Targeted lipid nanoparticle delivery of siRNA to treat preeclampsia

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Preeclampsia is a severe pregnancy complication responsible for >76,000 maternal deaths and >500,000 neonatal fatalities annually.[1] Central to its pathogenesis is placental dysfunction, with excess production and release of anti-angiogenic factors into the maternal circulation, driving disease progression.[1] Currently, the only way to halt disease progression is placenta delivery by inducing birth, which in early onset preeclampsia, is necessary to save the mother. Lipid nanoparticles (LNPs) offer a promising option to safely target delivery of gene silencing nucleic acid therapies to the dysfunctional placenta, to halt disease progression. LNPs are an ideal treatment strategy due to their biocompatibility and limited off target effects, reducing maternal and fetal risk.[2] We aim to establish LNPs that can target the dysfunctional placenta, delivering short interfering RNA (siRNA) targeting pathogenic drivers of preeclampsia. We first encapsulated siRNA tagged with Cyanine 5 (siRNA-Cy5) in LNPs, and incubated the LNPs (200ng siRNA/well) with human placental trophoblast cells for 24 hours. Confocal microscopy identified strong fluorescence in the cultured placental cells (Figure 1A, 1B), confirming successful siRNA uptake. We then decorated the LNP with endothelial growth factor receptor (EGFR) antibodies (highly expressed on the placental surface) via thiol-maleimide chemistry. CytoFLEX nano flow cytometry revealed highly efficient antibody conjugation, >97% antibody attachment; importantly, controls had no detectable signal (Figure 1C), confirming successful conjugation. These findings establish proof-of-principle data demonstrating targeted siRNA delivery to placenta, paving the way for *in vivo* validation. This demonstrates strong potential to revolutionise delivery of siRNA therapies to the placenta during pregnancy, which if successful, offers a major step forward for maternal-fetal medicine.

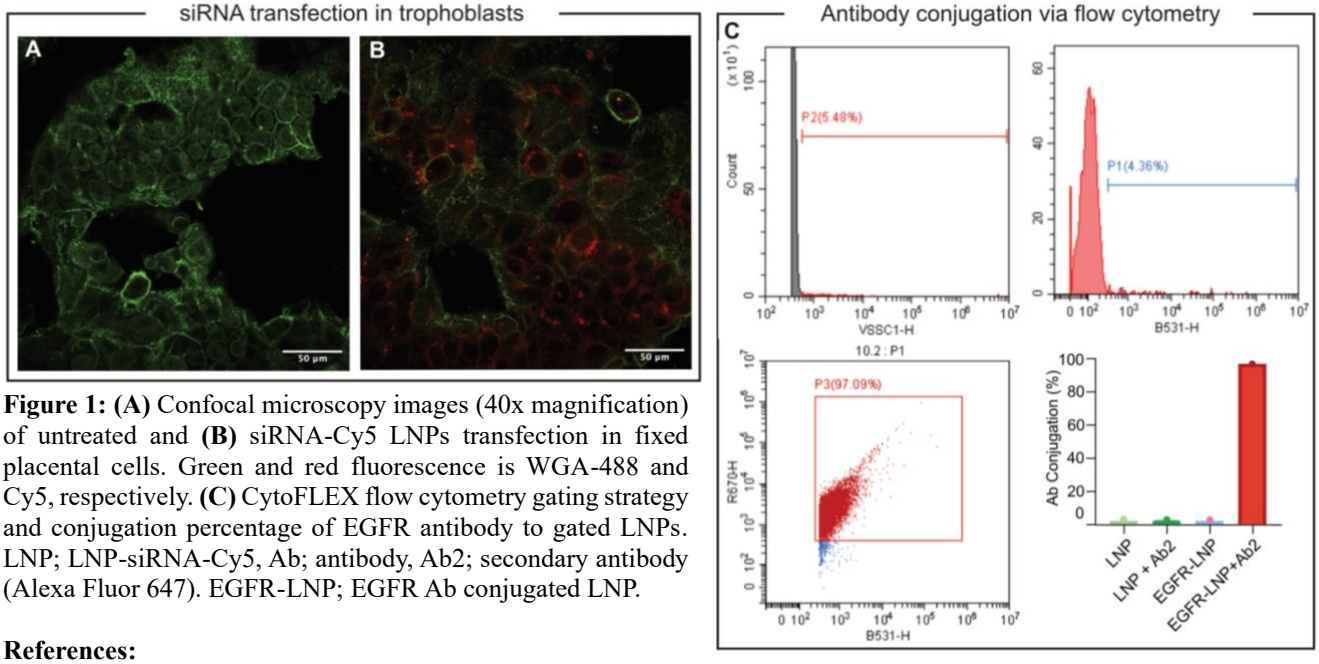

Figure 1: (A) Confocal microscopy images (40x magnification) of untreated and **(B)** siRNA-Cy5 LNPs transfection in fixed placental cells. Green and red fluorescence is WGA-488 and Cy5, respectively. **(C)** CytoFLEX flow cytometry gating strategy and conjugation percentage of EGFR antibody to gated LNPs. LNP; LNP-siRNA-Cy5, Ab; antibody, Ab2; secondary antibody (Alexa Fluor 647). EGFR-LNP; EGFR Ab conjugated LNP.

References:

[1] Brown, M.; et al. *Hypertension* **2018**, *72*, 24-43.
[2] Whitehead, K.; et al. *Nature* **2014**, *5,* 4277.