Optimization of Intracellular Genetic and Non-genetic Cargo Delivery Using Clinical Ultrasound and Microbubbles

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I. Introduction

Targeted delivery of drugs, genes and siRNA using ultrasound and microbubbles (USMB) has emerged as a promising approach for treating diseases such as cancer and acute respiratory distress syndrome (ARDS).[1] Optimization of ultrasound settings and microbubble concentration is critical for achieving high efficiency of intracellular cargo delivery. Most of the *in vitro* optimization studies on USMB-mediated cargo delivery have been done with non-clinical, research-grade ultrasound systems. However, such setups are not clinically approved and can only be used in research labs. Moreover, such laboratory setups offer tremendous flexibility in adjusting ultrasound settings while commercially available clinical ultrasound systems offer limited flexibility in adjusting ultrasound settings. Thus, it is essential to study the effect of different clinical ultrasound settings on intracellular delivery of cargoes to speed up the translation of USMB-mediated therapies to the clinic. To date, there are no systematic *in vitro* optimization studies done with commercially available, clinical ultrasound systems. In this work, we show how different clinical ultrasound settings and microbubble concentration affect intracellular delivery of model non-genetic and genetic cargoes, such as fluorescent dextran and fluorescent siRNA respectively, using a novel, 3D-printed, modular platform for USMB studies.

II. Methods

A 3D-printed, modular platform called ultrasound-microbubble-cell chamber (UMCC) was built for optimizing different cargo delivery parameters using a clinical ultrasound system.[1] UMCC consists of different pre-designed blocks that snap-fit into each other to form the complete platform, analogous to LEGO® blocks. We used the commercial Phillips SONOS 5500 ultrasound system with Phillips S3 transducer, as well as commercial DEFINITY® microbubbles for all experiments. As a model drug, we used 4 kDa FITC dextran and 70 kDA tetramethylrhodamine dextran. We also used Alexa Fluor 488 tagged-siRNA as a model genetic cargo. We studied the effect of different clinical ultrasound parameters, specifically mechanical index (MI), pulse interval (PI), ultrasound exposure time as well as DEFINITY® microbubble concentration on efficiency of cargo delivery. MI was varied from 0.1 to 1.3, PI was varied from 200 ms to 5000 ms, ultrasound exposure time was varied from 20 seconds to 80 seconds and microbubble concentration was varied from 8 x 10^5 bubbles/mL to 2.4 x 10^9 bubbles/mL. Ultrasound frequency was kept constant at 1.3 MHz. Experiments were done with HEK293 and CMT167 cell lines with cells either cultured on 22 mm x 22 mm square coverslips or suspended in DMEM cell culture media. Intracellular delivery of cargo (fluorescent dextran or siRNA) was confirmed using confocal imaging and delivery efficiency was evaluated using CytoFlex-LX flow cytometer. For flow cytometry analysis, cells were resuspended in flow cytometry buffer and the proportion of fluorescent cells within the viable cell population was quantified in control and USMB treated groups. Cell viability was assessed using membrane impermeable DAPI.

III. Results

We discovered that the proportion of fluorescent cells increased with increasing MI, with the highest proportion obtained at MI of 1.3. Increasing MI beyond 1.3 lead to significant detachment of cells from the coverslip, therefore, experiments were limited to MI values between 0.1 to 1.3. The proportion of fluorescent cells was observed to decrease with increasing PI with the highest proportion obtained at PI of 200 ms. Moreover, we found that the proportion of fluorescent cells peaked at ultrasound exposure time of 40 sec. Increasing exposure time beyond 40 seconds did not significantly change the proportion of fluorescent cells. Moreover, the proportion of fluorescent cells peaked in a narrow range of DEFINITY® microbubble concentration, ranging from 4 x 10^7 to 1.6 x 10^8 bubbles/mL.

IV. Conclusion

Our experiments showed that cargo delivery maximizes at MI of 1.3, PI of 200 ms and ultrasound exposure time of 40 sec at microbubble concentrations ranging from 4 x 10^7 to 1.6 x 10^8 bubbles/mL. These optimum parameters could be potentially used for maximizing delivery of cargo *in vivo* with commercially available clinical ultrasound systems

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References

1. K. Joshi, R. Sanwal, K. Thu, S.S.H. Tsai and W.L. Lee, "Plug and Pop: A 3D-Printed, Modular Platform for Drug Delivery Using Clinical Ultrasound and Microbubbles," Pharmaceutics, 14(11), 2516, 2022.