Image-guided phase change nanodroplets for the treatment of brain tumours

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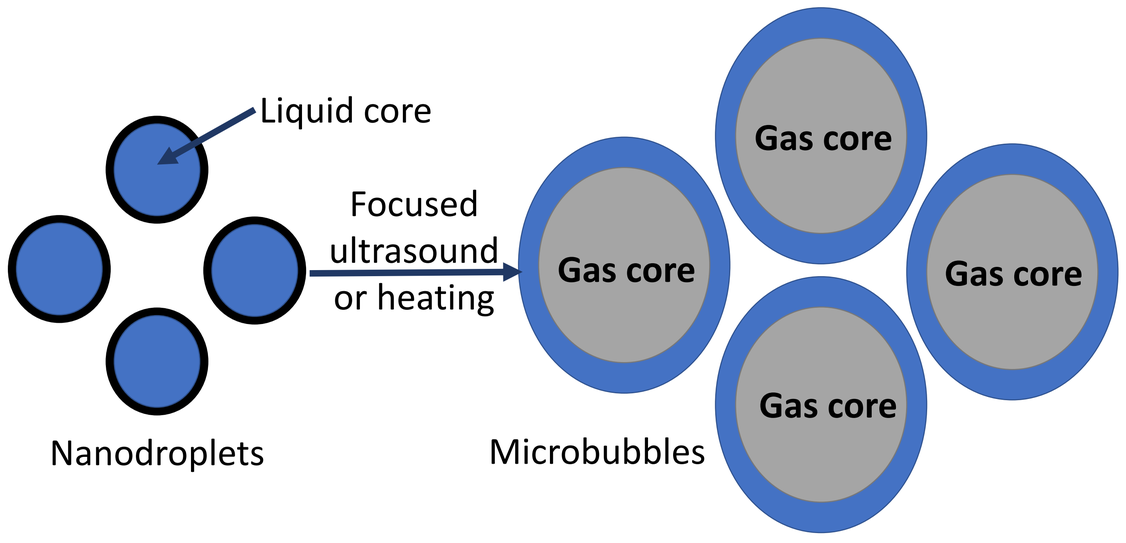
1. Introduction

High-Intensity Focused Ultrasound (HIFU) has attracted notable attention in the last years due to the ability to alter tissue characteristics and enhance the delivery of therapeutic molecules. In preclinical models (including non-human primates) HIFU has proved to intensify the permeability of macromolecules and nanoparticles through the Blood-Brain Barrier (BBB). The combination of HIFU with microbubbles (MB) cause cavitation and, potentially, a reversible permeability of the BBB for a short period occurs. However, the exact mechanism of brain delivery with the combination of HIFU and MB has not been discovered yet.



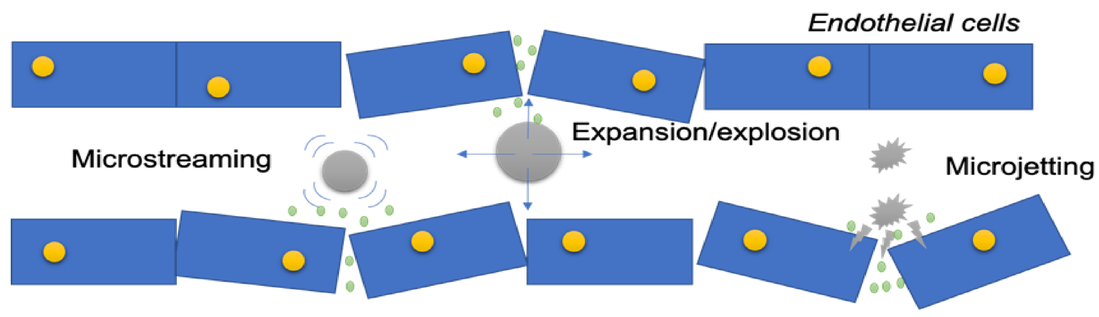
Scheme 1: Schematic representation of the phase changed nanodroplets into microbubbles after the application of focused ultrasound or heating

2. Aim of the study

This study will analyse the preparation of lipid-based phase change nanodroplets (NDs) labelled with fluorescent probes and/or loaded with therapeutic molecules which possess significant advantages than microbubbles.

3. Synthesis and characterisation

The nanodroplets formed from DPPC/DSPE-PEG_{2000}-MeO at 15 mg/mL total lipid concentration, containing 1% v/v perfluoropentane (PFP) or perfluorohexane (PFH) and 1-3% w/w SN-38. They stored in sealed glass vials with 20 mM HEPES/10% glucose, pH 7.4 at 4°C.



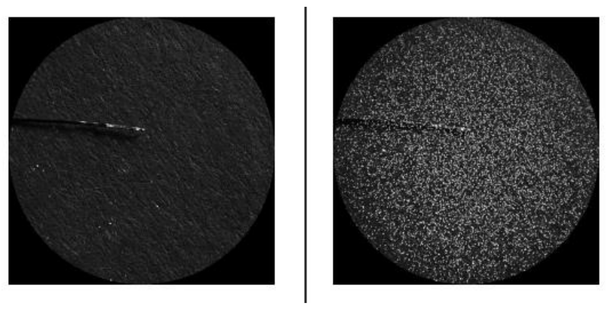
Scheme 2: Graphical representation of the acoustic cavitation induced biophysical effects on endothelial cells.

Sample (lipid ratio)	%PFC core, %drug	Size (nm)	Size in ND:FBS solution (nm)
SV501 (93:7)	1% PFP, -	221.3 ± 12.43	95.27 ± 1.1
SV502 (93:7)	1% PFP, 1% SN-38	143.9 ± 34.1	99.73 ± 1.08
SV503 (93:7)	1% mix, -	107.5 ± 2.77	93 ±1.64
SV504 in FBS	1% mix, 1% SM-38	123.87 ± 2.01	114.23 ± 2.93
SV505 (93:7)	1% PFH, -	88.29 ± 1.2	88.15 ± 10.01
SV506 in FBS	1% PFH, 1% SM-38	141.4 ± 1.9	118.0 ± 5.6

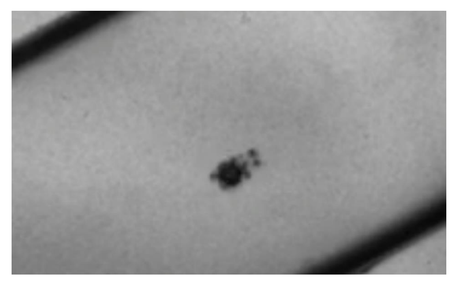
Table 1: Nanodroplet size measurement with different PFC core and/or drug loading. The size was also measured in an FBS solution to assess the stability after the *in vivo* injection

4. Physicochemical characterisation of Nanodroplets

The fabricated nanodroplets were tested with Dynamic Light Scattering (DLS) (Table 1). The encapsulated PFC was measured using fluorine ^{19}F NMR, while the SN-38 concentration was measured using fluorescent emission in 560 nm. Moreover, nanodroplet samples were heated at various temperatures for 10 min, tracking the evolution of gas bubbles with a camera set to collect images (Picture 1). Finally, using a high-speed camera (HSC) and a 692 kHz transducer, we were able to detect and capture the cavitation effects of the NDs (Picture 2).



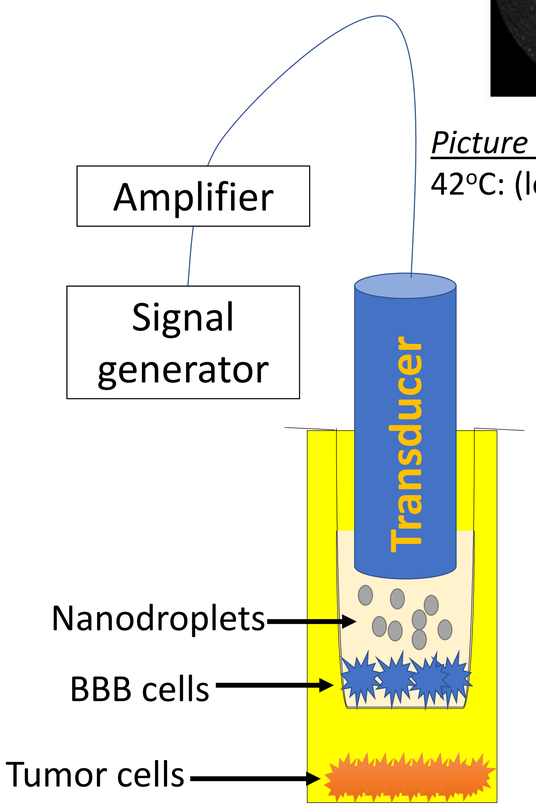
Picture 1: Nanodroplet images after heating in 42°C: (left) 0 min image; (right) 10 min image.



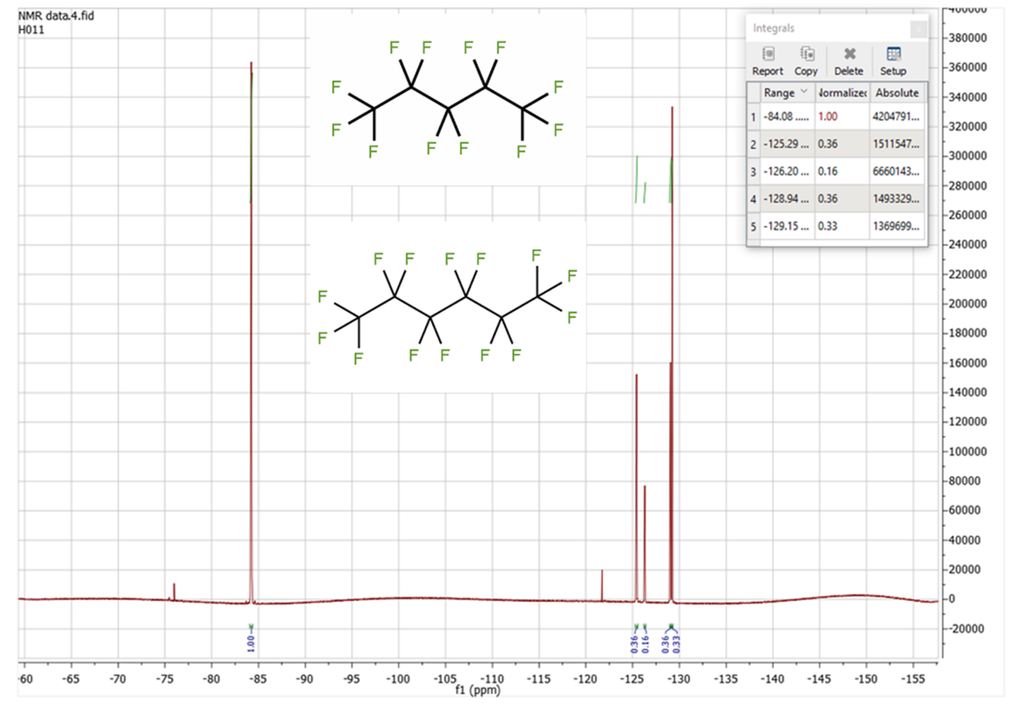
Picture 2: HSC image after FUS application in the ND solution. This is a single bubble capture.

5. Conclusion and Future Work

These experiments proved the concept that the ND core consists of PFC after the NMR analysis. Moreover, the NDs started cavitating after the FUS application and captured with HSC. Furthermore, the encapsulation efficiency of the SN-38 is around 100%. The preliminary cell culture experiments (Scheme 2) showed that there is an increase in the membrane permeability after the application of ultrasound in ND solution. Further experiments and troubleshooting need to be made.
Furthermore, NIRF dyes will be incorporated in the membrane to mark the NDs for *the in vivo* experiments.



Scheme 3: Cell culture setup to assess the BBB permeation after the administration of nanodroplets and the application of focused ultrasound.



Picture 3: NMR spectrum of the SN-38 NDs with a PFC mixture core. It is evident that both PFH and PFP coexist in the formulation core.