permission of [5]

Sheeran et al. [14, 15] suggested low boiling temperature PFC such as decafluorobutane (DFB, C_4F_{10} , boiling temperature of -1.7°C) and octafluoropropane (OFP, C_3F_8 , boiling temperature of -36.7°C) to synthesize the liquid droplets, which can be easily activated by low laser energy under the FDA limit and generates strong PA signal with high efficiency. This approach will enable nanoscale droplets to be used under the laser energy safety guidelines, which can have the benefit of EPR effect that tiny droplets would passively home to the tumor sites [16].

The droplets in the initial liquid phase are hypoechoic although they provide great PA contrast in response to the laser irradiation. Once vaporized, the liquid core of droplet undergoes a phase transition into the gas and becomes hyperechoic due to significant mismatch of acoustic impedance at the boundary interface, similar to microbubbles that have been widely used as US contrast agent in clinics. In this gas phase, it should be noted that PA signals would still be generated through the thermal expansion of the remaining chromophores with now reduced amplitude compared to that from the vaporization process. Therefore, the optically-triggered phase-transition droplets have potential to act as a dual mode contrast agent for a combined US and PA imaging (Fig. 1b) [5, 8].

1.2 Underlying physics of optically-triggered phase-transition droplets

The phase-transition droplets using high-intensity ultrasound as activation source have been extensively studied in the past decades [17]. A droplet emulsion vaporizes into a gas bubble much larger than its original size when the acoustic pressure applied to the droplet induces a temperature increase exceeding the vaporization threshold of the liquid core, called acoustic droplet vaporization (ADV) [18, 19]. For further understanding of ADV, the relationships among several key droplet design parameters, including vaporization temperature threshold, activation ultrasound frequency and intensity, shell properties, and droplet size have been thoroughly studied [20–22].

Along the same line, some significant efforts have been made to explore the strategy to efficiently achieve enhanced PA contrast using droplet vaporization triggered by a short pulse laser irradiation. Kolios' group fabricated micron-sized PFC droplets loaded with silica-coated lead sulfide (PbS) nanoparticles as a light absorber, which can produce PA signals through ODV that is several times higher efficient process than thermal-expansion based PA [9, 23]. They reported that the PA signal amplitude was proportional to the concentration of the light absorber [9, 23]. Wilson et al. [5] developed the optically-triggered droplets incorporated with gold nanorods as light absorber and demonstrated in vivo feasibility using a mouse model.

In addition, this study shows payload of incorporated plasmonic nanoparticles is linearly proportional to PA contrast. In the following study, Hannah et al. [24] developed PA nanodroplets conjugated with an organic FDA approved dye, ICG, instead of gold nanorods as dual-mode contrast agent for enhanced both PA and US imaging. In this study, enhanced PA contrast was found when the ambient temperature was increased. Lajoinie et al. [25] utilized an ultrafast camera to optically observe the ODV dynamics of micron-sized polymeric droplets, containing the Nile red dye as light absorber. They developed a vaporization dynamics model based on thermal diffusion and Rayleigh–Plesset equation shown in Eq. 2, to explain the ODV process, which showed a good agreement with their simultaneous optical and ultrasound measurement (Fig. 2) [25].

$$P(r, t) = \rho_l \frac{R(t)}{r} \left(2\dot{R}(t)^2 + R(t)\ddot{R}(t) \right), \quad (2)$$

where, $P(r, t)$ is generated PA pressure, ρ_l is the liquid density, $R(t)$ represents the droplet radius along to the time, the dot over droplet radius denotes the time derivative, and r is the distance between the sound source and the US transducer location. Yu et al. [12] also verified the Rayleigh–Plesset equation-based ODV dynamics model using the PFP-based droplets incorporated with ICG by comparing with concurrent acoustical and optical measurements using an acoustic transducer and an ultrafast camera, respectively. When the internal temperature of the droplet core exceeds its vaporization threshold, the droplet turns into a gas bubble with a shell. In some controlled cases, the vaporized droplet immediately recondensed and repeated the process of vaporization-recondensation in response to the following laser pulses. This repeated vaporization and recondensation might allow for continuous monitoring of the imaging target with strong PA signals, which can be useful in many biomedical applications. Asami et al. [26] firstly reported the repeated optical vaporization of droplets in sizes of $0.2\text{--}1.0\ \mu\text{m}$. Although small droplets generate reduced PA signals compared to larger droplets, the PA signal magnitude is still up to three times greater than that through thermal expansion. Subsequently, Yu et al. [12] investigated the optical vaporization and recondensation dynamics of PFP droplets containing ICG (Fig. 3). According to their observations, droplets with a smaller size that may have higher vaporization threshold temperature tend to be more stable and recondense consistently after vaporization [12]. For another way to achieve repeatability of droplet, using higher bulk boiling temperature of the liquid core could be considered to increase the vaporization threshold temperature of the droplet. This hypothesis is supported by other studies that made repeatable droplets using PFH, which has relatively higher

Fig. 2 Ultrafast imaging of optical droplet vaporization in response to low laser fluence of 70 mJ cm^{-2} (a) and high laser fluence of 1.4 J cm^{-2} (b). c, d Radius change of model and measurement shown

in the (a, b). e, f show corresponding estimated and measured acoustic pressure. Figures are reproduced with permission of [25]

bulk boiling temperature than PFP [8, 26]. However, it should be noted that PA signal amplitude would be less for small size droplets. This signal loss could potentially be compensated by increasing payload of the light absorber in the droplet core.

1.3 Applications of optically-triggered phase-transition droplets

PA nanodroplets hold a great potential in biomedical application including imaging, therapy and targeted drug delivery [27]. Various efforts in preclinical studies have been made for promoting such biomedical applications. Wilson et al. [5] successfully performed PA imaging using ODV of PA nanodroplets injected into the pancreas of a mouse. PA imaging of pancreas is challenging since the spleen located closely over pancreas is a highly optically absorbing organ. Both PA and US contrast were greatly enhanced in the target area (Fig. 4a–d). This in vivo study demonstrates that PA nanodroplets can be used as dual-

mode contrast agent for US and PA imaging. Subsequently, Yoon et al. [8] demonstrated contrast-enhanced US imaging technique with repeatable optically-triggered nanodroplets based on perfluorohexane (PFH, C_6F_{14} , Boiling temperature of 56°C) to identify mouse lymph node. They utilized repeatable vaporization that can provide transient and periodic change in US contrast with periodic laser irradiations. A probability function was utilized to measures how regularly the temporal US contrast fluctuates. This estimated probability was mapped to reconstruct droplet-localized, contrast-enhanced image overlaid onto B-scan image. With using similar imaging platform, Geoffrey et al. [28] presented super-resolution ultrasound imaging technique using stochastically recondensable laser-activated PFH-based nanodroplets. They demonstrated in vivo feasibility using a mouse brain in which a very high spatial resolution of $8\text{--}16 \mu\text{m}$ was achieved. Arnal et al. developed a novel sono-photoacoustic (SPA) technique for targeted molecular imaging by simultaneously transmitting ultrasound and laser pulses to

Fig. 3 Repeatability of optically droplet vaporization depending on size. **a** Repeated vaporization of a small sized droplet of 2.0 μm . **b** Vaporization without recondensation of a large sized droplet of 7.7 μm . Figures are reproduced with permission of [12]

nanodroplets. This technique hypothesizes that the vaporization threshold would be reduced due to the combination of photothermal heating of the PFC core from chromophores and homogenous cavitation from the acoustic-rarefaction phase. This idea was supported by previous studies [29–31]. In the sono-PA imaging, therefore, the required optical energy for ODV can be lowered under a safety limit [32, 33]. A thin-walled plastic tube containing polypyrrole-coated PFC nanoemulsions was embedded in chicken breast tissue to examine the efficiency of sono-PA imaging. Significantly enhanced PA signals were obtained compared to conventional PA imaging (Fig. 4e, f). Moreover, PA signals scattered from surrounding tissues were successfully suppressed using this technology.

Optically-triggered nanodroplets were also used for therapy. Wei et al. [34] explored the potential of using optically-triggered nanoemulsions with gold nanospheres for human blood clot disruption in an in vitro experiment. Fibrin gelatin was injected into a 2.5 mm tube to mimic the human blood clot. Nanoemulsions were added near the fibrin clot as dual contrast agent of both therapy and imaging. Under the activation of laser pulse, cavitation energy of bubbles vaporized from the droplets successfully ruptured the clot. This process can also be detected by PA and ultrasound imaging with the help of contrast created by

Fig. 4 **a–d** Dual contrast agent of optically triggered droplets for Combined US and PA imaging technique. **a, b** Contrast-enhanced PA overlaid on US imaging using optically triggered droplet. PA contrast based on vaporization is significantly improved compared to PA contrast based on thermal expansion. **c, d** Contrast-enhanced US imaging using optically triggered droplets. US contrast is only enhanced after optically triggered vaporization. **e, f** In vitro evaluation of Sono-PA imaging technique overlaid on the B-scan image using an optically-triggered phase-transition droplet with a comparison of traditional PA imaging overlaid on the B-scan image. Sono-PA image showed the improved SNR. Figures are reproduced with permission of [5, 33]

ODV. However, noting that the laser fluence applied in this study was much higher than human safety limit and the clot was not completely removed in this pilot study, further investigation is required to determine its usefulness in situ.

Looking into in vivo applications of ADV may provide insights for further in vivo applications of ODV. Kripfgans et al. [35, 36] investigated the acoustic droplet vaporization for occlusion therapy in kidney. Williams et al. [37] demonstrates the feasibility of extravascular ultrasound imaging with ADV in vivo mouse tumor model. Kagan et al. [38] successfully designed a micro-bullet that can deeply penetrate and deform kidney tissue by the means of ADV. Rapoport et al. [11] conducted a targeted tumor chemotherapy with acoustic activated droplets towards ovarian, breast, and orthotopic pancreatic tumor tissues. Since these biomedical applications using ADV are based

on the rapid and vigorous phase-transition process of the liquid core, ODV can also be adopted as an alternative and complementary method, especially for multi-modal approaches.

2 Conclusion and future direction

Optically-triggered phase-transition droplets are promising PA contrast agent that provide significantly improved PA contrast compared to the conventional PA contrast agents based on thermal expansion. In addition to some proof-of-concept in vitro study of ODV, some studies using animal models have demonstrated the in vivo feasibility. Some observational studies using an ultrafast camera provided further understanding of the ODV dynamics, which can eventually help guide optimal design of optically-triggered phase-transition droplets, especially with the capability of repeated vaporization-recondensation. It also has been shown that such droplets can be used as a dual-mode contrast agent for US and PA combined imaging as well as have a potential as a molecular theranostic agent conjugated with targeting molecules and therapeutics. Although ODV approach is novel and some feasibility and potential have been shown in animal study, there is still a quite long way off for the clinical translation. A complete understanding of the underlying physics including thermodynamic aspects of the phase transition between liquid and gas in optically-triggered vaporization is needed to design further optimized droplets. In addition, to eventually utilize PA droplets as contrast agent in human body, significant efforts to unveil and minimize their potential bio-effects that can be induced by vigorous vaporization dynamics as well as excessive laser irradiation under biologically relevant conditions should be followed.

Compliance with ethical standards

Conflict of interest Author Qiyang Chen declares that he has no conflict of interest. Author Jaesok Yu declares that he has no conflict of interest. Author Kang Kim declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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