Stably engineered nanobubbles for ultrasound-triggered drug delivery: formulation, characterization and *in vitro* evaluation

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Introduction

Nanobubbles (NB) are a novel delivery system comprising gas entrapped inside a nanosized carrier. When exposed to high intensity ultrasound (US), the vesicles transiently enhance neighbouring cell/tissue permeability (1, 2). While having shown success in maximizing the delivery of various therapeutics, NBs are plagued with low stability and large size variations (3).

Objectives

- To optimize the size, polydispersity and storage stability of an investigative NB formulation
- To evaluate the impact of US on NB echogenicity and integrity
- To assess NB+US combination toxicity *in vitro*
- To determine the impact of the NB+US combination on the cellular uptake of a model macromolecule

Methods

Formulation: Liposomes (DPPC and DSPE-PEG(2k)-OMe (94:6)) were prepared using the thin film hydration method (4). Particles were supercharged with perfluoropropane (PFP) gas in a pressurized environment and downsized by means of either filtration, extrusion or sonication to form NBs.
Characterization: Particle size and PDI were determined using a Malvern nanozetasizer. NBs were imaged (Ellex® EyeCubed) and burst on-demand (Johari Digital®), using the respective ultrasound instruments. NBs were visualized via SEM and cryo-TEM.
In vitro assays: The impact of NB+US on cellular toxicity and its impact on macromolecule internalization was evaluated in ARPE-19 cells.

Results

	Crude NB	Filtered	Extruded	Sonicated
Z-ave size (nm) Day 1	606.4 ± 132.4	379.2 ± 14.1	212.3 ± 15.9	136.1 ± 16.5
Z-ave size (nm) Day 28	522.3 ± 48.2	353.8 ± 7.08	220.8 ± 12.3	147.3 ± 22.7
PDI Day 1	0.496 ± 0.096	0.253 ± 0.010	0.293 ± 0.030	0.405 ± 0.102
PDI Day 28	0.471 ± 0.050	0.232 ± 0.019	0.284 ± 0.030	0.419 ± 0.142

Table 1 – Day 1 and day 28 *Z*-average size and polydispersity index profiles of NB formulations downsized using various techniques (n=3).

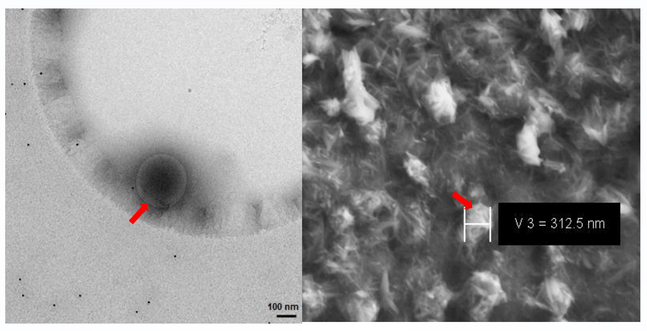

Figure 1 – Cryo-TEM (left) and SEM (right) images of extruded NBs with entrapped PFP gas indicated by arrows.

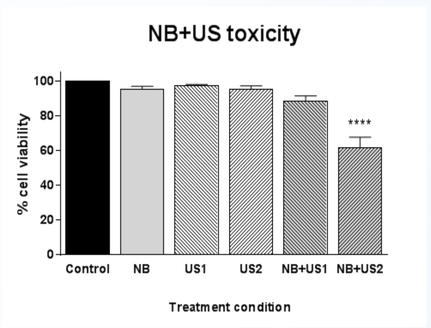

**Figure 2 – Impact of various treatment conditions on ARPE-19 cell viability.
US1: 1 MHz, 20% duty, 0.5 W/cm², 30 s
US2: 1 MHz, 20% duty, 1.0 W/cm², 30 s**

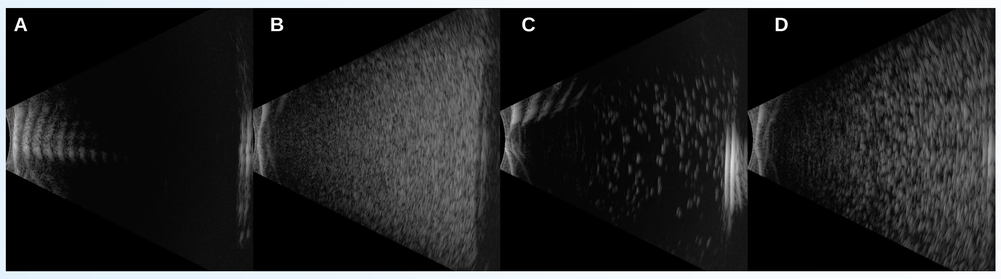

Figure 3 – Echogenic profiles of (A) standard liposomes, (B) crude NBs, (C) filtered NBs and (D) extruded NBs on day 28, with formulations (B) & (D) effectively retaining echogenicity.

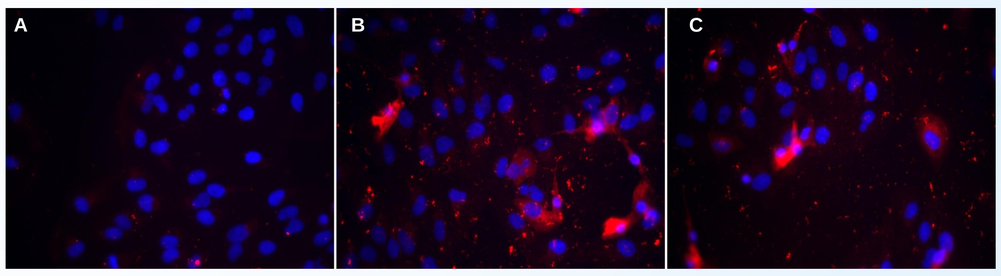

Figure 4 – Fluorescence microscopy images demonstrating ARPE-19 internalization of a model Cy3-labeled (red) antibody following (A) no treatment, (B) filtered NB+US1, (C) unfiltered NB+US1.

Conclusions/Future Work

1. Filtration and extrusion are ideal methods for reduction and standardization of particle size
2. Formulation storage echogenicity was maintained for at least 28 days, with gas localising inside the NBs
3. The NB+US combination can be effectively optimized to minimize treatment-related cell toxicity
4. NB+US can enhance the internalization of a co-administered model antibody into ARPE-19 cells
5. US optimization is underway to evaluate NB efficacy in *ex vivo* tissue models

References:

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2. *S Thakur et al. (2014) Intravitreal drug delivery in retinal disease: are we out of our depth? Expert Opin Drug Deliv [Early online].*
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