Ultrasound-Responsive Nanobubbles for Enhanced Posterior Eye Delivery of Therapeutics

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Introduction

Background

- The burden of posterior eye disease continues to increase due to an ageing global population
- Current treatments are only sparingly able to reach affected retinal tissue[1]
- Low efficacy of the intravitreal route has resulted in high treatment costs
- Ultrasound-responsive bubbles may help improve the outcomes of posterior eye delivery[2]
- While showing promise in the *in vitro* setting, earlier bubble-ultrasound strategies have failed in all clinically relevant *ex vivo* and *in vivo* retinal evaluations to-date[3,4]

Study Aims

We assessed the impact of trans-scleral ultrasound on intravitreal bubble migration. Rhodamine dyes were injected into porcine eyeballs either alone, or co-formulated with or entrapped within nanobubbles. Dye migration was evaluated in both fresh and aged models of the vitreous humour.

Methods

Preparation of nanobubbles: Particles were prepared using a method adapted from Suzuki et al[5]. Liposomes composed of DPPC and DSPE-PEG(2k) (94:6 mol ratio) were supercharged with perfluoropropane gas to form lipid-shelled bubbles. The formulation strategy was further optimised to improve bubble size homogeneity. Rhodamine dyes were either incorporated into the liposomal bilayer or simply mixed with the formulation at a final dye concentration of 2 mg/mL immediately prior to injection.

Intravitreal injection technique: Formulations were injected into the mid-vitreous through the pars plana on the temporal side of the eye. A clinically relevant dose was injected with the formulation manifesting as a bolus residing marginally posterior to the lens.

Ultrasound administration protocols: A Johari® JUS-2 ultrasound unit equipped with a 35 mm probe was utilised for experiments. Following dye ± nanobubble injection, eyes were subjected to extraocular ultrasound administration either at the pars plana or transcorneally (Figure 1).

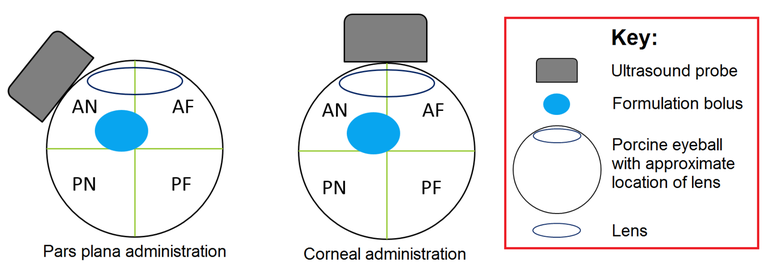

Figure 1 – Schematic depicting investigated modes of therapeutic ultrasound administration to the eye. Injection was made through the AN region.

Evaluation of dye distribution: Eyes were snap-frozen in liquid nitrogen immediately following ultrasound administration[6]. Following complete freezing, eyeballs were divided into four distinct regions (Figure 1). Thawed vitreous humour from each section was centrifuged at 16,000 *g* for 25 minutes at 4 °C to sediment the collagen and all residual retinal material. Supernatant comprising all dye was collected and dye concentrations were evaluated by absorbance (554 nm, rhodamine B) and fluorescence (Ex/Em 530/590, 5-TAMRA) spectroscopy.

Results and Discussion

Impact of injection depth on ultrasound responsiveness

Clinically relevant depths of 4 and 8 mm resulted in distinct intravitreal dye distribution profiles. Namely, dye injected at the shorter 4 mm depth distributed irregularly within the AN and PN regions whereas dye injected at 8 mm remained a bolus primarily within the AN region (Figure 2). Particles injected at a 4 mm depth also remained unaffected by ultrasound exposure and so the 8 mm depth was promoted.

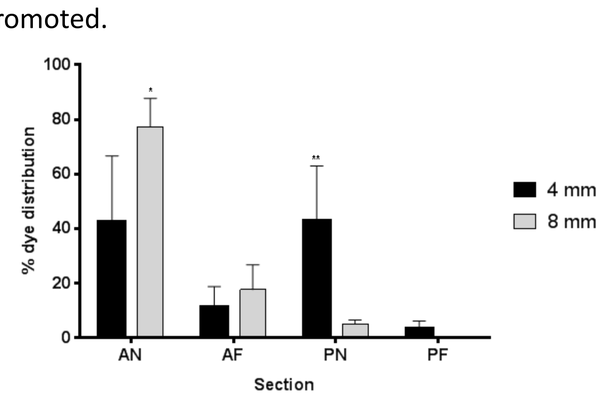

Figure 2 – Regional distribution of rhodamine B around porcine eye following 4 mm or 8 mm injection.

References

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Impact of vitreous age on technique efficiency

Ultrasound failed to impact intravitreal distribution of rhodamine dye in absence of nanobubbles (Table 1). Dye co-formulated with nanobubbles was not impacted by ultrasound in fresh eyes, however this could be significantly migrated in aged eyes with liquefied vitreous ($p<0.05$, Figure 3).

Table 1 – Summary of rhodamine migration behaviour in fresh and aged (liquefied) vitreous humour following subjections to various combinations of ultrasound +/- nanobubble treatment.

Treatment	Fresh vitreous	Aged vitreous
Ultrasound + rhodamine	No migration	No migration
Ultrasound + rhodamine/nanobubble co-formulation	No migration	Significant migration
Ultrasound + nanobubble-entrapped rhodamine	Significant migration	Significant migration

Influence of probe position on directional nanobubble migration

Pars plana ultrasound administration led to significant lateral dye movement from the AN to AF region of the vitreous humour ($p<0.0001$, Figure 4). Conversely, corneal ultrasound administration resulted in significant dye movement from the AN to PN region ($p<0.0001$).

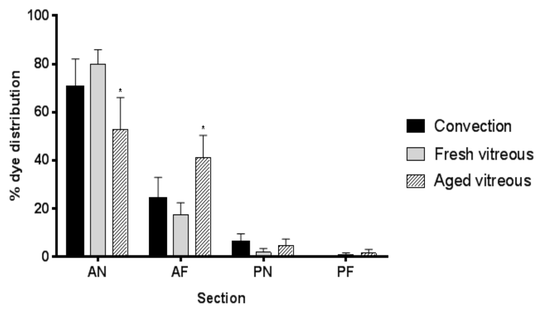

Figure 3 – Regional distribution of rhodamine B coformulated with nanobubbles following pars plana ultrasound administration to fresh and aged porcine eyes.

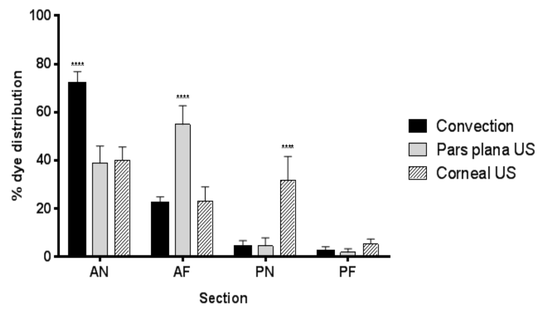

Figure 4 – Regional distribution of rhodamine-tagged nanobubbles following convection, and pars plana and corneal ultrasound administration.

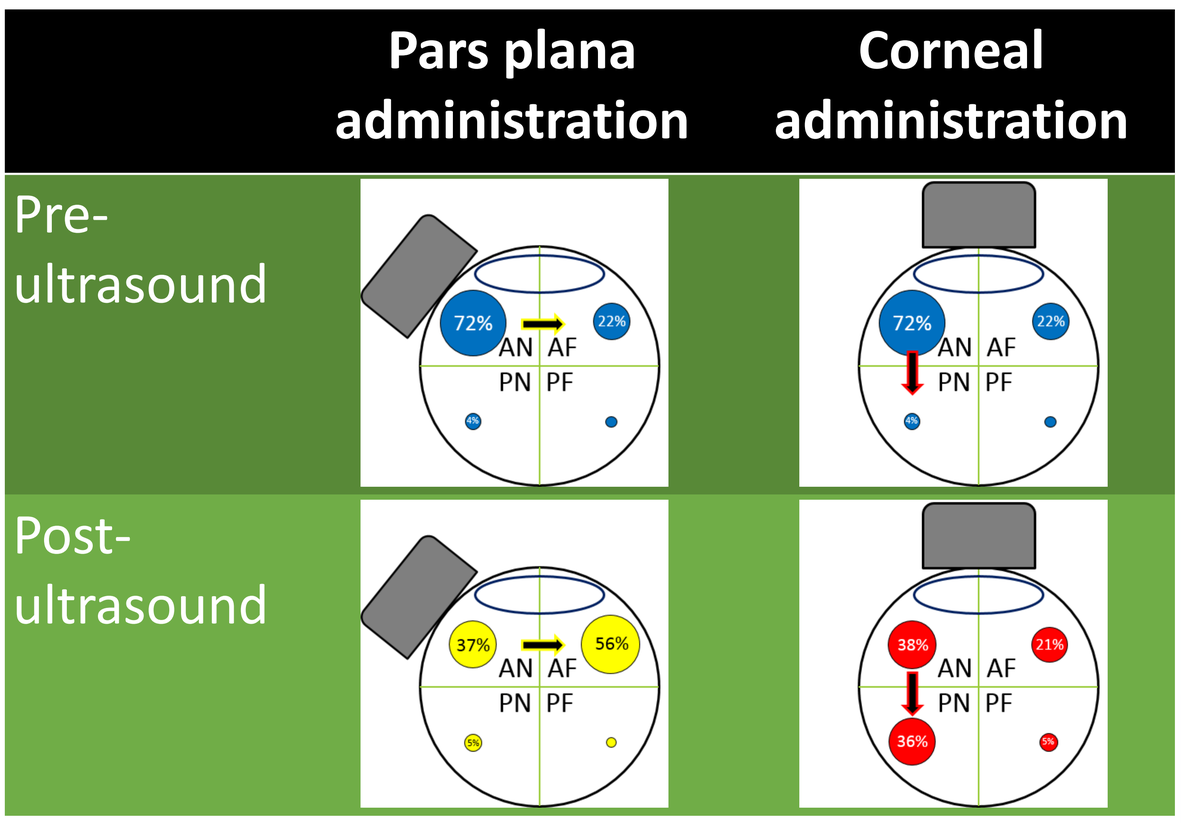

Figure 5 – Nanobubble-entrapped rhodamine localisation in porcine vitreous pre- and post-ultrasound administration via the pars plana and corneal route. Arrows show primary direction of dye migration following ultrasound application pars plana or transcorneally.

Conclusions

1. Depth of intravitreal injection and location of bolus impacts particle distribution and responsiveness to ultrasound.
2. Vitreous liquefaction has a substantial impact on efficacy of ultrasound-assisted dye migration, this phenomenon only being achievable in presence of echogenic nanobubbles.
3. Ultrasound probe position dictates ultimate migration path of nanobubble-entrapped rhodamine dye, with nanobubbles traveling away from the applied ultrasound.

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For more information & collaborative opportunities contact Dr H.S. Parekh
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