Effective ultrasound-triggered drug release from stable nanocarriers

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Selective delivery of concentrated medication into specified targets would enable personalized medicine while minimizing side effects associated with systemic administration. Low-intensity ultrasound provides noninvasive and practical means to release drugs from nanocarriers selectively into its focus. However, which nanocarriers and ultrasound parameters can provide effective and safe drug delivery has been unclear. We found that nanocarriers with highly stable perfluorocarbon cores mediate effective release so long as they are activated by ultrasound of relatively low frequencies. We further demonstrated a repeatable method for manufacturing these nanocarriers to help facilitate future work in this area. This study guides the formulation and release parameters for effective and safe drug delivery into specific parts of the body or brain regions.

Introduction

Treatments of mental, neurological, and other disorders are often curbed by intolerable side effects or low effectiveness of drugs.[1–7] Consequently, millions of patients remain resistant to treatments and suffer from poor quality of life. There is a critical need for approaches that deliver medication selectively into the desired target at a high concentration while sparing surrounding tissues.

Pioneering work in this domain devised temperature-sensitive liposomes[8] that can be activated by heat or radiation. However, localized heating at depth has been challenging to achieve safely and in a controlled manner. Due to this issue, recent efforts have shifted to using low-intensity ultrasound as a safe and practical form of energy for localized drug release. Ultrasound can be remotely focused into a biological target at depth. Impacting circulating drug carriers, ultrasound triggers targeted release with minimal off-target effects (Fig. 1).

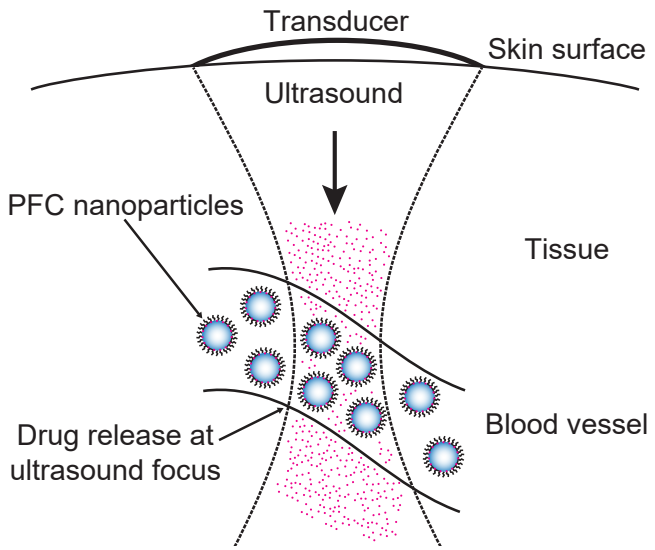

Figure 1. Controlled drug release in a specific body location. Drug-filled biodegradable nanoparticles are introduced into the circulation. Brief pulses of focused, low-intensity ultrasound are applied from the outside of the body using a standard transducer. At the ultrasound target, the focused pressure wave impacts the nanoparticles, which leads to a release of the drug specifically at the target and not elsewhere.

Several groups have shown that ultrasound can trigger drug release from nano-sized structures stabilized with biocompatible polymeric shells.[9–21] These nano-sized structures have been commonly filled with perfluorocarbon (PFC) cores. PFCs are highly inert and bestow the resulting nanoparticles with sensitivity to ultrasound. When

exposed to ultrasound, the PFC core has been hypothesized to change phase from liquid to gas, greatly expanding in volume, and thus mediating drug release.[13,22–25] Harnessing this understanding, a majority of previous studies used nanoparticles with PFC boiling points below body temperature.[9–14,16–20,26] However, these nanocarriers have suffered from instability issues, which has hampered successful translation. By contrast, PFCs with high boiling points are considered to be highly safe and stable and have been approved for use in large quantities by the FDA.[27,28] Nonetheless, such nanoparticles have provided relatively ineffective release.[15,21] In this study, we tested whether such nanoparticles could be activated with specific ultrasound parameters.

In particular, this study was designed to assess whether low frequency ultrasound could enable drug release from stable, high boiling point drug carriers. According to the primary hypothesis of drug release, ultrasound delivered at the target induces vaporization of the PFC core.[13,22–24] This vaporization may be related to either the mechanical or thermal effects of ultrasound, which vary depending on frequency, with high frequencies more likely to engage thermal mechanisms by depositing more energy at the target. PFCs with high boiling points are unlikely to be influenced strongly by thermal effects, and thus are expected to more effectively release drug in response to low frequency ultrasound. By contrast, higher frequency ultrasound more often studied is likely to favor low boiling point PFCs. In this paper, we demonstrate that the specific combination of nanoparticle formulation and ultrasound parameters could provide remotely controlled drug delivery that is both effective and safe and provide methods to consistently reproduce these results.

Results

In vitro ultrasound-triggered drug release

We prepared PFC-based, copolymer-stabilized nanoparticles using a similar approach as described in previous studies (see Methods for details). We loaded the nanoparticles with the neuromodulatory drug propofol[16,29] and quantified the effectiveness of its release using an approach in which drug is released from the nanoparticles into an organic solvent.[17] Uniquely, we tested how the critical component of the nanoparticle—its core—governs the amount of drug released as a function of specific ultrasound parameters. Specifically, we tested the release effectiveness of three different PFC cores—perfluoropentane (PFP), decafluoropentane (DFP), and perfluorooctylbromide (PFOB). These PFCs have boiling points of 29°C, 55°C, and 142°C, respectively. Nanoparticles with these cores had comparable sizes: mean±SD of 543.3±23.7, 550.8±91.7, and 473.0±28.4 nm for PFP, DFP, and PFOB-based nanoparticles, respectively.

We used an ultrasonic transducer capable of operating at low (300 kHz) and high (900 kHz) frequency focused on vials containing the nanoparticle solutions.[30] We found (Fig. 2, left) that 300 kHz ultrasound triggered more drug release from the nanoparticles than 900 kHz. The difference in the percentage of drug released at the two frequencies (31.7% and 20.3%, respectively) was significant when averaged across all cores and ultrasound pressures ($t(130) = 3.3$, $p = 0.0013$, two-sample two-tailed t-test). In line with our hypothesis, we found (Fig. 2, right) that at the higher 900 kHz frequency, the release effectiveness strongly depends on the boiling point of the PFC core. The scaling is in the expected direction—the lower the boiling point of the PFC, the higher the release effectiveness. An omnibus ANOVA model (Table 1) that incorporated all factors tested (core, ultrasound frequency, ultrasound pressure) as well as all possible interactions, detected a significant core × frequency interaction ($F(2,90) = 8.05$, $p = 0.00061$).

The scaling of the release effectiveness by the PFC boiling point (red bars in Fig. 2, right) suggests the engagement of a thermal mechanism at the higher frequency, in agreement with previous propositions.[13,18,23,31] If this is the case, the release should also scale with the average ultrasound intensity I delivered into the nanoparticles. For longitudinal waves, it holds $I = \frac{P^2}{2Z}$, where P is the ultrasound pressure amplitude, and Z is the acoustic impedance of the medium.[32] Thus, a thermal effect scales with pressure squared. We indeed found that drug release at 900 kHz showed a quadratic dependence on pressure (Fig. 3, right). Specifically, quadratic fits to the data (solid curves)

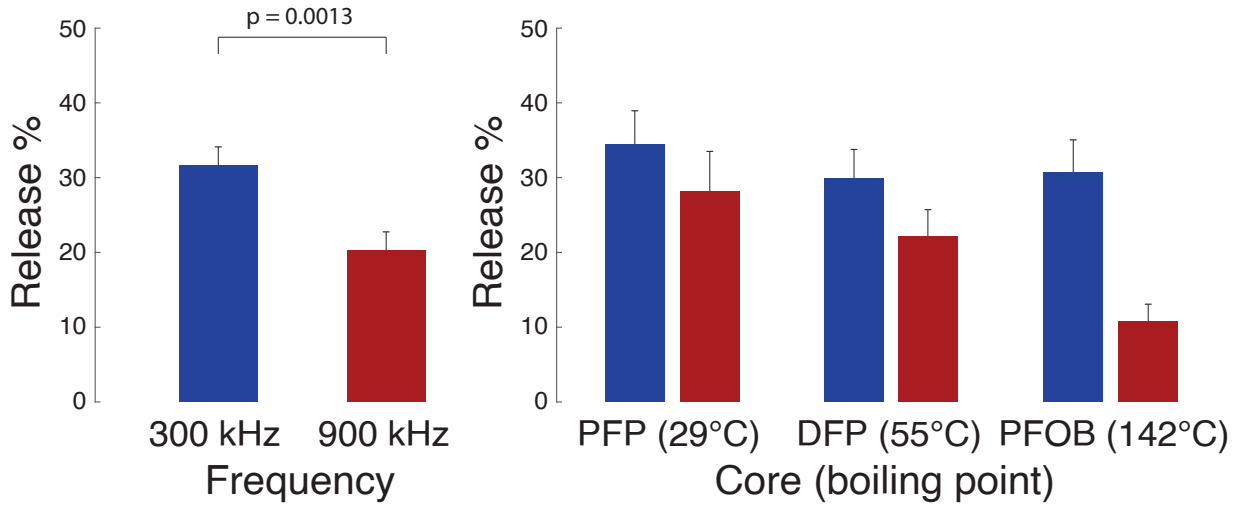

Figure 2. Release from nanoparticles with distinct cores under two ultrasound frequency modes. Mean±s.e.m. propofol release relative to the amount encapsulated (see Methods for details) for the two ultrasound frequencies (left, combined across all cores) and the three cores (right) tested. The ultrasound was delivered in 100 ms pulses repeated 60 times over the period of 1 minute. The p-value denotes the significance of a two-sample two-sided t-test. A complete statistical analysis of the effects is provided in Table 1.

Factor	Significance
Core	**< 0.001**
F	**< 0.001**
P	**< 0.001**
Core × F	**< 0.001**
$F \times P$	**< 0.001**
Core × P	**0.030**
Core × $F \times P$	**0.049**

Table 1. Summary of the effects. The effects of the nanoparticle core, ultrasound frequency (F), and ultrasound pressure (P). These effects were assessed using a three-way ANOVA that featured the three main effects and all possible interactions. Bold entries are significant ($p < 0.05$).

explained 96.5, 93.5, and 94.8% of the variance in the PFP, DFP, and PFOB data points. In comparison, linear fits only explained 80.3, 80.5, and 69.3% of the variance, respectively. The difference in the mean variance explained by the quadratic and linear fits (94.9% versus 76.7%) was significant ($t(2) = 4.83$, $p = 0.040$, two-sided t-test). In contrast, the lower 300 kHz frequency showed a linear dependence of the release on pressure (Fig. 3, left). A quadratic fit did not significantly ($p > 0.16$) differ from the linear fit in terms of variance explained (89.5% versus 89.3%). The linear dependence on pressure at 300 kHz is consistent with a mechanical rather than a thermal effect (see Discussion for details).

We summarize the effects of the three factors tested (core, ultrasound frequency, and ultrasound pressure) as well as all possible interactions in an omnibus ANOVA model (Table 1). This analysis confirms that both the core and ultrasound parameters (frequency and pressure) are important considerations for effective release. In addition, the factors interact: the selection of a specific core must be performed in conjunction with suitable ultrasound parameters.

Stability of PFC nanoparticles

From a safety perspective, cores with higher boiling points can be expected to be more stable, thus minimizing the risk of spontaneous drug release and embolism when injected into the bloodstream. To quantify this notion, we measured the sizes of PFP and PFOB nanoparticles over the course of 24 hours (Fig. 4, left). PFP-based nanoparticles more than doubled in size over the 24 hours, from mean±SD of 428±27 to 914±108 nm ($t(5) = 11.9$, $p < 0.001$, paired two-tailed t-test). In contrast, the size of PFOB-based nanoparticles remained steady, increasing

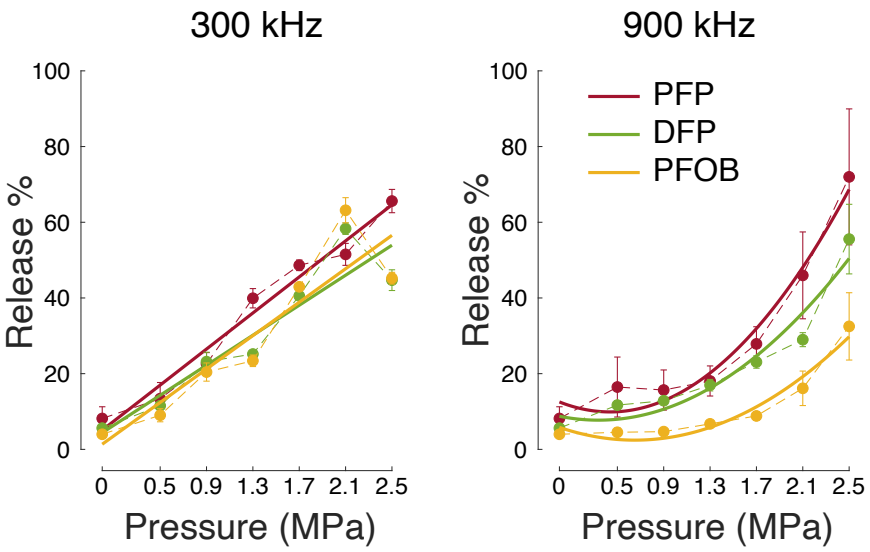

Figure 3. Release across all tested factors. Mean±s.e.m. percentage of the released propofol for the two ultrasound frequencies (left versus right), ultrasound pressure amplitude (abscissa), and the three cores tested (color; see inset). n=3 samples for each core and ultrasound parameter except 0MPa, where n=4. The thick lines represent quadratic fits to the data. Notably, for the 300 kHz data, linear fits were as explanatory as the quadratic fits (see text).

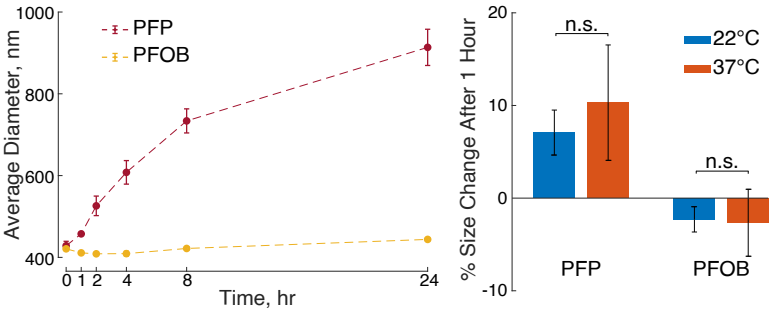

Figure 4. Stability of PFOB and PFP-based nanoparticles over time. Left: Mean±s.e.m. diameter measured at the times as indicated on the abscissa. The times were measured relative to the time of the completion of the nanoparticle production. The data include $n = 6$ samples of PFOB nanoparticles and $n = 6$ samples of PFP nanoparticles. The error bars for PFOB are smaller than the symbols. Right: PFP and PFOB nanoparticle average size change after one hour at 22°C or 37°C. There was no significant difference in either case (see text).

only by 23 nm over the 24 hours (from 421±10 to 444±10 nm; $t(5) = 6.3$, $p = 0.0015$). A repeated measures ANOVA detected a highly significant interaction of PFC core and time ($F(5,50) = 43.54$, $p < 0.001$), confirming that the PFP-based nanoparticles expanded at a higher rate than PFOB-based nanoparticles.

We additionally tested how the stability depends on temperature. Specifically, fresh PFP and PFOB nanoparticles were incubated at 37°C for one hour. After one hour, most nanoparticles are likely to have been cleared from the bloodstream as observed by previous studies.[14,33] We found that temperature did not have a significant effect on the sizes of either PFP ($t(10) = 0.10$, $p = 0.93$, two-sided t-test) or PFOB ($t(10) = -0.48$, $p = 0.64$) particles (Fig. 4, right).

A versatile platform for drug delivery

In addition to propofol, we loaded the PFOB nanoparticles with the immunosuppressant drug mycophenolate motefil to demonstrate their versatility as drug carriers. We observed a similar sensitivity to ultrasound as propofol-loaded nanoparticles (Fig. 5). Indeed, an ANOVA model incorporating ultrasound pressure and drug type indicated no significant effect of drug ($F(1,46) = 0.88$, $p = 0.4$). Mycophenolate motefil-loaded nanoparticles did release more drug without ultrasound (($t(5) = 6.5$, $p = 0.0013$, two-sample two-tailed t-test). This may be attributed to the lower hydrophobicity compared to propofol leading to less effective encapsulation inside the nanoparticles.[34,35] Further, mycophenolate motefil is incorporated in the nanoparticles from a solid form, so free drug may be less effectively removed during centrifugation.

In order to identify a reproducible process that yields ultrasound-sensitive PFOB nanoparticles, we systematically studied the variables in the manufacturing process. This included the ratio of nanoparticle components (drug and

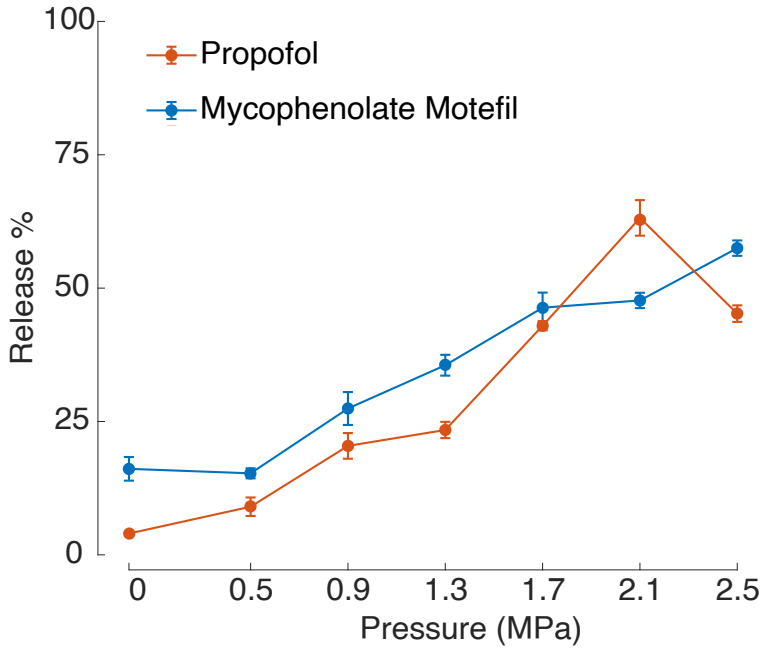

Figure 5. Propofol and mycophenolate motefil released by PFOB-based nanoparticles. Mean±s.e.m. percent drug released under ultrasound stimulation under the same experimental conditions as in Fig. 3.

	Significance			
Factor	Encapsulation	Release US	Release no US	Size
Drug:polymer ratio	**0.008**	**< 0.001**	**< 0.001**	**< 0.001**
PFOB:polymer ratio	**0.004**	**0.002**	**< 0.001**	**0.015**
Sonication time	0.232	**0.004**	**0.003**	0.070
Sonication temperature	**0.035**	**< 0.001**	**0.005**	0.514
Centrifuge time	0.052	0.144	0.074	**0.011**
Centrifuge speed	0.761	0.379	**0.008**	**0.026**

Table 2. Summary of manufacturing parameters. The effects of nanoparticle manufacturing parameters assessed using a one-way ANOVA for each factor and metric tested. Bold entries are significant ($p < 0.05$).

PFOB to polymer), sonication bath parameters (temperature and duration), and centrifuge parameters (speed and duration). Results are summarized in Fig. 6, which shows how each variable affects drug encapsulation and release. Table 2 summarizes the statistical analysis of this study, indicating which manufacturing parameters affect nanoparticle size, drug encapsulation, and release. Ratios of polymer shell to drug and PFOB core strongly influenced the resulting nanoparticle composition and thus must be carefully tuned to specific applications. For example, increasing the ratio of drug to polymer shell can significantly increase the rate of encapsulation and drug release, but also increases the rate of spontaneous drug release and the average particle size from 780±80 nm to 3300±600 nm in size. A full summary of size results can be found in Supplementary Fig. S1. Further analysis of sizes across all trials found no significant linear correlation between particle size and encapsulation ($p = 0.61$) or drug release with ($p = 0.47$) or without ultrasound ($p = 0.89$) (Supplementary Fig. S2).

Discussion

This study investigated the effectiveness and safety of drug release from biocompatible nanoparticles triggered by focused ultrasound. Uniquely, we evaluated the release efficacy from nanoparticles filled with PFCs with distinct boiling points, including boiling points above the body temperature, and investigated the relevant ultrasound parameters that activate them. We found that low frequency ultrasound (300 kHz in particular) was more effective at mediating release in general and that low frequencies are paramount for effective release from PFCs with a high boiling point.

The core has been hypothesized to be a critical factor in governing the effectiveness of ultrasound-based drug

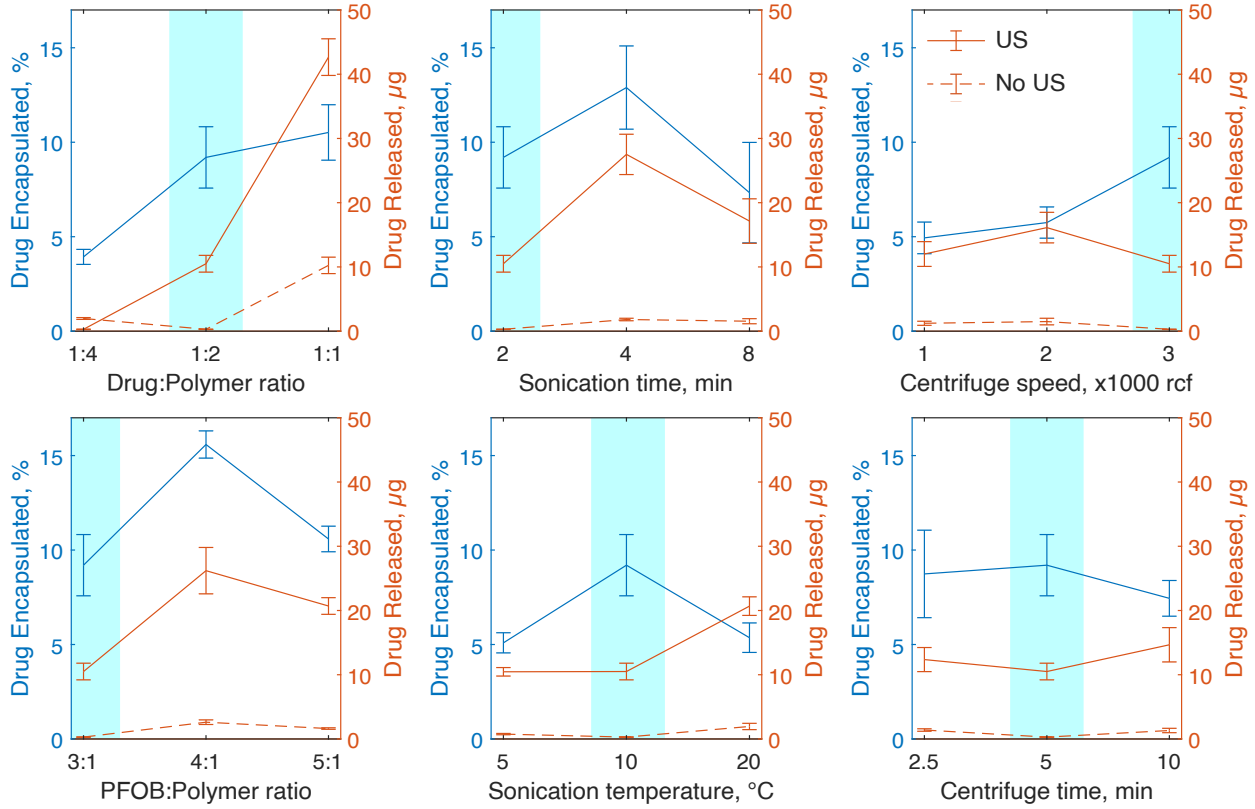

Figure 6. Manufacturing methods effects on drug encapsulation and release. Each plot shows the mean±s.e.m. propofol encapsulated as a percentage of propofol used in blue and total drug released with (solid line) and without US (dotted line). 300 kHz ultrasound was applied for 60 seconds in 10 ms bursts at a rate of 10 Hz and peak negative pressure of 1.5 MPa. 5 samples were prepared for each parameter tested. Baseline parameters highlighted in blue were held constant while other parameters were varied.

release.[11,18,21] Indeed, our 900 kHz data show (Fig. 2, right, red) that at the range of commonly applied ultrasound frequencies ($\geq$ 1 MHz), the core is a key determinant of release effectiveness. We found that the higher the boiling point of the encapsulated PFC, the lower the release effectiveness. This finding is in accord with previous results.[13,18,23,31] Critically, we found that lowering the frequency to 300 kHz increased the release effectiveness at a general, core-independent level (Fig. 2). This effect can also be found in a study that used PFP.[17] The application of low-frequency ultrasound thus opens the path to using high boiling point cores (e.g., PFOB) as release actuators. A frequency of 300 kHz provides spatial resolution on the order of several millimeters, which approximates the focal volume of the drug released in tissue by ultrasound.[16,19] While the focal size is larger than ultrasound of higher frequencies, the focal size remains applicable for targets in the brain including the amygdala, which is around 1 cubic centimeter in volume[36] or glioblastoma tumors, which can reach dozens of cubic centimeters.[37]

The majority of studies that applied ultrasound to PFC-based nanoparticles used PFCs with boiling point below the body temperature.[9–14,16–20,26] Although the Laplace pressure of sub-micron particles can effectively increase the boiling point,[38] PFCs with low boiling points may suffer from instability issues, which may raise safety concerns. Our data show that PFP-based nanoparticles not exposed to ultrasound spontaneously increased in size over time, whereas PFOB nanoparticles remained stable (Fig. 4). These data suggest that PFP-based nanoparticles should be used near-immediately following their production and that their time in the blood stream should be minimized, if possible. Further, low boiling point PFCs can form persistent microbubbles after vaporization by ultrasound.[10,13,20] In fact, with respect to PFC safety, the FDA has thus far approved PFCs with a high boiling point for large-scale use in humans—PFOB in particular. Specifically, PFOB has been used in liter- quantities in humans as agents that deliver oxygen into tissues. The PFOB-based products have included LiquiVent—an oxygen-carrying liquid drug, and Oxygent (both Alliance Pharmaceutical Corporation, San Diego, CA, USA)—a blood substitution agent.[27,28,39] Our finding that PFOB can be used for effective release from nanoparticles at low ultrasound frequencies (Fig. 2, Fig. 3) should mitigate the boiling-point associated concerns.

The *in vitro* release data presented here contribute to the understanding of the release mechanism. Thus far, the predominant hypothesis of action has been the vaporization of the PFC droplet upon the impact of focused ultrasound.[11,18,21–24] In this mechanism, the thermal and mechanical aspects of propagating ultrasound exceed the vaporization threshold governed by the PFC boiling point and the Laplace pressure. The PFC core increases in size (up to a 5-fold increase in diameter[38]), which contributes, in addition to any superimposed effects of ultrasound, to the drug release. Indeed, our data at the common range of ultrasound frequencies, 900 kHz, provide two lines of evidence in support of this hypothesis. First, we found that the release increases with decreasing boiling point (Fig. 2, right), as expected from the vaporization hypothesis. Second, thermal energy delivered by ultrasound is known to be proportional to pressure squared.[32] We indeed found a quadratic dependence of the release on pressure (Fig. 3, right).

Our and a previous study[17] suggest that ultrasound of frequencies lower than those in the common diagnostic range may mediate more effective drug release. Lower frequencies are known to accentuate mechanical effects, which can take two forms. First, ultrasound can induce cavitation, the formation of gaseous nuclei from dissolved gasses under the low-pressure epochs of the ultrasound pressure wave.[40] Cavitation can provide useful mechanical forces until it exceeds a threshold at which the formed gaseous nuclei collapse and cause damage.[40] From the FDA's 510(k) Track 3 standpoint,[41] cavitation is unlikely to occur for mechanical index—defined as ultrasound pressure divided by the square root of frequency—values below 1.9. In our hands, a 300 kHz, 1.0 MPa pressure at target yields a mechanical index of 1.83. Despite being below the value of 1.9, this pressure level already drives significant release (Fig. 3, left). Thus, if cavitation is involved, it is either in the safe range or just one of several mechanisms. Another potential mechanism for drug release is particle displacement. The maximal displacement of a particle in the ultrasound path, ξ_m, is linearly proportional to the ultrasound pressure amplitude

P: $\xi_m = \frac{P}{Z}\int_0^{T/2}\sin(2\pi Ft)dt = \frac{P}{Z\pi F}$, where F is the ultrasound frequency and Z the acoustic impedance. Indeed, our 300 kHz data show a linear dependence of the release on pressure (Fig. 3, left). This mechanism is supported by high-speed imaging, which did not detect a persistent phase change despite effective release in rodents.[17] Together, our findings indicate that both thermal and mechanical effects can be at play, depending on the applied ultrasound frequency. Higher frequencies accentuate thermal effects (PFC vaporization), whereas lower frequencies accentuate mechanical effects (cavitation or cycle-by-cycle particle displacement).

Interestingly, at 300 kHz, we observed a marginal decrease in drug release from DFP and PFOB nanoparticles at the highest ultrasound pressure tested (Fig. 3, left). This effect has been observed in another study.[19] These high pressures may lead to cavitation—the formation of bubbles—which may consequently block the propagation of ultrasound into the target.[19] PFP did not exhibit this effect, possibly due to the lower boiling point. Complete vaporization and subsequent collapse of PFP bubbles may prevent shielding effects with the repeated pulsing scheme used here.

The study has three limitations. First, we tested the effects of ultrasound frequency and pressure, but not the ultrasound waveform. For instance, we have not tested the effects of the duty cycle and only used a fixed value of 10%. Increases in the duty cycle are known to lead to stronger, cumulative effects.[14,17] Second, we quantified the release of nanoparticles using a single copolymer shell (PEG:PDLLA). This limitation is mitigated by a previous study that found little variability in the release due to specific forms of the polymeric shell.[17] Third, the effectiveness of drug release from stable, high boiling point drug carriers remains to be verified *in vivo*. Future studies will investigate the behavioral effects of propofol delivered in deep brain targets of nonhuman primates using the experimental paradigm developed in the lab .[42]

Conclusions

In summary, we found that low-frequency ultrasound can effectively release drugs from stable PFC-based nanocarriers and identified methods critical to their manufacture. This study informs the use of specific PFC-based nanoparticles and ultrasound parameters for effective, safe, and targeted drug release in humans.

Methods

Materials

Methoxy poly(ethylene glycol)-b-poly(D,L-lactide) (PEG-PDLLA) co-polymers with 2,000 : 2,200 g/mol molecular weights, respectively, were obtained from PolyScitech (USA). 2H,3H- decafluoropentane and perfluorooctyl bromide were obtained from Tokyo Chemical Industry Co. (Japan). Perfluoro-n-pentane was obtained from Strem Chemicals (USA). Propofol was obtained from Sigma Aldrich (Millipore Sigma, Canada). Infrared dye IR800RS NHS Ester was obtained from LI-COR Biosciences (USA). HPLC-grade tetrahydrofuran (THF) and methanol were obtained from Fisher Scientific (USA). Phosphate buffer solution (PBS) was obtained from Gibco (Thermo Fisher Scientific, USA) and Cytiva (USA). Mycophenolate Motefil was obtained from Akorn, Inc. (USA).

Nanoparticle production

The process of manufacturing the drug-encapsulating, ultrasound-responsive PFC particles is illustrated at a conceptual level in (Fig. 7) and described in detail in previous studies.[9,17] The process converts small ($<$ 30nm) micelles into much larger ($>$ 300nm) PFC-filled nanoparticles. First, the PEG-PDLLA polymer constituting the basis of the nanoparticle shell is dissolved in THF at a rate of 1mL THF : 16 mg polymer. For the biodistribution and blood clearance studies, infrared dye is added at a ratio of 1:32 (dye:polymer) for the rats and marmoset and 1:110 or 1:89 for the macaques 1 and 2, respectively. THF is then evaporated under vacuum until a gel-like layer remains. PBS is added at a rate of 1 mL PBS : 8 mg polymer and placed on a shaker table at 120 rpm to dissolve for 15 minutes. The addition of PBS orients the hydrophilic copolymer, PEG, toward the water and the

hydrophobic, PDLLA, copolymer away from the water, and as a consequence, micelles are formed. Next, the PFC core and propofol are added and emulsified. A ratio of 1 mg propofol : 2 mg polymer was used in all cases. The nanoparticles' diameter can be controlled by the ratio of PFC to polymer, as reported previously.[11] For PFOB and DFP nanoparticles, a ratio of 4.5 μL PFC : 1 mg polymer was used. The ratio for PFP was scaled up to 6.25 μL : 1 mg to account for PFC lost to vaporization before being emulsified. A 20 kHz, 500W sonicator with a cup horn attachment (VCX500, Sonics) was used to perturb the thermodynamic equilibrium of the micellar system, which leads to the incorporation of PFOB into the micelles and the formation of stable nanodroplets or nanoparticles.[43] The PFC and drug are added to 15 mL centrifuge tubes and gently shaken to combine before adding 8 mL of the micelle solution. The samples are then sonicated in a cold bath at 20% power in 30-second intervals until the solution is cloudy and drug and PFC are fully emulsified (1-3 minutes in total). A custom temperature-controlled cooling system maintained the bath temperature during sonication at 2°C for PFP and 10°C for DFP and PFOB. PFP must be kept colder to minimize vaporization before emulsification, while DFP and PFOB require higher temperatures to emulsify successfully without vaporizing. We found this controlled temperature approach to maximize the consistency of the nanoparticle sizes, drug encapsulation, and release properties. The resulting solution contains the desired nanoparticles in addition to remaining micelles, dissolved polymer, and free propofol. Nanoparticles are isolated using three cycles of centrifugation at 3,000 relative centrifugal force (RCF) at 4°C. After each cycle, the supernatant is discarded and the pellet dissolved in 5 mL fresh PBS. If the resulting solution contains larger particles than needed, these were removed by a slower centrifuge cycle for 1 minute at 800 RCF, this time keeping the supernatant. Larger particles contained in the pellet are discarded.

Hydrophobic drug preparation

The propofol used was already in its hydrophobic form, but mycophenolate motefil had to be modified from a hydrochloride salt to be encapsulated in the nanoparticles. To do so, the drug is first dissolved in deionized water in a centrifuge tube at a concentration of 10 mg/mL. Then, 3N NaOH is added dropwise at an equal molar ratio with the HCl until the drug precipitate forms. The solution is then centrifuged for 5 minutes at 600 RCF and the supernatant discarded. Centrifugation is repeated for a total of three cycle, resuspending in deionized water after each time. After the final centrifuge cycle, the drug is dissolved in methanol then filtered through a 0.2 micron syringe filter. Methanol is then dried under a gentle nitrogen stream, leaving the hydrophobic drug in a powdered form which can be incorporated into nanoparticles.

Nanoparticle characterization

The sizes were measured using a Zetasizer Nano S (Malvern Panalytical, UK), which reports the intensity-weighted size distribution. The size values reported in the Results section describe the mean ± standard deviation of the distribution of the intensity values measured by the device. To quantify the amount of drug encapsulated, a 50 μL solution of nanoparticles is added to 450 μL of methanol to dissolve all components.[14] A UV-Vis spectrophotometer (NanoDrop 2000, Thermo Scientific) is used to quantify the concentration by comparing the absorbance to a standard curve at 276 nm for propofol and 305 nm for mycophenolate motefil.

Apparatus

As in a previous study,[17] drug release is quantified in standard 1.5 mL microcentrifuge tubes. Each tube with freshly produced nanoparticles is placed into a plastic holder. A focused ultrasonic transducer (H-115, 64 mm diameter, 52 mm focal depth, Sonic Concepts) was positioned 52 mm below the holder so the sample was within the ultrasound focus. Degassed water (AIMS III system with AQUAS-10 Water Conditioner, Onda) mediated coupling between the ultrasound face and the vial. The transducer was operated at 300 kHz and the third harmonic, 900 kHz. Stimuli were generated using a function generator (33520b, Keysight). The signals were amplified using a 55-dB, 300 kHz–30 MHz power amplifier (A150, Electronics & Innovation).

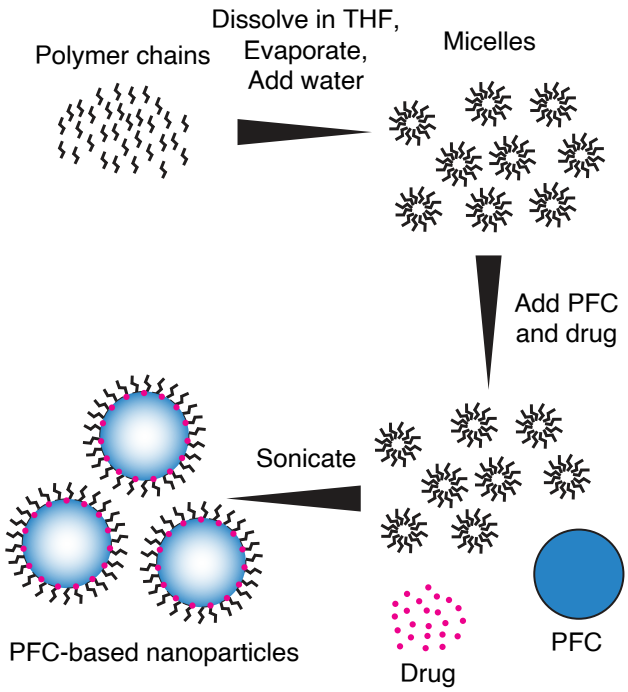

Figure 7. Production of the nanoparticles. The conversion of polymeric micelles into PFC-core-based nanoparticles using ultrasound. The three steps are described in detail in the text.

Ultrasound parameters

The ultrasound carrier frequencies for *in vitro* experiments were 300 kHz and 900 kHz. For the assessment of different PFC cores and drug encapsulated, continuous pulses 100 ms in duration were repeated once per second for a total of 60 seconds.[14,17] The pressure levels at the vial location, measured in degassed water, were 0, 0.5, 0.9, 1.3, 1.7, 2.1, and 2.5 MPa. During the nanoparticle manufacturing study, ultrasound was applied in 10 ms pulses 10 times per second for one minute. The pressure fields were measured using a capsule hydrophone (HGL-0200, Onda) calibrated between 250 kHz and 40 MHz and secured to 3-degree-of-freedom programmable translation system (Aims III, Onda).

Drug release characterization

100 μL of hexane was placed on top of 200 μL nanoparticle solutions prior to sonication to act as a sink for released drug.[14] After 1 minute, 45 seconds of total incubation time, 50uL of hexane was extracted. The amount of dissolved propofol was quantified using UV-Vis spectrophotometry as described previously. The percent release efficacy is defined as the amount of the drug released into the hexane relative to the amount encapsulated. Each datapoint in Fig. 3 included 3-4 distinct samples.

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Author contributions

J.K. and M.G.W. designed the studies. M.G.W. synthesized the nanoparticles and carried out core and frequency drug release and stability experiments. A.P. and M.G.W. carried out manufacturing experiments. J.K. and M.G.W. wrote and edited the manuscript.

Competing interests

The authors declare no conflict of interest.

Data availability

Correspondence and requests for materials should be directed to J.K. (jan.kubanek@utah.edu). The methods described herein are subject to a provisional patent.

References

1. Bystritsky, A. Treatment-resistant anxiety disorders. *Molecular psychiatry* **11**, 805–814 (2006).
2. Lyons, K. E. & Pahwa, R. Deep brain stimulation and essential tremor. *Journal of clinical neurophysiology* **21**, 2–5 (2004).
3. Zesiewicz, T. A., Chari, A., Jahan, I., Miller, A. M. & Sullivan, K. L. Overview of essential tremor. *Neuropsychiatric disease and treatment* **6**, 401 (2010).
4. Al-Harbi, K. S. Treatment-resistant depression: therapeutic trends, challenges, and future directions. *Patient preference and adherence* **6**, 369 (2012).
5. Elias, W. J. *et al.* A pilot study of focused ultrasound thalamotomy for essential tremor. *New England Journal of Medicine* **369**, 640–648 (2013).
6. Elias, W. J. *et al.* A randomized trial of focused ultrasound thalamotomy for essential tremor. *New England Journal of Medicine* **375**, 730–739 (2016).
7. Jaffe, D. H., Rive, B. & Denee, T. R. The humanistic and economic burden of treatment-resistant depression in europe: a cross-sectional study. *BMC psychiatry* **19**, 247 (2019).
8. Needham, D. & Dewhirst, M. W. The development and testing of a new temperature-sensitive drug delivery system for the treatment of solid tumors. *Advanced drug delivery reviews* **53**, 285–305 (2001).
9. Rapoport, N., Gao, Z. & Kennedy, A. Multifunctional nanoparticles for combining ultrasonic tumor imaging and targeted chemotherapy. *Journal of the National Cancer Institute* **99**, 1095–1106 (2007).
10. Rapoport, N. Y., Kennedy, A. M., Shea, J. E., Scaife, C. L. & Nam, K.-H. Controlled and targeted tumor chemotherapy by ultrasound-activated nanoemulsions/microbubbles. *Journal of Controlled Release* **138**, 268–276 (2009).
11. Rapoport, N. *et al.* Ultrasound-mediated tumor imaging and nanotherapy using drug loaded, block copolymer stabilized perfluorocarbon nanoemulsions. *Journal of Controlled Release* **153**, 4–15 (2011).
12. Rapoport, N., Gupta, R., Kim, Y.-S. & O'Neill, B. E. Polymeric micelles and nanoemulsions as tumor-targeted drug carriers: Insight through intravital imaging. *Journal of Controlled Release* **206**, 153–160 (2015).
13. Rapoport, N. Drug-loaded perfluorocarbon nanodroplets for ultrasound-mediated drug delivery. In *Therapeutic Ultrasound*, 221–241 (Springer, 2016).
14. Airan, R. D. *et al.* Noninvasive targeted transcranial neuromodulation via focused ultrasound gated drug release from nanoemulsions. *Nano Letters* **17**, 652–659 (2017).
15. Boissenot, T. *et al.* Ultrasound-induced mild hyperthermia improves the anticancer efficacy of both taxol® and paclitaxel-loaded nanocapsules. *Journal of Controlled Release* **264**, 219–227 (2017).
16. Wang, J. B., Aryal, M., Zhong, Q., Vyas, D. B. & Airan, R. D. Noninvasive ultrasonic drug uncaging maps whole-brain functional networks. *Neuron* **100**, 728–738 (2018).

17. Zhong, Q. *et al.* Polymeric perfluorocarbon nanoemulsions are ultrasound-activated wireless drug infusion catheters. *Biomaterials* **206**, 73–86 (2019).

18. Wu, S.-Y. *et al.* Focused ultrasound-facilitated brain drug delivery using optimized nanodroplets: vaporization efficiency dictates large molecular delivery. *Physics in Medicine & Biology* **63**, 035002 (2018).

19. Lea-Banks, H. & Hynynen, K. Sub-millimetre precision of drug delivery in the brain from ultrasound-triggered nanodroplets. *Journal of Controlled Release* **338**, 731–741 (2021).

20. Lea-Banks, H. *et al.* Ultrasound-sensitive nanodroplets achieve targeted neuromodulation. *Journal of Controlled Release* **332**, 30–39 (2021).

21. Somaglino, L. *et al.* In vitro evaluation of polymeric nanoparticles with a fluorine core for drug delivery triggered by focused ultrasound. *Colloids and Surfaces B: Biointerfaces* **200**, 111561 (2021).

22. Sheeran, P. S., Matsunaga, T. O. & Dayton, P. A. Phase-transition thresholds and vaporization phenomena for ultrasound phase-change nanoemulsions assessed via high-speed optical microscopy. *Physics in Medicine & Biology* **58**, 4513 (2013).

23. Doinikov, A. A., Sheeran, P. S., Bouakaz, A. & Dayton, P. A. Vaporization dynamics of volatile perfluorocarbon droplets: a theoretical model and in vitro validation. *Medical physics* **41**, 102901 (2014).

24. Shpak, O. *et al.* Acoustic droplet vaporization is initiated by superharmonic focusing. *Proceedings of the National Academy of Sciences* **111**, 1697–1702 (2014).

25. Kripfgans, O. D., Fowlkes, J. B., Miller, D. L., Eldevik, O. P. & Carson, P. L. Acoustic droplet vaporization for therapeutic and diagnostic applications. *Ultrasound in medicine & biology* **26**, 1177–1189 (2000).

26. Sheeran, P. S., Luois, S. H., Mullin, L. B., Matsunaga, T. O. & Dayton, P. A. Design of ultrasonically-activatable nanoparticles using low boiling point perfluorocarbons. *Biomaterials* **33**, 3262–3269 (2012).

27. Castro, C. I. & Briceno, J. C. Perfluorocarbon-based oxygen carriers: review of products and trials. *Artificial organs* **34**, 622–634 (2010).

28. Li, X. *et al.* Perfluorooctylbromide nanoparticles for ultrasound imaging and drug delivery. *International journal of nanomedicine* **13**, 3053 (2018).

29. Orser, B., Wang, L., Pennefather, P. & MacDonald, J. Propofol modulates activation and desensitization of gabaa receptors in cultured murine hippocampal neurons. *Journal of Neuroscience* **14**, 7747–7760 (1994).

30. Riis, T. & Kubanek, J. Effective ultrasonic stimulation in human peripheral nervous system. *IEEE Transactions on Biomedical Engineering* (2021).

31. Zhang, M. *et al.* Initial investigation of acoustic droplet vaporization for occlusion in canine kidney. *Ultrasound in medicine & biology* **36**, 1691–1703 (2010).

32. Cobbold, R. S. *Foundations of biomedical ultrasound* (Oxford university press, 2006).

33. Cao, Y. *et al.* Drug release from phase-changeable nanodroplets triggered by low-intensity focused ultrasound. *Theranostics* **8**, 1327 (2018).

34. Propofol. URL https://pubchem.ncbi.nlm.nih.gov/compound/4943.

35. Mycophenolate motefil. URL https://pubchem.ncbi.nlm.nih.gov/compound/5281078.

36. Weniger, G., Lange, C. & Irle, E. Abnormal size of the amygdala predicts impaired emotional memory in major depressive disorder. *Journal of affective disorders* **94**, 219–229 (2006).

37. Bette, S. *et al.* Prognostic value of tumor volume in glioblastoma patients: size also matters for patients with incomplete resection. *Annals of surgical oncology* **25**, 558–564 (2018).

38. Rapoport, N. Phase-shift, stimuli-responsive perfluorocarbon nanodroplets for drug delivery to cancer. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* **4**, 492–510 (2012).

39. Cohn, C. S. & Cushing, M. M. Oxygen therapeutics: perfluorocarbons and blood substitute safety. *Critical care clinics* **25**, 399–414 (2009).

40. Apfel, R. E. & Holland, C. K. Gauging the likelihood of cavitation from short-pulse, low-duty cycle diagnostic ultrasound. *Ultrasound in medicine & biology* **17**, 179–185 (1991).

41. FDA. Marketing clearance of diagnostic ultrasound systems and transducers. *Food and Drug Administration* **FDA-2017-D-5372** (2019).

42. Webb, T. D., Wilson, M. G., Odéen, H. & Kubanek, J. Remus: System for remote deep brain interventions. *iScience* **25**, 105251 (2022). URL https://www.sciencedirect.com/science/article/pii/S2589004222015231.

43. Gupta, R., Shea, J., Scaife, C., Shurlygina, A. & Rapoport, N. Polymeric micelles and nanoemulsions as drug carriers: therapeutic efficacy, toxicity, and drug resistance. *Journal of Controlled Release* **212**, 70–77 (2015).

Supplementary Material

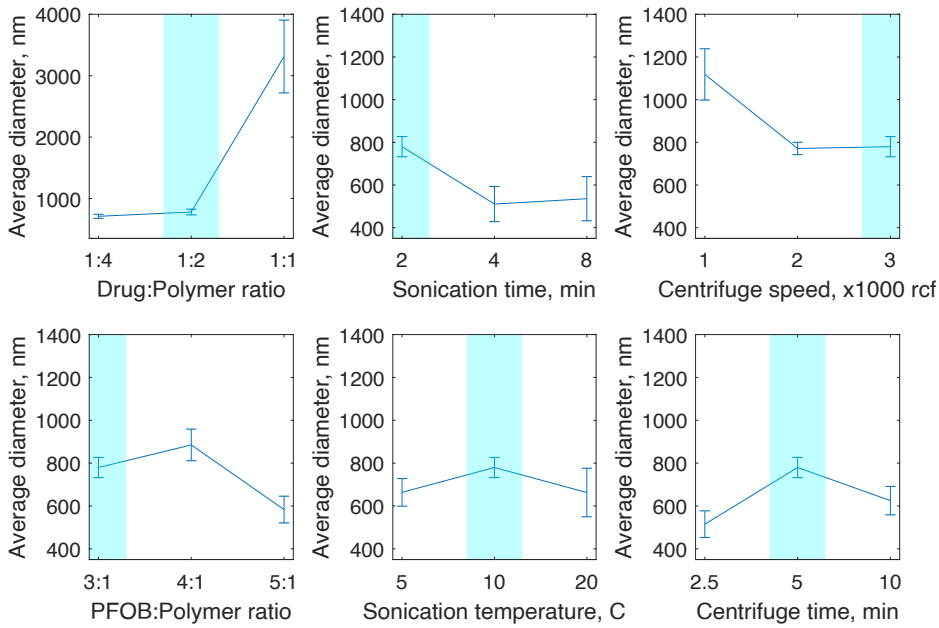

Figure S1. Manufacturing methods effects on particle size.

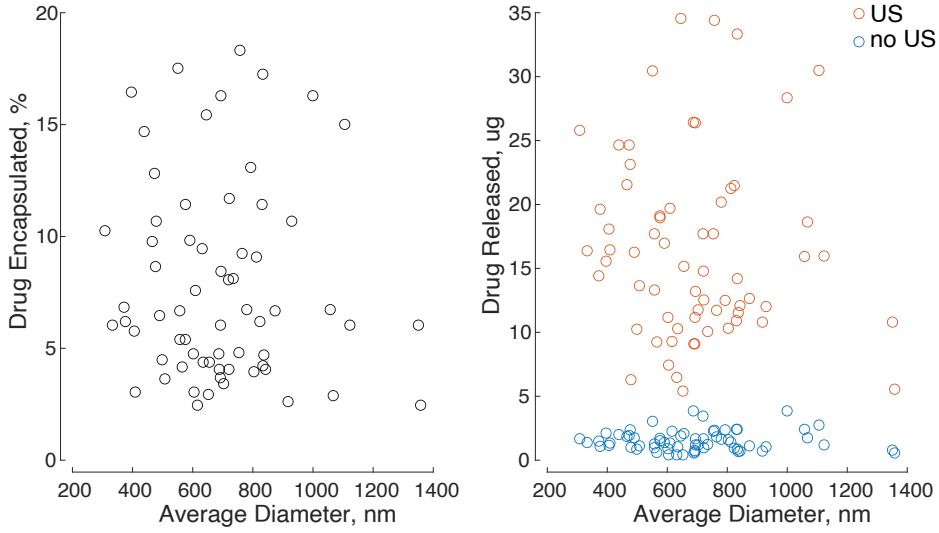

Figure S2. Particle size effects on drug encapsulation and release.