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Supplementary information

Tutorial: using nanoneedles for intracellular delivery

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Supplementary Table 1: Summary of cargoes nanoinjected into biological systems in literature

Type of cargo		Biological system		Nanoneedle device – geometry	Interfacing method	Delivery efficiency	Functionality	Ref.
Nucleic acids	siRNA	Stem cells	hMSC	Porous nanocones – Tip diameter: 50 nm, Base diameter: 600 nm, Height: 5 μm, Pitch: 2 μm	Spontaneous membrane disruption	38%	GAPDH-siRNA knockdown (43%)	108
		Primary immune cells	Naïve primary mouse T cells (T_H17)	Solid nanowires – Diameter: < 150 nm, Height: 2–3 μm, Density: 0.3–1 μm^{-2}	Spontaneous membrane disruption	> 95 %	efficient siRNA knockdown (> 40%) of 34 genes	145
			$CD19^+$, $CD4^+$, NK, BMDC, DC, macrophage	Solid nanowires – Diameter: < 150 nm, Height: 1–3 μm, Density: 0.15–1 μm^{-2}	Spontaneous membrane disruption	Not quantified	LEF1-siRNA knockdown (≥ 69%)	135
		Cell lines	MCF-7	Solid nanowires – Tip diameter: 100 nm, Base diameter: 700 nm, Height: 7 μm, Pitch: 4 μm	Assisted membrane disruption (electroporation)	82%	Cav3.1-siRNA knockdown (71%)	143
			GPE86	Hollow nanotubes – Inner/outer diameter: 300/500 nm, Height: 1.5–2 μm, Pitch: 5 μm	Assisted membrane disruption (centrifugation)	Not quantified	Triobp-siRNA knockdown	13
			HeLa	Porous nanocones – Tip diameter: 50 nm, Base diameter: 600 nm, Height: 5 μm, Pitch: 2 μm	Spontaneous membrane disruption	> 90%	GAPDH-siRNA knockdown (80%)	26
			HeLa, PC3, MDA-MB-231, MCF-7, T47D, HepG2	Hollow nanostraws (nanosyringes) – Inner diameter: 45 nm, Height: 100–120 nm	Assisted membrane disruption (centrifugation)	Not quantified	VEGF-siRNA knockdown (40–60%)	122
		Neural cells	Rat hippocampal neurons	Solid nanowires – Diameter: 100–200 nm, Height: 3 μm	Spontaneous membrane disruption	Not quantified	$Na_V1.X$ channel-siRNA knockdown	14
	mRNA (GFP, mCherry)	Stem cells	hiPSC-CMs	Hollow nanostraws – Diameter: 150 nm, Height: 1.5–3 μm, Density: 1 μm^{-2}	Assisted membrane disruption (electroporation)	80%	—	12
			HSCs			60%	—	
		Cell lines	HEK293			75–90%	—	
			GPE86	Hollow nanotubes – Inner/outer diameter: 300/500 nm, Height: 1.5–2 μm, Pitch: 5 μm	Assisted membrane disruption (centrifugation)	57.3±2.8%	—	13
		Neural cells	Mouse primary glia cells	Hollow nanostraws – Diameter: 150 nm, Height: 1.5–3 μm, Density: 1 μm^{-2}	Assisted membrane disruption (electroporation)	65%	—	12
			Mouse primary neurons			60%	—	
	ssDNA–FAM	Cell lines	GPE86	Hollow nanotubes – Inner/outer diameter: 300/500 nm, Height: 1.5–2 μm, Pitch: 5 μm	Assisted membrane disruption (centrifugation)	78%	—	13
	dsDNA90	Cell lines	A549	Solid nanowires – Diameter: 300 nm, Height: 5 μm		Not quantified	STING activation	16
		Neural cells	Primary hippocampal neurons					

Type of cargo		Biological system		Nanoneedle device – geometry	Interfacing method	Delivery efficiency	Functionality	Ref.
Nucleic acids	pGFP, pRFP	Stem cells	hDPSCs	Porous nanowires – Diameter: 400 nm, Height: 3.5 μm, Density: 1 μm^{-2}	Spontaneous membrane disruption	85%	—	39
		Primary immune cells	Naïve primary mouse T cells	Solid nanocones – Tip diameter: 100 nm, Height: 3.2 μm, Pitch: 3 μm	Assisted membrane disruption (centrifugation)	30%	—	18
			Primary mouse lymphocytes	Hollow nanostraws/ nanosyringes – Inner diameter: 45 nm, Height: 100–120 nm		46%	—	122
		Cell lines	HEK293	Porous nanowires – Diameter: 400 nm, Height: 3.5 μm, Density: 1 μm^{-2}	Spontaneous membrane disruption	86%	—	39
				Hollow nanostraws – Diameter: 250 nm, Height: 1.5 μm, Density: 0.2 μm^{-2}	Assisted membrane disruption (electroporation)	67%	—	30
				Hollow nanostraws – Inner/outer diameter: 250/500 nm, Height: 5 μm, Pitch: 5 μm	Assisted membrane permeation (saponin)	Not quantified	—	28
			GPE86	Solid nanocones – Tip diameter: 100 nm, Height: 3.2 μm, Pitch: 3 μm	Assisted membrane disruption (centrifugation)	22%	—	18
			L1.2			25%	—	
			Jurkat			5%	—	
			HeLa	Porous nanowires – Diameter: 400 nm, Height: 3.5 μm, Density: 1 μm^{-2}	Spontaneous membrane disruption	7%	—	39
				Porous nanocones – Tip diameter: 50 nm, Base diameter: 600 nm, Height: 5 μm, Pitch: 2 μm		> 90%	—	26
			Spleen T lymphocytes (from mouse cell line)	Solid nanowires – Diameter: 100 nm, Height: 10 μm	Assisted membrane disruption (optoporation)	80%	—	100
			CHO	Hollow nanostraws – Diameter: 250 nm, Height: 1.5 μm, Density: 0.2 μm^{-2}	Assisted membrane disruption (electroporation)	81%	—	30
			MCF-7	Branched hollow nanostraws – Diameter: 250 nm, Height: 1.5 μm, Density: 2 μm^{-2}		64.2%	—	54
		Neural cells	Primary hippocampal neurons	Solid nanowires – Diameter: 326±110 nm, Height: 4.55±0.68 μm, Density: 0.07 μm^{-2}	Assisted membrane disruption (centrifugation)	45%	—	15
			Rat neural progenitor cells	Solid nanowires – Diameter: 100–200 nm, Height: 3 μm	Spontaneous membrane disruption	> 95%	—	14
	hVEGF165	Tissues	Murine muscle	Porous nanocones – Tip diameter: 50 nm, Base diameter: 600 nm, Height: 5 μm, Pitch: 2 μm	Mechanically assisted (thumb pressure)	Not quantified	pVEGF-induced neovascularisation and 6-fold increase in blood perfusion	26

Type of cargo		Biological system		Nanoneedle device – geometry	Interfacing method	Delivery efficiency	Functionality	Ref.
Proteins	GFP, RFP	Cell lines	T47D	Hollow nanostraws/ nanosyringes – Inner diameter: 45 nm, Height: 100–120 nm	Assisted membrane disruption (centrifugation)	70.3% (GFP)	—	122
			HepG2			79.9% (RFP)	—	
	Caspase-3		Raji B cells			Not quantified	Caspase-3-induced apoptosis (36.9% in 12h)	
	Cytosolic fragment of STIM1		HEK293	Hollow nanostraws – Diameter: 150 nm, Height:1.5–3 μm, Density: 1 μm^{-2}	Assisted membrane disruption (electroporation)	Not quantified	Binding and colocalisation of STIIM1 with Orai calcium channel	12
	Fluorescentlly tagged antibodies		GPE86	Hollow nanotubes – Inner/outer diameter: 300/500 nm, Height: 1.5–2 μm, Pitch: 5 μm	Assisted membrane disruption (centrifugation)	> 80% (IgG-AF647/-AF488)	—	13
		Neural cells	Primary hippocampal neurons	Solid nanowires – Diameter: 326±110 nm, Height: 4.55±0.68 μm, Density: 0.07 μm^{-2}		35.5±4.4% (IgG-AF647)	—	15
Peptides	Ac-DEVD-CHO (inhibitor of caspase 3/7)	Cell lines	HeLa	Solid nanowires – Diameter: 100–200 nm, Height: 3 μm	Spontaneous membrane disruption	Not quantified	Successful apoptosis inhibition	14
CRISPR gene editing tools	Cas9 RNP		GPE86	Hollow nanotubes – Inner/outer diameter: 300/500 nm, Height: 1.5–2 μm, Pitch: 5 μm	Assisted membrane disruption (centrifugation)	14.7%	6% cleavage efficiency of targeted Hprt gene	13
			HEK293	Hollow nanostraws – Diameter: 150 nm, Height: 1.5–3 μm, Density: 1 μm^{-2}	Assisted membrane disruption (electroporation)	> 90%	33% induced knockout of PPIB targeted gene	12
Nanoparticles	QDS, Polystyrene NPs		HeLa	Porous nanocones – Tip diameter: 50 nm, Base diameter: 600 nm, Height: 5 μm, Pitch: 2μm	Spontaneous membrane disruption	Not quantified	—	136
		Neural cells	Primary hippocampal neurons	Solid nanowires – Diameter: 326±110 nm, Height: 4.55±0.68 μm, Density: 0.07 μm^{-2}	Assisted membrane disruption (centrifugation)	> 60% (QDs)	—	15
						14.8±2.9% (Polystyrene NPs)		
		Tissues	Murine muscle, skin and ear	Porous nanocones – Tip diameter: 50 nm, Base diameter: 600 nm, Height: 5 μm, Pitch: 2 μm	Mechanically assisted (thumb pressure)	Not quantified	—	136

Type of cargo		Biological system		Nanoneedle device – geometry	Interfacing method	Delivery efficiency	Functionality	Ref.
Other molecules	Fluorescent dyes (PI, EthD-1, calcein green)	Cell lines	HeLa	Hollow nanostraws – Diameter: 400 nm, Height: 1.5 μm, Density: 1 μm^{-2}	Assisted membrane disruption (electroporation)	82.9 ± 3.3% (PI)	—	34
				Solid nanocones (nanopyramids) – Base diameter: 2.4 μm, Height: 1.4 μm, Pitch: 1.2 μm	Assisted membrane disruption (optoporation)	95% (calcein green)	—	141
			MCF-7	Solid nanowires – Tip diameter: 100 nm, Base diameter: 700 nm, Height: 7 μm, Pitch: 4 μm	Assisted membrane disruption (electroporation)	85% (PI)	—	143
				Branched hollow nanostraws – Diameter: 250 nm, Height: 1.5 μm, Density: 2 μm^{-2}		83.8% (PI)	—	54
			CHO	Hollow nanostraws – Diameter: 250 nm, Height: 1.5 μm, Density: 0.2 μm^{-2}	Assisted membrane disruption (electroporation)	> 95% (PI)	—	30
			NIH 3T3	Hollow nanotubes – Inner/outer diameter: 90/180 nm, Height: 1.1 μm, Pitch: 1.5 μm	Assisted membrane disruption (optoporation)	≥ 95% (PI)	—	164
			NIH 3T3	Solid nanowires – Diameter: 326±110 nm, Height: 4.55±0.68 μm, Density: 0.07 μm^{-2}	Assisted membrane disruption (centrifugation)	80% (EthD-1)	—	15
		Neural cells	Primary hippocampal neurons					
		Tissues	Murine skin, ear, muscle	Porous nanocones – Tip diameter: 50 nm, Base diameter: 600 nm, Height: 5 μm, Pitch: 2 μm	Mechanically assisted (thumb pressure)	80%	—	26
	Dextrans (fluorescently tagged; various M.W.)	Stem cells	rBMSCs	Solid nanowires – Tip diameter: 100 nm, Base diameter: 700 nm, Height: 7 μm, Pitch: 4 μm	Assisted membrane disruption (electroporation)	51% (70 kDa)	—	143
		Cell lines	MCF-7			86% (10k Da)		
						49% (70 kDa)		
			HeLa			63% (70 kDa)		
			HeLa	Solid nanocones (nanopyramids) – Base diameter: 2.4 μm, Height: 1.4 μm, Pitch: 1.2 μm	Assisted membrane disruption (optoporation)	73.9% (150 kDa)	—	141
						24% (150 kDa)		
						16% (200 kDa)		
			HEK293	Hollow nanostraws – Inner/outer diameter: 250/500 nm, Height: 5 μm, Pitch: 5 μm	Assisted membrane permeability (saponin)	70±15% (3 kDa)	—	28
		Tissues	Murine skin	Solid nanowires – Tip diameter: 100 nm, Base diameter: 700 nm, Height: 7 μm, Pitch: 4 μm	Assisted membrane disruption (electroporation)	Not quantified	—	143
	Co^{2+} ions	Cell lines	CHO	Hollow nanostraws – Diameter: 100 nm, Height: 1-2 μm, Density: 1 μm^{-2}	Spontaneous membrane disruption	70.3%	—	29