Microbubble-assisted Crispr/Cas9 delivery in ARPE-19 cells

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I. Purpose

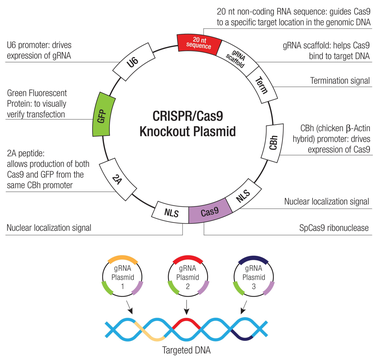
- To develop an ultrasound (US) guided and microbubble (MB) assisted approach to delivering Crispr/Cas9 in the eye for gene therapy of ocular diseases.
- Crispr-mediated gene editing is particularly appealing in the treatment of retinal diseases involving dominant-negative and gain-of-function mutations, as it offers a promising therapeutic strategy to repair the disease-causing gene, thereby removing the abnormal protein. The Crispr/Cas9 delivery technology we are developing is minimally invasive and not limited to any specific gene defects.

sonoporation, ultrasound, microbubbles, gene delivery, gene therapy

II. Methods

The GTS Sonoporation System (ST-GTS, Nepagene) equipped with a plane wave transducer module (PW-1.0-15 mm, 15 mm diameter tip, center frequency of 1 MHz) was used as the US source to transfect plasmid DNA into cultured human embryonic kidney 293 (HEK) cells and the human retinal pigment epithelial cell line (ARPE-19).

Fig. 1. A schematic of the *in vitro* US setup to carry out MB facilitated DNA transfection. The transducer is positioned in a tank, submerged in distilled water heated to 37°C. The cell monolayer is cultured on the cover slip, which is placed afloat in a polydimethylsiloxane (PDMS) well filled with the cell culture medium supplemented with the MBs and the plasmid DNA. The well is placed approximately 34-35 mm above the transducer.



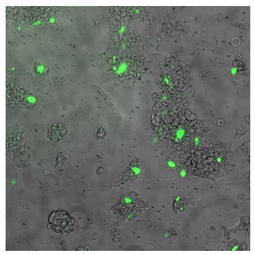


Fig. 2. The RPE65 Crispr/Cas9 plasmid construct (left) and the transfection in Att20 cells with the use of the transfection reagent (right). The Crispr/Cas9 plasmid was obtained from Santa Cruz Biotechology, CA, and it consists of a pool of three constructs each encoding the Cas9 nuclease and a RPE65-specific 20 nt guide RNA (gRNA).

III. Results

a) Delivery of the GFP plasmid in the HEK cells

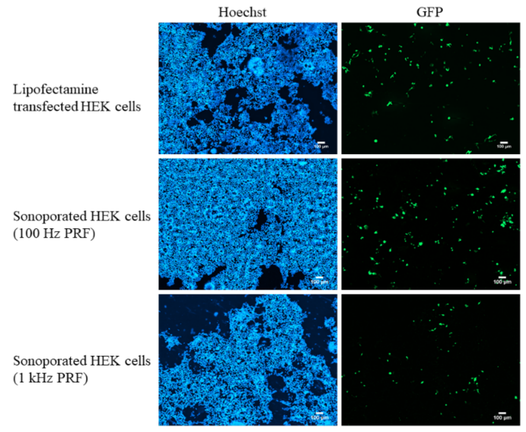


Fig. 3. Confocal imaging of the HEK cells transfected with the GFP plasmid (structure shown below) by sonoporation at 100 Hz (top panel), and 1 kHz PRF (bottom panel)

b) Sonoporation of ARPE-19 cells with varied parameters

Table 1. Variations in the sonoporation parameters in the delivery of the GFP plasmid into ARPE-19 cells under 100 Hz PRF and 30% DC.

	Control	Power	Duration	Plasmid	MB
Power intensity (W/cm²)	2	4	2	2	2
Sonication duration (min)	3	3	6	3	3
GFP plasmid (ug/mL)	4	4	4	8	4
MB%(V/V)	0.4	0.4	0.4	0.4	4

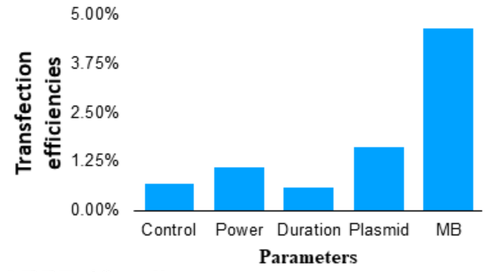


Fig. 4. The effect of changing different sonoporation parameters on transfection rate of GFP in ARPE-19 cells. Percentage of green fluorescent ARPE-19 cells = (number of green fluorescent cells / number of Hoechst stained cells) × 100%.

c) Delivery of the Crispr/Cas9 DNA plasmid in the HEK cells and ARPE-19 cells

- These experiments were carried out under 1 MHz acoustic frequency, 100 Hz pulse repetition frequency (PRF) and 30% duty cycle (DC), a combination that has been shown to yield better transfection rate than the 1 kHz RPF.

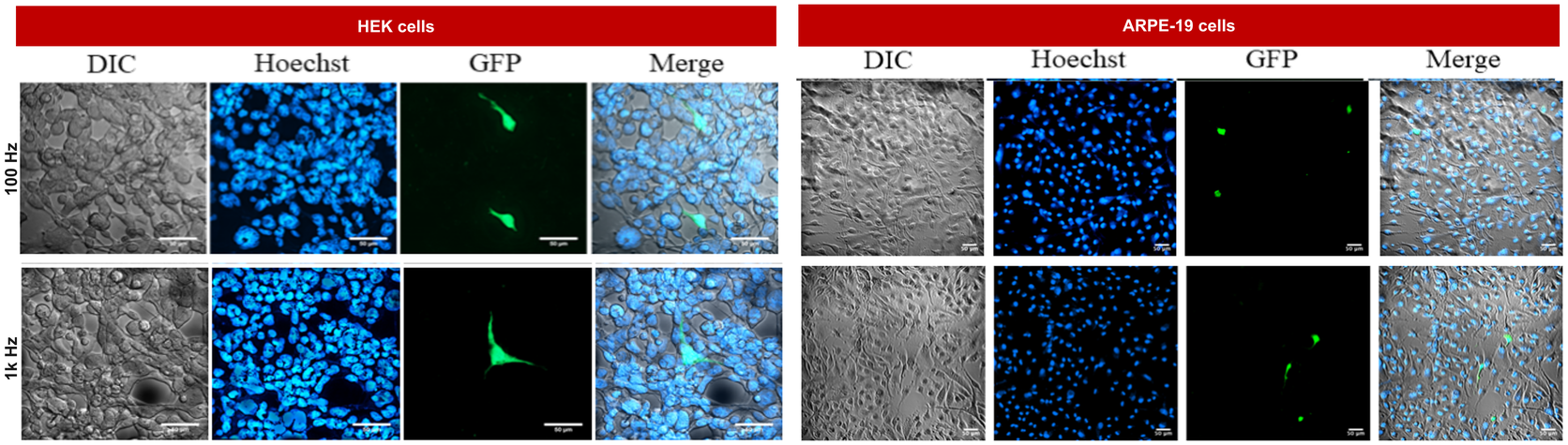


Fig. 5. Confocal imaging of the HEK cells and ARPE-19 cells transfected with the RPE65 Crispr/Cas9 knockout plasmid by sonoporation at 100 Hz (top panel), and 1 kHz PRF (bottom panel)

IV. Discussion and Conclusion

- This study explores the feasibility of developing an MB-assisted Crispr/Cas9 sonoporation method in cultured human HEK cells and RPE cells. The preliminary data shows the possibility of introducing the GFP plasmid and the commercial RPE65 Crispr/Cas9 knockout plasmid into the cells by our *in vitro* experimental setup.
- Transfection efficiency of the Crispr/Cas9 plasmid is low compared with that of the GFP under similar sonoporation conditions, likely resulting from the bulky size of the Crispr/Cas9 plasmid.

Next steps:

- Increase the delivery efficiency by developing more versatile Crispr/Cas9 constructs for sonoporation, e.g., separate the all-in-one vector containing both the Crispr and the gRNA into multiple vectors.
- Examine both the transfection and gene-editing efficiency of the new constructs.

V. Acknowledgement

This work is supported by the NSF Biophotonics #1805210; Dean's Pilot Funding Program, USC Keck School of Medicine; Unrestricted Grant to the Department of Ophthalmology from Research to Prevent Blindness, New York, NY; and funding from USC Ginsburg Institute for Biomedical Therapeutics (GIBT).