Microbubble-Mediated Sonoporation Induces a Multitude of Downstream Bioeffects on HL-60 Leukemia Cells

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Background, Motivation and Objective

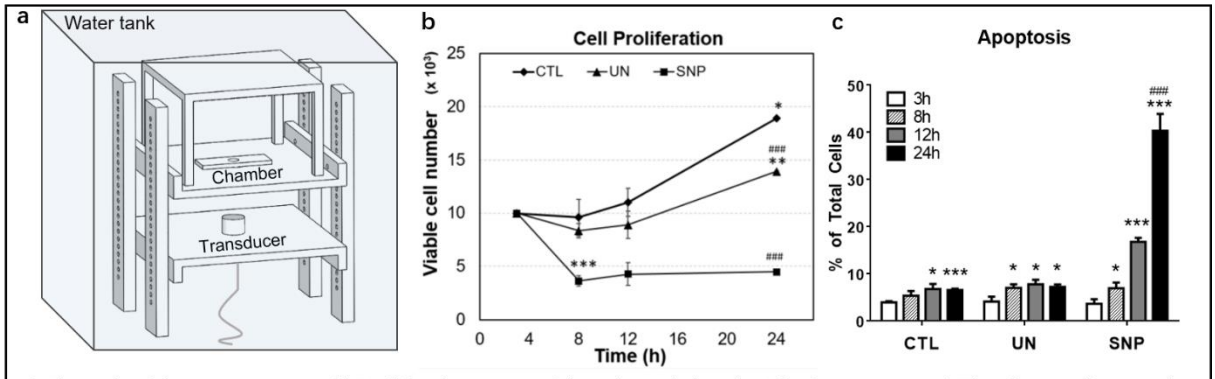
Sonoporation has demonstrated potential in transiently perforating cell membrane to facilitate drug delivery. However, the multitude of cellular bioeffects that may be induced by sonoporation has not been properly substantiated with convincing evidence. One caveat with many bioeffect experiments is that cells sonicated in the presence of microbubbles were presumed to be all sonoporated, but this is not necessarily true. Here, we devised a population-based experiment protocol that can specifically identify cell groups of interest and applied it to obtain robust observations of downstream bioeffects induced on sonoporated (SNP) cells that initially remained viable.

Contribution/Methods

We designed an immersion-based exposure setup with in-situ field calibrations (Fig. a). Its transducer delivered 1-MHz ultrasound pulses (100-cycle duration, 1 kHz PRF, 0.5MPa peak negative pressure) to the cell chamber, which was a 600μL-volume sealable labware with cover layers thinner (0.13-0.18 mm) than the ultrasound wavelength. In each trial, HL-60 leukemia cells and microbubbles ($2x10^7$/ml, 1:1 cell-bubble ratio) were suspended in RPMI-1640 medium and were added to the cell chamber together with calcein (sonoporation tracer). After 30s exposure, the cells were sorted using flow cytometry that analyzed the fluorescence of calcein and PI (viability indicator; added after exposure). Two cell groups were thus isolated: SNP (calcein+, PI-) and unsonoporated (UN) (calcein-, PI-). Two bioassays – cell proliferation (CCK-8), apoptosis (Annexin-V/PI flow cytometry) – were conducted on SNP and UN groups at 3, 8, 12, 24h after exposure. Real-time qPCR was also performed on cells 3 h after exposure to study the genetic expression of heat shock protein-70 (HSP70) (activated during cellular stress).

Results/Discussion

SNP and UN groups exhibited different downstream bioeffects. Over 24 h, the SNP group barely restored a cell proliferation trend (Fig. b) and showed significant apoptosis (Fig. c). In contrast, the UN group had limited apoptosis and restored cell proliferation 8h after exposure. Genes of HSP70 (HSPA1A, HSPA1B, HAPA6) were significantly upregulated for the SNP group after 3 h, whereas the corresponding genes had no significant change for the UN group. These findings serve to substantiate the notion that sonoporation poses prolonged stress on living cells.



a) Skematic of the exposure setup. b) Proliferation curves of the subpopulations in 24h after exposure. c) Time-lapse cell apoptosis of each subpopulation after exposure. Data were presented as mean±SEM. *,**,***, $p<0.05$,$p<0.01$,$p<0.001$ vs. the previous time point of the same group. #,##,###, $p<0.05$, $p<0.01$, $p<0.001$ vs. sham control group at the same time point.