ISTU On-Air Webinar Series: July 23,2020 Q & A

Alfred Yu, Ph.D. presenting "Sonoporation: It's More Than a Poke"

#	Question	Answer
1	Is is possible to use the sonoporation without adding the microbubble?	In principle, it is possible to achieve sonoporation without adding synthetic microbubbles to the cell's vicinity, if cavitation nuclei already pre-exist prior to ultrasound exposure. Also, the application of HIFU in the absence of microbubble is known to be able to generate cavitation activities (although the use of HIFU inevitably would lead to heating). Having acknowledged the above, let me point out that one key advantage of using synthetic microbubbles is that sonoporation may then be achieved using low-intensity ultrasound pulsing. Doing so would allow a high degree of spatiotemporal control over where and when sonoporation is realized. This is a big plus for controlled drug delivery applications.
2	In slide 13, Compared to sham group, morphology of sonoporated cells was changed in some degrees, which may cause eventually cell viability. Have you examined the sonoporated cells at least 1 or 2 days after sonoporation? Also, have you examined cell viability experiments with Live/dead imaging kit or LDH assay?	Admittedly an in-situ time-lapsed imaging study on the same sonoporated cell was not performed in that study. Though we did study the time-lapse viability of sonoporated cells using a population-based flow cytometry analysis approach. Corresponding data is presented in Slide 16 of the webinar. In brief, we have observed that a portion of sonoporated cells became non-viable over a prolonged period.
3	Great talk. Can we understand the sonoporation as an application type of cavitation? Thank you!	Yes, sonoporation can be regarded as a bio-oriented application of cavitation. After all, we are making use of forces generated over the course of acoustic cavitation to achieve cell membrane perforation.
4	How far are we in unravelling the mechanism of microbubble-mediated drug delivery? And what do we need to do to further unravel the mechanism?	I think the topic is still wide open for new contributions on the mechanisms of sonoporation and ultrasound-mediated drug delivery. While we seem to have mature knowledge on the physics of ultrasound and cavitation, the community still has not managed to achieve sonoporation and drug delivery with high controllability. One root cause is that our current understanding of sonoporation biophysics is still quite rudimentary. As a community, we need to do more to bridge the gap between cavitation physics and cell biology.
5	How does the cellular phenotype following sonoporation compare to the phenotypes found after other types of poration (e.g. electroporation, microfluidic squeezing)?	There should be similarities and differences in the cellular phenotypes of sonoporated cells and electroporated cells (and microfluidically squeezed cells). In terms of similarities, all these types of porated cells involve disruption of membrane integrity, so they will likely show similar acute biophysical phenomena, such as calcium ion influx to trigger membrane resealing. In terms of differences, because various poration techniques rely on different physical principles to achieve membrane perforation, the membrane's recovery dynamics and signaling pathways involved would likely be different.
6	Actin is very impirt for auditory sensiory cells. Is this a safety issye?	Based on our actin disruption results (Slides 11-13), we believe it would be necessary to further evaluate whether sonoporation affects the long-term functional activness of the actin machinery, since actin itself plays important functional roles in various cell types, including auditory sensory cells. Such a further investigation would help to address biosafety concerns of sonoporation. For this investigation, it would be worthwhile to determine the recovery time that a sonoporated cell needs to restore normal functioning of its actin machinery (if at all).
7	Thank you for the clear and well-organized presentation! 1. What might be the clinical application of sonoporation? If the mechanisms of sonoporation on a single-cell level become more clear, how does it help to apply sonoporation to clinical use? 2. Are there differences of sonoporation rate among cell types? Can we control the sonoporation rate (pore numbers) by control the ultrasound parameters?	Re Q1: Sonoporation is clinically relevant to ultrasound-mediated drug delivery in various application domains (as mentioned on Slide 3). Our mechanistic findings will be important to more rationally realize ultrasound-mediated drug delivery. For instance, through investigating poration dynamics and post-sonoporation recovery approaches, strategies can be designed to enhance the efficiency of ultrasound-mediated drug delivery. Re Q2: Based on our findings, we hypothesize that cell-type variations exist for sonoporation dynamics. It will be worth investigating these variations in the future, especially in connection to relevant biophysics parameters such as intracellular pressure and membrane tension. As for the second part of the question, in principle, the number of pores generated in each sonoporation episode depends on the number of bubbles in the vicinity of the cell. This is why cell-to-bubble ratio is known to be a parameter that influences the extent of sonoporation. Our experiments were mostly done at a 1:1 cell-to-bubble ratio to realize single-site sonoporation episodes.
8	Whether the repair time of the cell membrane depends on the size of the pore?	Yes, we found that repair time (pore resealing time) in general is longer for larger pores. Also, for larger pores, subcellular stress (actin disassembly) tends to persist for a longer period (as discussed on Slide 12), and membrane blebs tend to be larger (as discussed on Slide 22).

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9	How does the cell internal pressure affect sonoporation? Is the cellular membrane of the cell under tension, neutral, or compressively stressed?	One direct role that intracellular pressure would play here is that it would regulate blebbing dynamics during post-sonoporation recovery (as discussed on Slide 20). Intracellular pressure also plays a role in regulating membrane tension. Thus, it is likely a biophysical factor that influences perforation and resealing dynamics in a sonoporation episode. This will be a great avenue for further investigations on sonoporation dynamics. Note that our experiments were done on various cell types, including fibroblasts (whose membrane tends to be under tension) and breast carcinoma cells (whose membrane is more compliant). We have observed sonoporation in these different cell types. In the future, it would be worthwhile to further characerize the cell-type variation of sonoporation dynamics.
10	is it possible to "kill" selected cells from a bulk of different cells by choosing different parameters??	Perhaps one way of selectively realizing irreversible sonoporation to "kill" certain cells is to design targeted microbubbles that have strong binding preference to antigens expressed on a specific cell type. Also, depending on how the bubble shell is designed, the cavitation dynamics may also be tuned.
11	Which contrast microbubbles are you using and can you comment on the impact of using different types/sizes of microbubbles on your results	We have mainly used commercial microbubbles (Targeson, now Trust Biosonics) for most of our experiments. For the actin and blebbing studies, we used targeted microbubbles that were fabricated in house (based on protocol published by Sasha Klibanov). We found that larger bubbles tend to generate larger pores and larger blebs (Slide 22).
12	Would you like to comment on the ultrasound frequency dependence of sonoporation?	Ultrasound frequency surely plays a role in influencing the extent of cavitation activities. So there is a mild dependence of sonoporation on ultrasound frequency. Nevertheless, the relationship is somewhat indirect because it is the cavitation-induced physical forces that instigate a sonoporation episode.
13	Are there theoretical analyses of the viscoelastic dynamics? It looks like there might be a critical stress between self-healing and rupturing.	To our knowledge, nothing much has been done regarding theoretical modeling of membrane dynamics during sonoporation. It is well possible that a critical force exists beyond which membrane rupturing starts to occur. This is a research topic that is well worth looking into, perhaps in collaboration with membrane biophysicists.
14	How all of these results like the fragmentation of actin etc. can be employed in the context of drug delivery?	By identifying the subcellular impact of sonoporation, and the dependence of these mechanistic effects on different parameters, we can spark new efforts to take into account the biophysical facet of sonoporation to more controllably and effectively achieve ultrasound-mediated drug delivery while curtailing its downstream bioeffects. These further investigations will be important to establish the application merit of ultrasound-mediated drug delivery.
15	How reliable is FACS for assessing cell viability post sonoporation? The centrifugation step will blow the cells which are damaged (sonoporated) and these will not be accounted for when counting cells positive for PI.	The FACS procedure itself does not seem to have a significant impact on cell viability. This inference can be drawn based on our viabiility data obtained from sham exposure cells and unsonoporated cells (both underwent cell sorting within the flow cytometer). These two cell groups generally remained viable over the course of time, and few apoptotic and necrotic cells are detected in these two groups (Slide 16). In contrast, a more significant fraction of sonoporated cells became apoptotic and necrotic over a prolonged period.
16	Excellent talk presenting detailed studies of sonoporation in the cellular and sub-cellular levels. I really enjoyed it. Thanks to the presenter and to ISTU for this webinar.	Thanks for tuning in. Hopefully, through this webinar, we can help the therapeutic ultrasound community to establish a deeper understanding on the science of sonoporation.
17	How would this sonoporation effect on a cell be extended to tissue level? Do you have any study or guess?	From various insights that we have gained from single-cell sonoporation studies, such as subcellular stress and recovery signaling pathways, we can design new experiments to investigate the impact of sonoporation at the tissue level and how tissues recover from sonoporation episodes. One avenue in which research can move forward in this direction is to design vascular network models and to use them in sonoporation biophysics investigations.