

Mechanistic insights into theranostic ultrasound-mediated brain gene delivery

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Understanding the mechanisms of focused ultrasound (FUS)-mediated brain gene delivery is of utmost importance for successful translation of gene therapy technologies. Our group has developed theranostic ultrasound (ThUS), which enables a flexible sequence design with accurate, real-time cavitation monitoring in a cost-effective setting [1-2].

This study aimed to explore two aspects of ThUS-mediated gene delivery in mice. The first objective was to understand whether co-injection of microbubbles and adeno-associated viruses (AAV) during ThUS-BBBO enhances transduction efficiency compared to asynchronous injections, where the AAV were injected 10 minutes after ThUS+microbubbles. The second objective was to elucidate whether microbubbles with different stiffness, based on the ratio of modifiers in the lipid solution (80 %v/v PBS plus either 20 %v/v glycerol for stiff microbubbles or 20 % v/v propylene glycol for compliant microbubbles [3]) affect the resulting BBBO volume, cavitation dose and gene transduction. The ThUS system consisted of a P4-1 imaging phased array, which was driven by a Verasonics Vantage system at 1.5 MHz. Male C57BL/6 mice aged 6-8 weeks were used throughout the study, and a ThUS sequence was programmed to deliver 10- and 1.5-cycle pulses to the right and left hemispheres of mice's brains respectively while the AAV9-CAG-GFP construct was intravenously administered.

For the first objective, BBBO volumes and cavitation doses did not differ between synchronous and asynchronous injection groups, however fluorescence imaging showed approximately 2.4 times increased AAV transduction in the case of microbubble and AAV co-injection. This result demonstrates the applicability of the microbubble pump effect previously shown by our group [4] in the context of ThUS-mediated gene therapy. For the second objective, the stiffer microbubbles showed about 1.6 times increased cavitation levels compared to the compliant ones, with increased inertial cavitation levels, however the compliant microbubbles were more effective at BBB opening, with 1.3 times larger BBBO volumes on average. Preliminary AAV transduction results did not show substantial differences between the two microbubble groups. Overall, our results provide new mechanistic insights into ThUS-mediated brain gene delivery and highlight the influence of injection timing and microbubble stiffness on BBB opening and viral transduction.

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Conflicts of interest: None; **References:** [1] Batts et al., *Theranostics*, 2023; [2] Tsitsos et al, *J Control Release*, 2025; [3] Jafari Sojahrood et al., *ACS Nano*, 2021; [4] Chen et al., *Sci. Rep.*, 2016

