Effect of the Suspending Medium on the Stability and Acoustic Behavior of Phospholipid-Coated Microbubbles

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BACKGROUND

Microbubbles (MBs) are gas-core, shelled colloids that oscillate under ultrasound

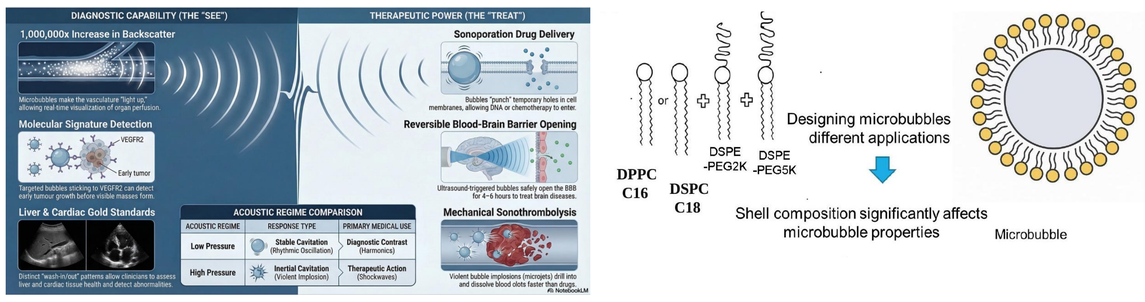


Fig. Schematics of typical phospholipid shell MBs with different PEG-emulsifiers. Shell composition governs MB stability and acoustic response. Gas core: perfluorobutane (PFB).

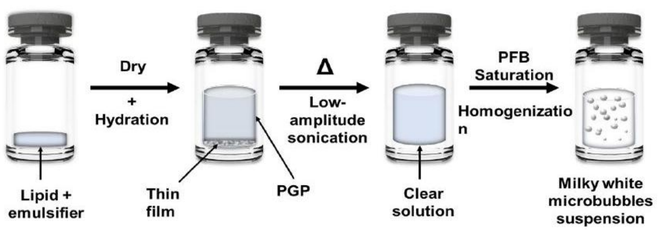
Knowledge Gap: MB characterisation is almost exclusively performed in PBS — which does not represent physiological conditions.

OBJECTIVE

Compare stability and acoustic behaviour of three phospholipid-shell MB formulations in PBS, FBS, and porcine whole blood (37°C).

METHODS

Synthesis Process



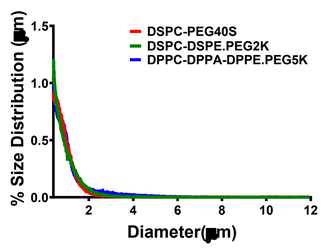


Fig. Lipid-film hydration + PFB gas saturation (×5) → homogenization (Precellys, 10,000 rpm, 45 s) → centrifugal wash → resuspension in PGP buffer (PBS:glycerol:propanediol 80:10:10 v/v). RHS panel show size distribution plot as obtained from standard characterization electro-sensing zone technique using Multisizer 4e.

Three Formulations

Formulation	Main Lipid	Gas	Mean Size (μm)	Concentration (#/mL)
DPPC-based (Definity® like)	DPPC, DPPA, DPPE-PEG5000	PFB	1.1	~2E10
DSPC	DSPC-PEG40S	PFB	1.6	~4E09
DSPC	DSPC-DSPE.PEG2000	PFB	1.5	~9E09

Initial Size Distributions

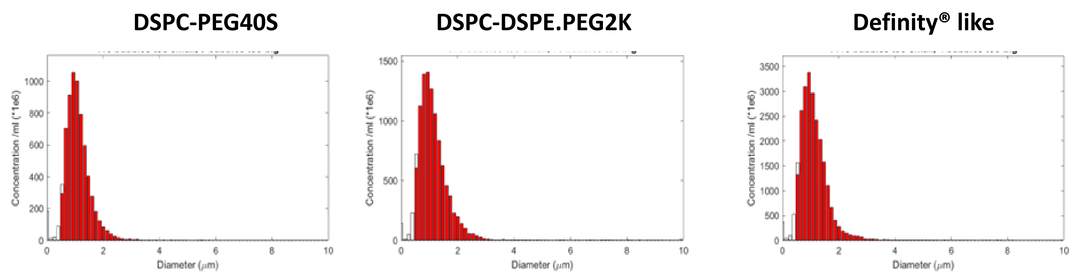
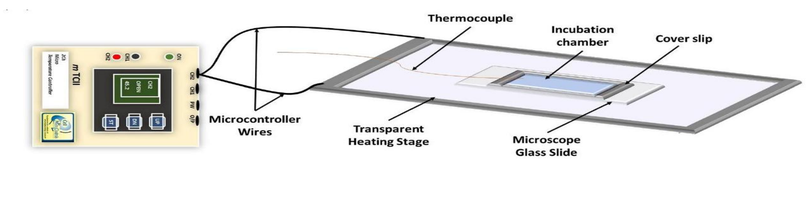


Fig.: Size distribution plot of all three formulations. Custom MATLAB script was used to analyze microscope images to count >1000 MBs. As prepared MBs have very similar size distribution profile across formulations with narrow peak at 1–2 μm.

Stability Testing Setup (37°C, 60 min)



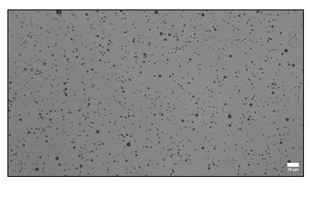
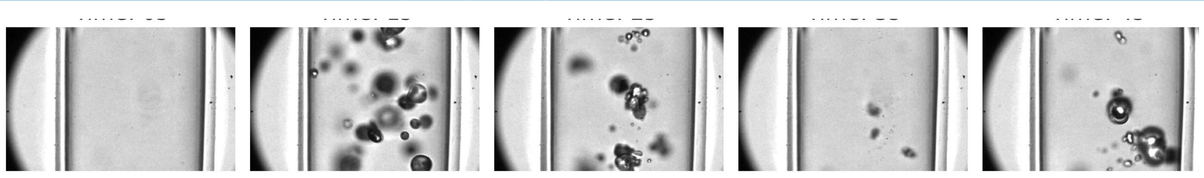


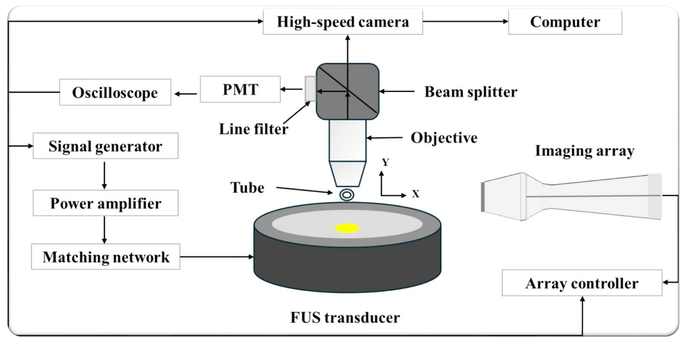
Fig.: Heated transparent stage (37°C ± 0.2°C) with coverslip incubation chamber. Optical tracking at 40×; size and concentration measured at t = 0, 5, 10, 15, 20, 30, 45, 60 min. RHS, microscope image of a typical MB suspension

Passive Cavitation Detection (PCD) Setup



PCD Parameters

- 0.5 MHz FUS
- 50 cycles
- PNP 0.1–1.0 MPa
- 7.5 MHz PCD receiver
- Flow: 0.1 mL/min



MB conc: ~10^7/mL · Blood diluted 10% v/v in PBS; Broadband SNR integrated 2–4 MHz ·

RESULTS

1. In Vitro Stability (37°C, 60 min)

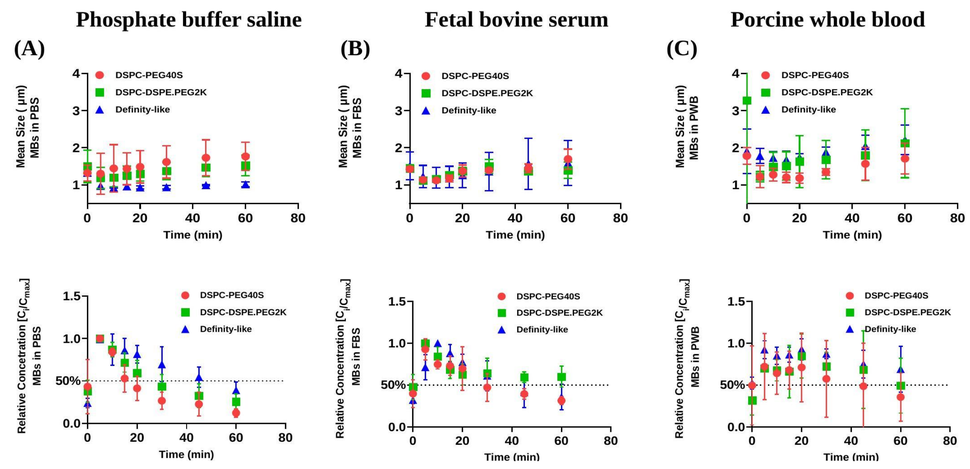


Fig. Mean size (top row) and relative concentration C/C_{max} (bottom row) in PBS (A), FBS (B), and porcine whole blood (C) at 37°C over 60 min. Dotted lines = 50% threshold.

Key finding: Stability follows whole blood > FBS > PBS. DSPC-PEG40S loses ~88% concentration in PBS within 1 hr. Definity-like shows greatest persistence across all three media.

2. Shelf-Life Stability (4°C, 28 days)

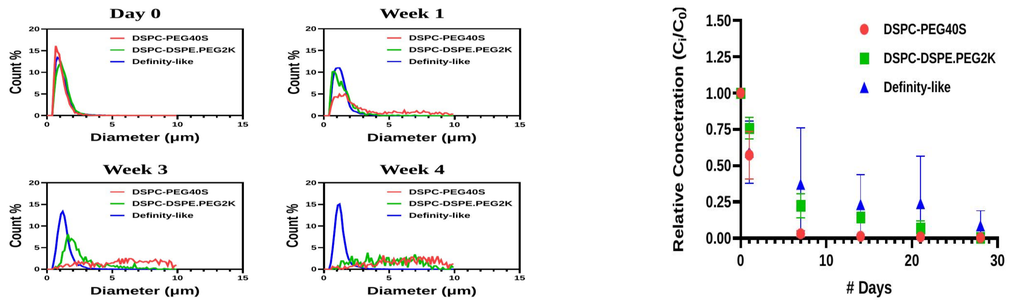


Fig. Size distributions (left, Days 0–28) and relative concentration over 4 weeks (right). Definity-like retains ~24% at Day 21 vs ~6% (DSPC-DSPE-PEG2000) and ~1% (DSPC-PEG40S).

3. Acoustic Emissions — Broadband SNR (0.5 MHz, PCD)

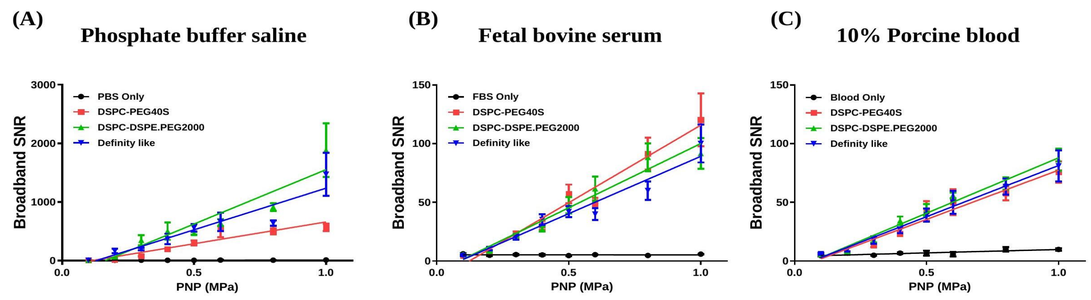


Fig. Broadband SNR vs PNP (0.1–1.0 MPa) in (A) PBS, (B) FBS, and (C) 10% v/v porcine blood. Note y-axis scale: PBS up to 3000 vs 150 for FBS/blood — a ~20× difference.

Key finding: Broadband SNR ~20× lower in FBS and blood vs PBS across all formulations and PNP levels. Protein corona formation and increased viscosity increase shell rigidity and damp oscillatory response.

4. Ultrasound Contrast Imaging (7 MHz, MI 0.5, iU22 Philips)

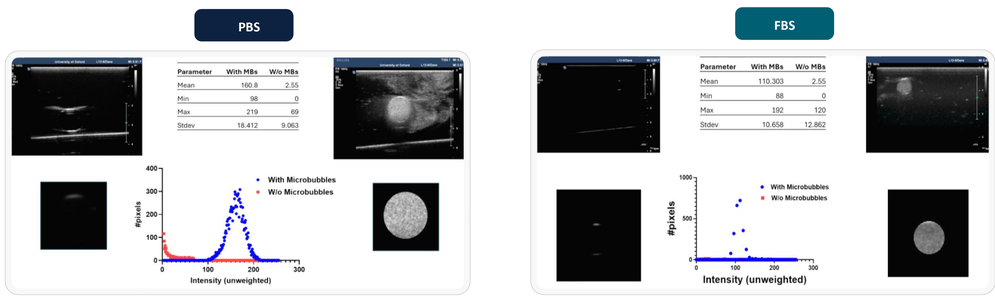


Fig. B-mode images of Definity-like MBs in PBS (left, mean grey = 161) and FBS (right, mean grey = 110) vs blanks (~2.6). Both confirm enhancement; signal lower in FBS, consistent with acoustic data.

CONCLUSIONS

1. Suspending medium governs MB stability: whole blood > FBS > PBS. Protein corona and increased viscosity provide a protective effect — independent of formulation.
2. Broadband SNR is ~20× lower in FBS and blood vs PBS, across all formulations and PNP levels (0.1–1.0 MPa), due to shell rigidification and damped MB oscillatory response.
3. DPPC-based (Definity® like) is the most stable formulation: greatest persistence across all three media, and ~24% MBs retained at Day 21 vs <7% for DSPC-based formulations.
4. PBS-only characterisation overestimates acoustic activity and underestimates in vivo persistence. Physiologically relevant media (FBS/blood) are essential for translational accuracy.

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Selected References

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Poster

Contact