Plasmid-loaded Magnetic Nanodroplets: Potential Nonviral Vector for Plasmid Delivery to Tumor Cells

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Background, Motivation, and Objective:

Gene therapy has a potential for cancer treatments but has been hindered by vector-related limitations, including toxicity and inefficient gene delivery to tumor cells after intravenous administration. In this study, we sought to construct plasmid-loaded magnetic nanodroplets (PLMNDs) as a nonviral vector for plasmid delivery, that could be magnetic targeted to tumor tissue and further transform into microbubbles after ultrasound (US) activation to enhance gene delivery to tumor cells both *in vitro* and *in vivo* due to sonoporation.

Statement of Contribution/Methods:

Preparation of PLMNDs consists of four steps, as shown in Fig. 1A. 25 kDa branched polyethylenimine (PEI) was added to protect the plasmids from enzymatic degradation in serum-conditioned media. PLMNDs were activated with a 1.2 MHz focused transducer using peak negative pressure (2-6 MPa), pulse duration (3-60 cycles), pulse repetition frequency (10-100 Hz) and exposure time (0.5-5 s) for inducing *in vitro* plasmid delivery to SMMC-7721 cells. Expression of green fluorescent protein (GFP) in cells was detected by fluorescence microscopy and flow cytometry. T2 weighted MRI images were collected to show the effect of *in vivo* accumulation of PLMNDs in mouse xenograft tumor model. Then plasmid delivery effect was demonstrated by GPF expression in tumor after US exposure.

Results/Discussion:

The prepared PLMNDs had a mean size of 270.6 ± 60 nm, each carrying 15 plasmids (Fig. 1B). The plasmids loaded on PLMNDs could avoid enzymatic decomposition in 10% serum PBS solution at least 4 h. The PLMNDs had a good magnetic targeting effect (Fig. 1C), and could efficiently permeate into the tumor vessel mimic agarose gel phantom with a pore size range of 200-800 nm under magnetic field. GFP expression in cells demonstrated that plasmids were successfully delivered into cells by magnetic-assisted targeting of PLMNDs at 3 MPa, 30 cycles, 80 Hz and 2 s (Fig. 1D), achieving 4-fold improvement in plasmid delivery efficacy than the nanodroplets without SPIO. More intratumoral accumulation of PLMNDs was demonstrated in right tumor (with magnet) than left one (without magnet) in T2 weighted images (Fig. 1E). Then appreciable GFP fluorescence was produced in the tumor tissue.

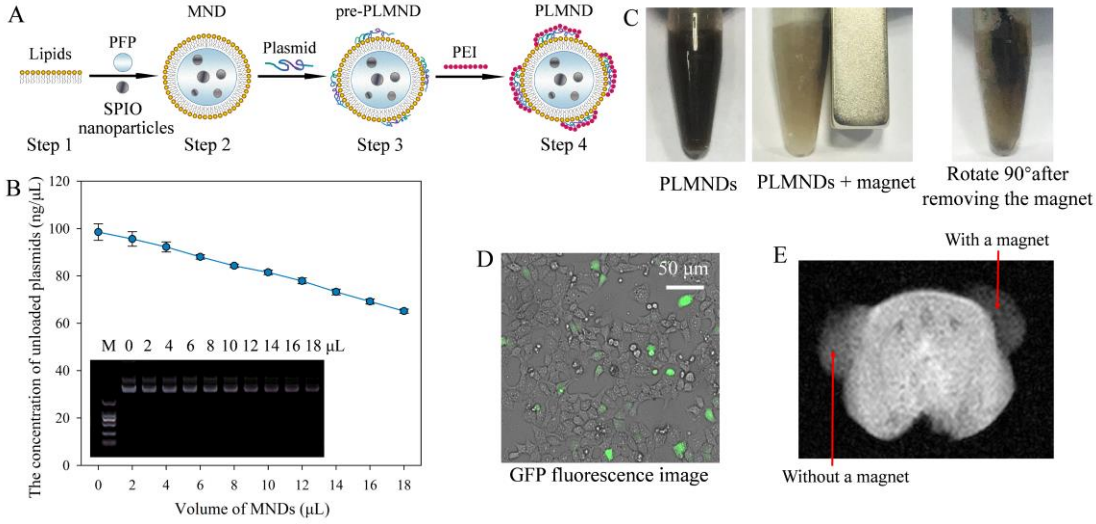

Fig. 1 A: schematic illustrating PLMNDs preparation. Step 1: evaporate a solution of lipids to form lipid film; Step 2: sonicate the mixture of hydrated lipid, PFP and fluorinated SPIO nanoparticles to form magnetic nanodroplet (MND); Step 3: absorb miRNA sponge by electrostatic interaction (pre-PLMNDs); Step 4: coat PEI on the surface of pre-PLMNDs. B: unloaded plasmids decreased with the increase of MND. C: magnetic adsorption effect of PLMNDs. D: fluorescence microscopic images of cells after PLMNDs treated with US under magnetic field. E: T2 weighted MRI image of mouse tumor model, left: without magnet, right: with magnet