Electronic Supplementary Material (ESI) for Nanoscale.

Micro Cell Vesicle Technology (mCVT): A Novel Hybrid System of Gene Delivery for Hard-To-Transfect (HTT) Cells

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Supporting Information

Table S1. Examples of current non-viral transfection methods[1–3]

	Technologies	Mechanism	Advantages	Disadvantages	Ref
Chemical Methods	Cationic lipid based Eg. Lipofectamine Transfectin, cationic liposomes	Forming a complex (lipoplex) with DNA, which enters the cells via endocytosis	• Numerous commercially available products • Applicable to many cell types • Easy procedures	• Cytotoxicity • Variable transfection efficiency • Unable to target specific cells • Mostly Inhibited in the presence of serum.	4,5
	Cationic polymer based Eg. Denderimer, polyamine	Forming a complex (polyplex) with DNA, which enters the cells via endocytosis	• Numerous commercially available products • Applicable to many cell types • Fully tunable structure • Easy procedures	• High cytotoxicity • Variable transfection efficiency • Unable to target specific cells	5,6
	Calcium phosphate mediated	Forming an insoluble precipitate with DNA, which enters the cells *via* endocytosis	• Easy procedures • Lower cytotoxicity • Low cost	• Low transfection efficiency • Unable to target specific cells	7,8
	Peptide mediated Eg. Cell penetrating peptides, cell targeting peptides	Forming a peptide-DNA complex, which may enter the cells *via* endocytosis or direct membrane translocation	• Lower cytotoxicity • Potential targeting ability (if cell specific targeting peptides is used)	• Low transfection efficiency • Poor endosomal escape • Unable to protect DNA against nucleases	9,10
Physical Methods	Magnetic beads mediated	DNA is associated with magnetic beads and magnetic force is used to direct the beads towards the cells.	• High transfection efficacy • Targeting ability	• Difficult to transfect suspension cells	1112
	Electroporation	Electric current is used to temporarily destabilize the cell membrane, which created pores for DNA to enter the cells.	• High transfection efficacy	• Difficult to transfect suspension cells • Tendency for excessive cell death • Required for cell dependent optimization of procedures and parameters	12

Table S2. Transfection efficiency of HTT with plasmids using commercially available reagents

Cells	Transfection methods	Transfection efficiency	Ref
3T3-L1	Cationic lipids Cationic polymers	> 10% ~ 35%	13 14
U937	Cationic lipids	< 30%	15
Jurkat cells	Cationic lipids	< 30%	15
HUVEC	Cationic lipids /polymers	~ 30-50%	16
Embryonic fibroblast	Cationic lipids/polymer	< 30%	17
Embryonic Stem cells	Cationic lipids	~ 20-25%	18,19

Table S3. Formulations for the proof-of-fusion assay and optimization. The optimized formulation **c** is in bold. The formulation was chosen based on the size and protein retention of produced mCVTs.

Formulations	Components	Amount of DOTAP (mg)	Number of $CG_{3T3\text{-}L1}$
a	DOTAP liposome	2.5	-
b	mCVT 0.5x	2.5	0.5×10^7
c	**mCVT 1x**	**2.5**	**1×10^7**
d	mCVT 2x	2.5	2×10^7
e	DOTAP (cells)	2.5	1×10^7 cells
f	CG 1x	-	1×10^7

Figure S1. Example histogram of the size distribution of (A) DOTAP Liposomes and (B) $mCVT_{3T3\text{-}L1}$.

A **B**

Formulations	Size (nm)	PDI	Zeta Potential
DOTAP Liposomes	489.5 ± 52.6	0.284 ± 0.024	54.7 ± 1.2
$mCVT_{3T3\text{-}L1}$	353.9 ± 16.2	0.429 ± 0.132	37.9 ±4.7

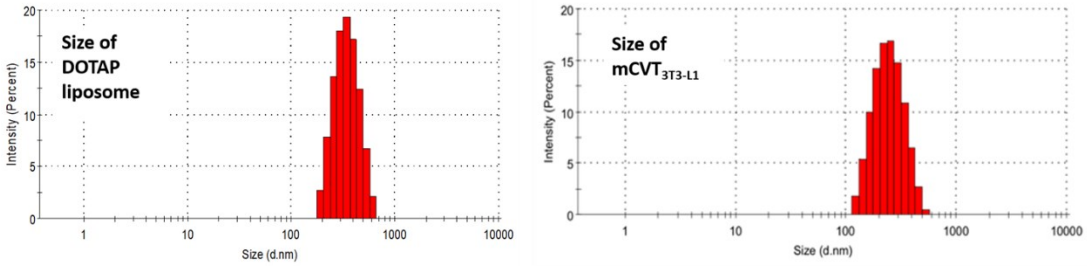


Figure S2. Zeta potential of $mCVT_{3T3\text{-}L1}$ with different amount of plasmid DNA.

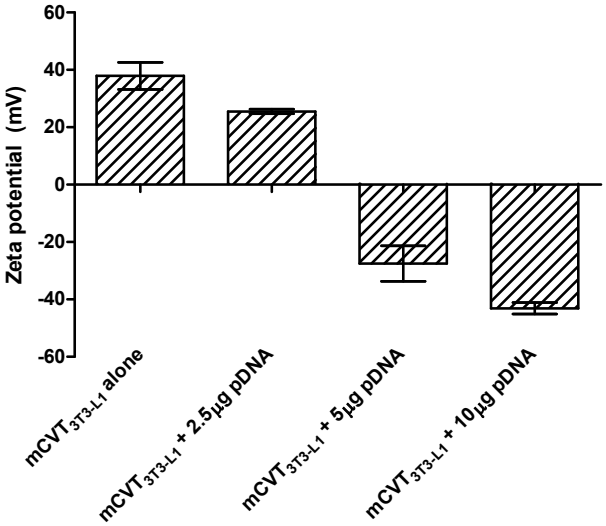


Figure S3. Transfection efficiency of 3T3-L1 cells and their respective cell viability after 24h post-treatment with various doses of mCVTs

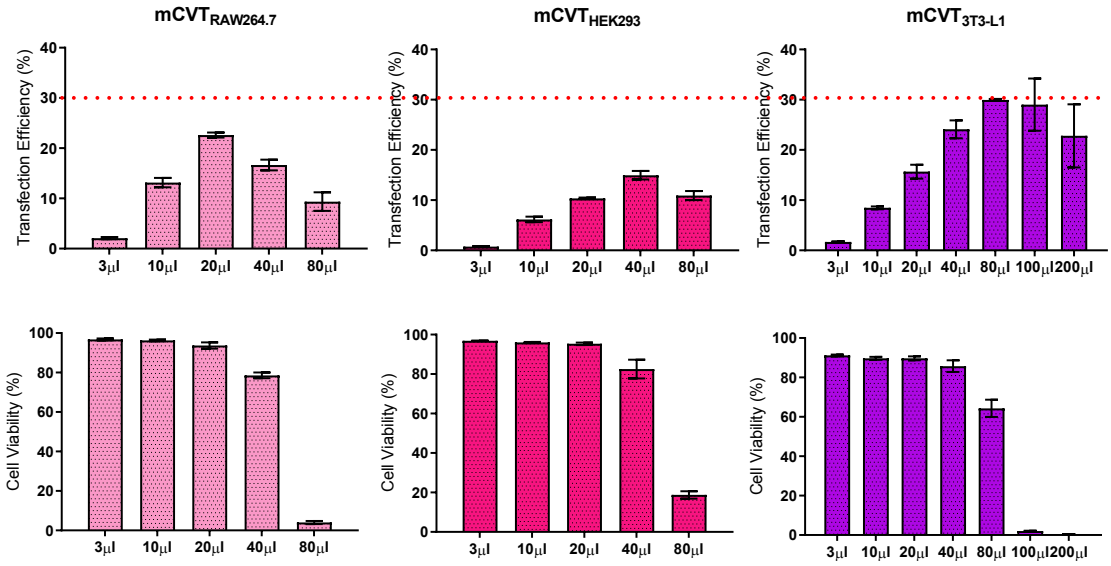


Figure S4. The effect of serum for transfection of 3T3-L1. 3T3-L1 cells were transfected with mCVTs with and without serum at 24 h, 48 h and 72 h, respectively. Both (a) transfection efficiency and (b) cell viability were recorded. Data represented means ± SD (n=3). * $P < 0.1$, *** $P < 0.001$.

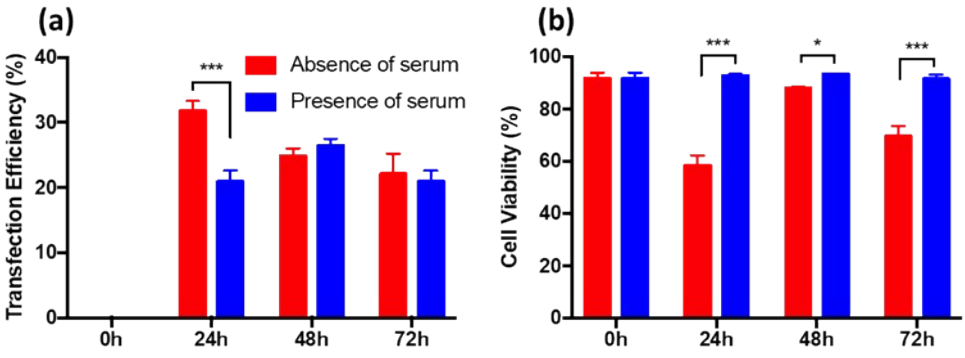


Figure S5. Transfection efficiency of 3T3-L1 and HEK293 (measured at 24h post-transfection) with mCVTs from respective cell lines.

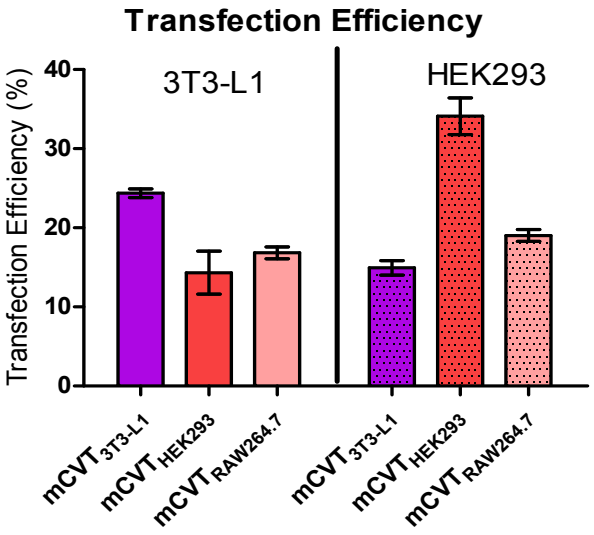
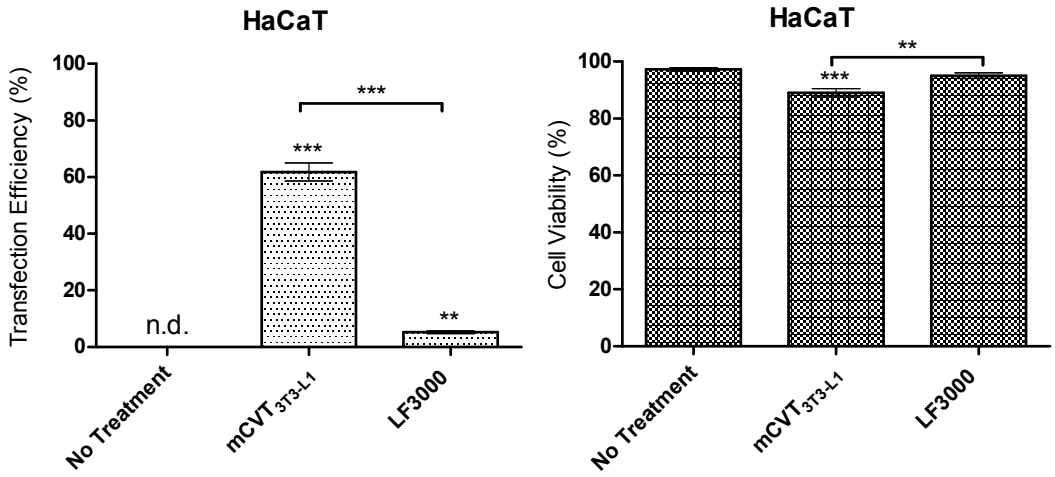


Figure S6. Transfection efficiency and cell viability of HaCaT using mCVTs from 3T3-L1 CGs, compared to commercially available transfection reagent, LF3000. n.d. indicated as not detectable, ** indicated $p<0.01$, *** indicated $p<0.001$.



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