Intracellular calcium fluctuations due to sonoporation in microbubble-mediated drug delivery imaged at high temporal and spatial resolution

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Background, Motivation and Objective

The vasculature is a barrier hindering efficient drug delivery to diseased tissue. Nevertheless, drug delivery can be locally enhanced with ultrasound (US) insonification of gas-filled microbubbles (MB) [Kooiman et al, Adv Drug Del Rev, 2014]. If we want to control and optimize the drug delivery pathways we need to understand their underlying biological mechanisms. We monitored intracellular calcium (Ca_i), since it plays a crucial role in membrane resealing, intercellular signaling, and integrity of cellular junctions [Qin et al, J Control Release, 2018]. To both visualize cellular effects and resolve MB oscillation, a unique optical imaging system was used consisting of a custom-built confocal microscope coupled to the Brandaris 128 ultra-high speed camera.

Statement of Contribution/Methods

Homemade MB were targeted to an angiogenic receptor ($\alpha_v\beta_3$) of human umbilical vein endothelial cells, grown to full confluency in a CLINIcell (50 µm, Mabio). The cellular response caused by single MB (n=138) was imaged with confocal microscopy up to 4 min after US (2 MHz, 100-250-400 kPa, 10 cycles). Sonoporation was monitored with Propidium Iodide (PI), integrity of cellular junctions with Cell Mask, and Ca_i fluctuations with Fluo-4. MB oscillation was recorded with the Brandaris 128 camera at ~16 Mfps and MB excursion was defined as the difference between maximum and resting radius.

Results/Discussion

Sonoporated cells always showed simultaneous influx of PI and Ca_i, while no influx was noted when no pore was formed. The Ca_i influx was assumed reversible when Ca_i returned to basal levels within 3 min, suggesting membrane resealing. On the other hand, Ca_i influx was considered irreversible when it increased for >3 min or clustered into vesicles (Fig. 1A). Upon sonoporation, adjacent cells showed a delayed and reversible Ca_i influx. The median MB excursion (1.3 µm) was significantly ($p<0.05$) higher when Ca_i influx was irreversible, than when reversible (0.8 µm) (Fig. 1B). Additionally, cellular junctions opened more often for irreversible Ca_i influx (54%), than for reversible influx (28%) (Fig. 1C). In conclusion, we found a clear correlation between Ca_i influx, MB excursion, sonoporation, and opening of cellular junctions. Understanding this relationship between drug delivery pathways and cell recovery will aid development of MB-mediated drug delivery.

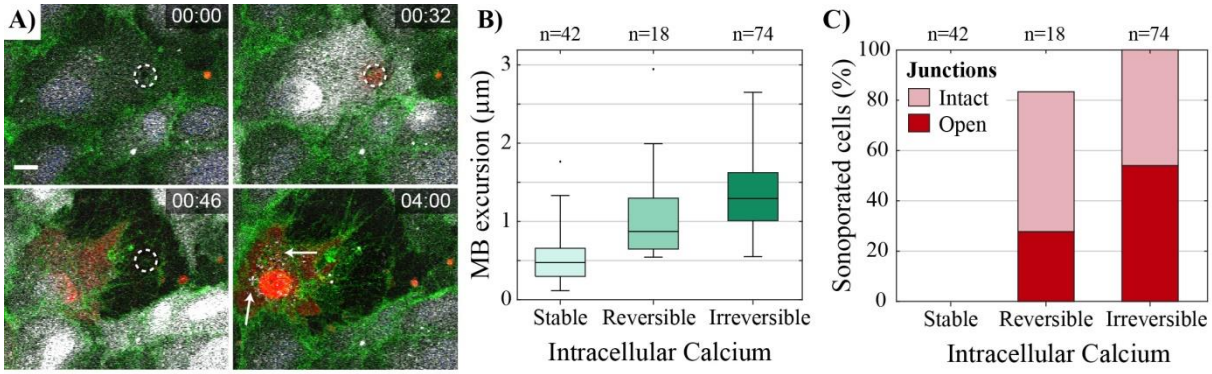

Fig. 1 A) Confocal microscopy of cellular response to ultrasound insonification (400 kPa at 00:30) of a single MB (dashed) showing PI uptake (red) and Ca_i influx (white) clustering into vesicles (arrows). Scale bar 10 µm. **B)** Median MB excursion for each Ca_i influx. **C)** Amount of sonoporated cells and opening of cellular junctions.

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