

Phase-change peptide-Cas9 nanoemulsions for ultrasound-guided 3D gene editing

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CRISPR gene editing technologies have transformed the study of genetic disease and development of therapeutics. Present techniques rely on viral or non-viral transfection of nucleic acids (e.g., DNA, mRNA) into cells to encode the production of Cas proteins and their paired guide RNA (gRNA). This transfect-produce-edit methodology is efficient in single-cell and monolayer cultures *in vitro*, but is challenging to translate into three-dimensional organoid cultures and *in vivo* tissues due to poor spatiotemporal control over delivery and limited diffusion through the extracellular matrix.

Here, we report acousto-responsive nanoemulsions that can be guided and activated by ultrasound to deliver ribonucleoproteins (RNPs) into cells within the bulk of three-dimensional tissue. This advance is realized through the development of peptide-stabilized perfluorocarbon nanodroplets that bind to cell surface integrins, and can be vaporized using traditional diagnostic ultrasound to mechanically drive surface-bound RNPs into target cells to affect gene editing. Using three-dimensional kidney organoids as a model, we demonstrate this technology improves the efficiency and depth of gene editing in the organoid mass relative to standard lipid-based transfection reagents, without compromising tissue structure or cellular viability. On-going studies focus on demonstrating spatiotemporal control of particle-mediated RNP delivery under acoustic guidance, thereby enabling opportunities to use ultrasound imaging to direct, monitor, and control gene editing *in vivo*.

This technologic paradigm may open new opportunities for imaging-guided, precision gene editing in tissues, thereby expanding the potential for CRISPR-based therapeutics in the clinic.