Biomolecular Cavitation Nuclei for Targeted and Cell-Based Therapy

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Background, Motivation and Objective:

Despite the achievements of ultrasound-enhanced drug delivery, this technology is currently limited by the physical properties of microbubbles as cavitation nuclei, and their synthetic nature. Due to their micron-scale size, microbubbles are purely intravascular and cannot interact with cells outside the vasculature, nor with the extracellular matrix. Furthermore, their short circulation times do not match those of many anti-cancer therapeutics. In this work, we explored the use of gas vesicles (GVs), a family of structurally stable and genetically encoded gas-filled protein nanostructures, as cavitation nuclei. We studied the use of GVs as both targeted cell disruptors, and genetically encoded cavitation nuclei produced by tumor homing cells.

Statement of Contribution/Methods:

The ability of GVs to nucleate cavitation and porate membranes of tumor cells was explored *in vitro* by engineering the GVs' outer shell proteins to express RGD peptides, targeting them to U87 cells. The cavitation of attached GVs was documented using a high frame rate (5Mfps) camera, while sonoporation was validated using fluorescence microscopy. Cavitation of GVs expressed by engineered tumor-homing *Salmonella Typhimurium* cells was detected by a Verasonics scanner, configured as a passive cavitation detector (PCD). Then, the ability of these cells to facilitate controlled cell lysis and release co-expressed therapeutics was evaluated using a bioluminescent protein as a payload model.

Results, Discussion and Conclusions:

The high frame rate movies demonstrated the capabilities of GVs to seed cavitation while attached to tumor cells, resulting in a significantly higher cavitation rate, compared to the control cells (Fig. 1a, $P<0.0001$). The sonoporation of these cells resulted in an increase in the uptake of propidium iodide following insonation. The insonation of Salmonella cells co-expressing GVs and the Nanoluc luminescent protein (Fig. 1b) produced wideband PCD signals and resulted in controlled cell lysis (42%, $p < 0.001$), and payload release (Fig. 1c). The use of GV cavitation, as part of molecular or cell-based therapies could improve the efficacy and selectivity of drug delivery, while mammalian expression of GVs could enable the integration of ultrasound cavitation with immunotherapy.

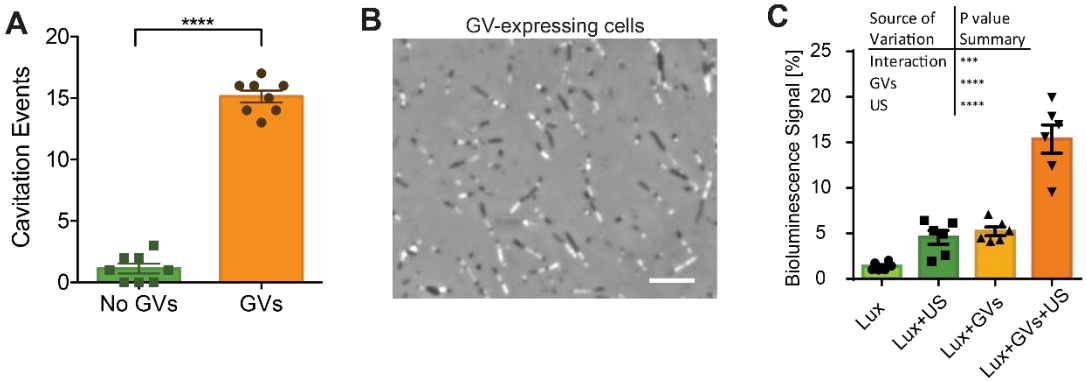

Fig. 1. Number of cavitation events observed in samples containing U87 cells with and without GVs (A). Phase microscopy images of GV expressing *S. typhimurium* cells. GVs are shown in white, while the rest of the cell cytoplasm appears black (B). Controlled payload release from *S. typhimurium* cells using GV cavitation (C).