Stretching-based cell stimulation enhances non-viral gene delivery effectiveness: insights into internalization pathways and YAP signaling dynamics

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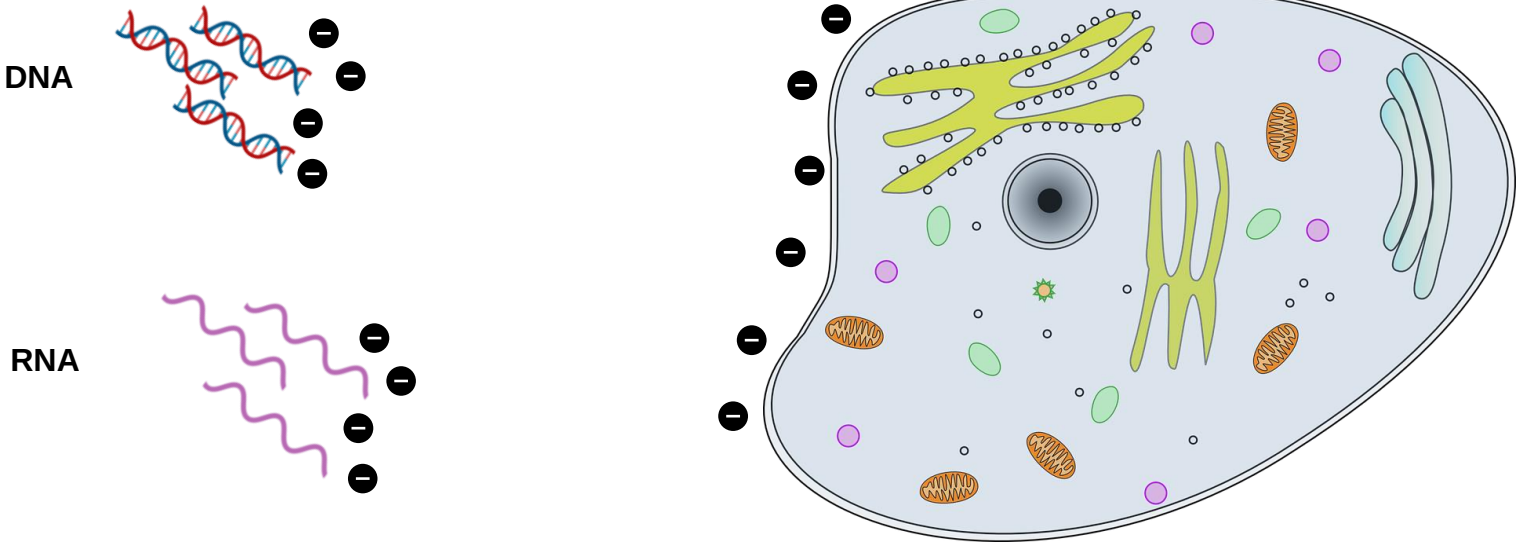
CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

Background: Gene delivery

GENE DELIVERY: introduction of exogenous genetic material into host cells to modulate gene expression patterns[1].



[1] Bono N. et al., *Pharmaceutics* 2020, *12*(2), 183

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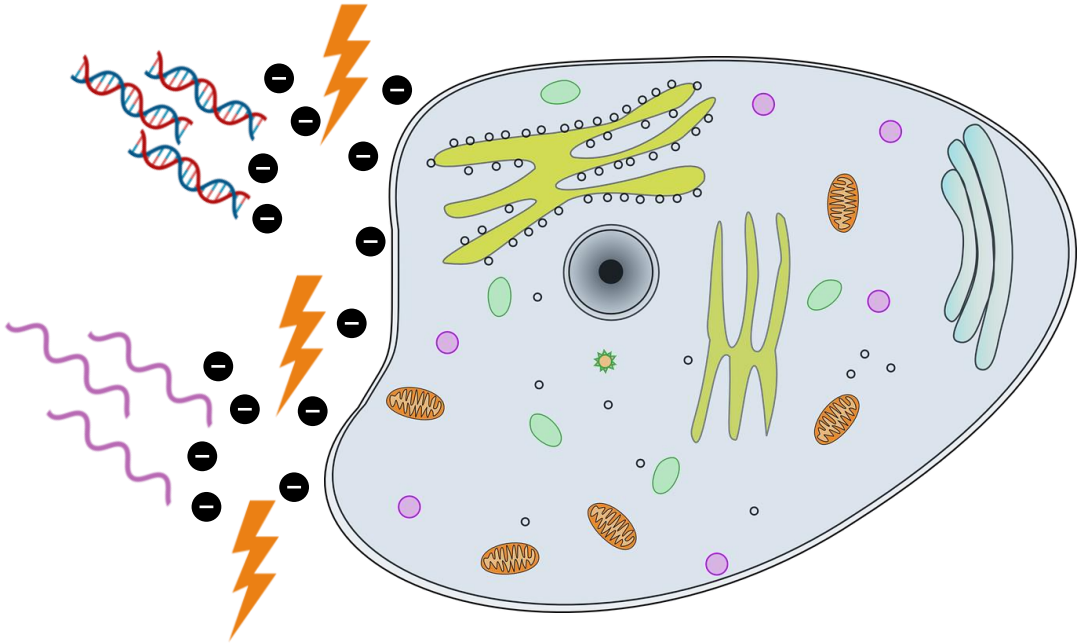
DNA

RNA



DRAWBACK
electrostatic repulsion between anionic nucleic acids and the negatively charged cell membrane

[1] Bono N. et al., *Pharmaceutics* 2020, *12*(2), 183

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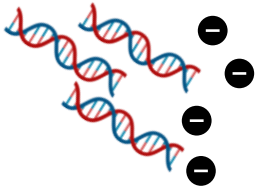
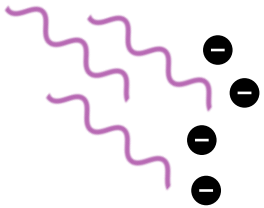
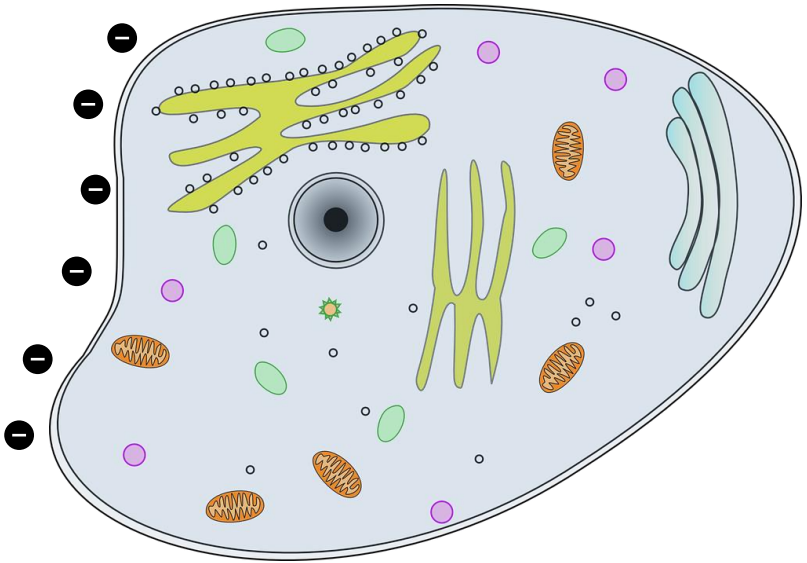


RNA





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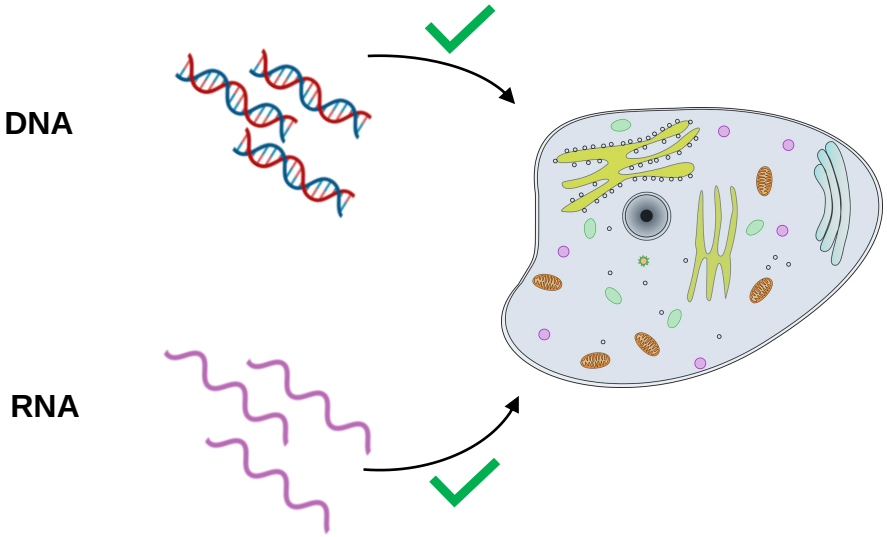

GENE DELIVERY STRATEGIES

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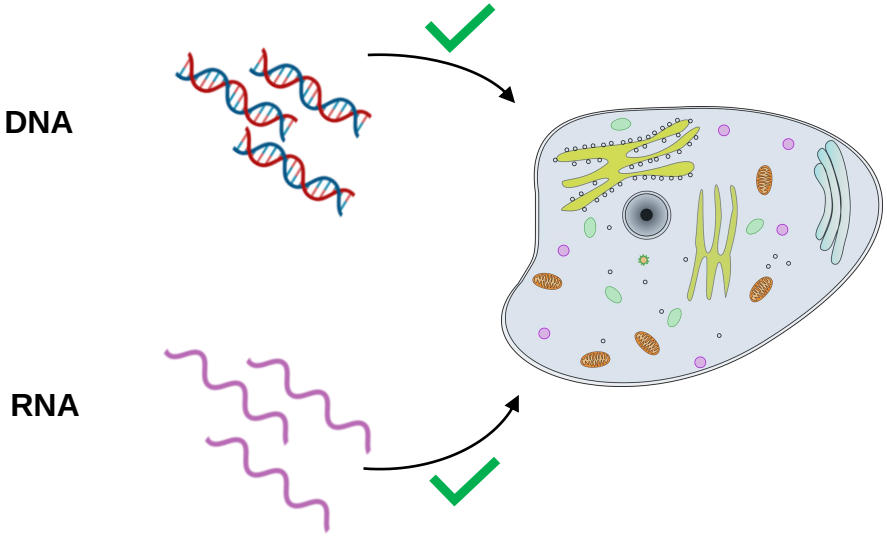
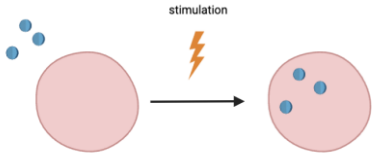


GENE DELIVERY STRATEGIES

PHYSICAL METHODS



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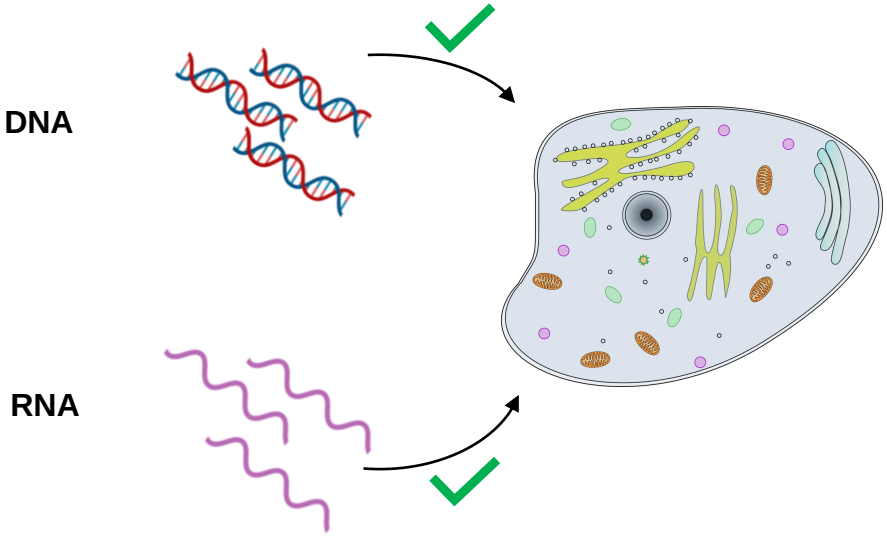

GENE DELIVERY STRATEGIES

VECTORS

VIRAL

CHEMICAL

lipoplexes polyplexes

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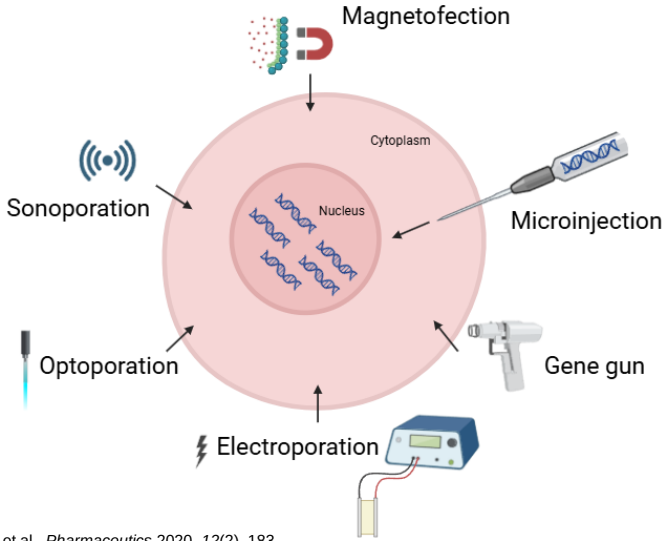
Background: Physical methods

PHYSICAL METHODS: application of **exogenous physical stimuli** that lead to a transient disruption and/or permeabilization of the plasma membrane



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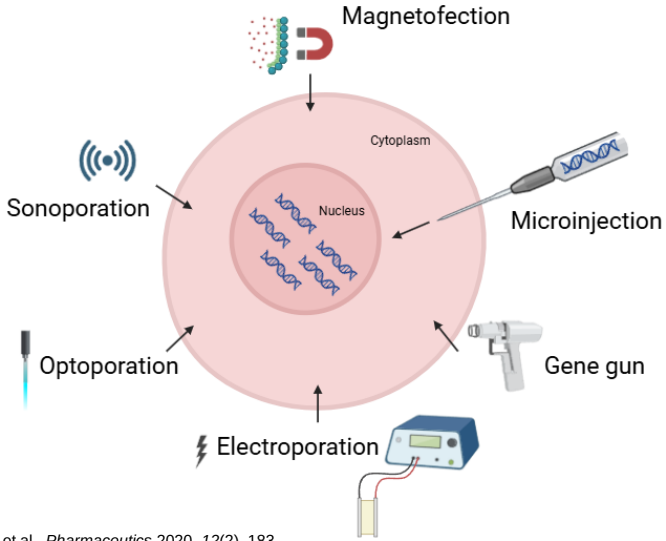

Advantages

- ✓ High **efficiency**
- ✓ High **reproducibility**

Drawbacks

- ✗ High **cytotoxicity**
- ✗ **Invasiveness**
- ✗ High **cost**

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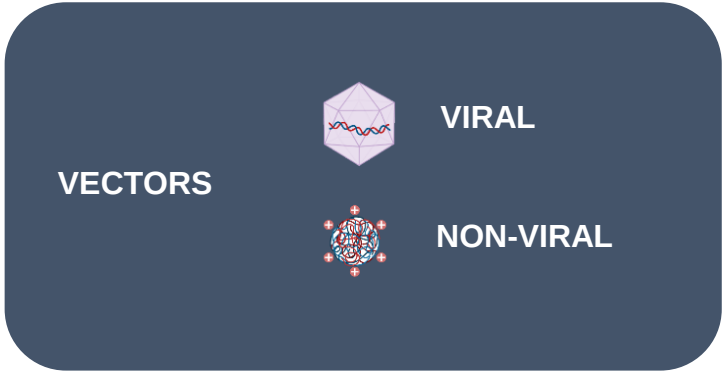

Lipoplexe
Cationic lipids
DNA
Polyplexes
Cationic polymers
DNA
N/P ratio

[1] Bono N. et al., *Pharmaceutics* 2020, *12*(2), 183

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Background: Vectors

VIRAL

NON-VIRAL

Lipoplexes

Cationic lipids + DNA → *Electrostatic interactions* → **Lipoplexes**

Polyplexes

Cationic polymers + DNA → *Electrostatic interactions* → **Polyplexes**

N/P ratio

$$\frac{N}{P} = \frac{[\text{amine groups of transfection reagent}]}{[\text{phosphate groups of NAs}]}$$

Advantages

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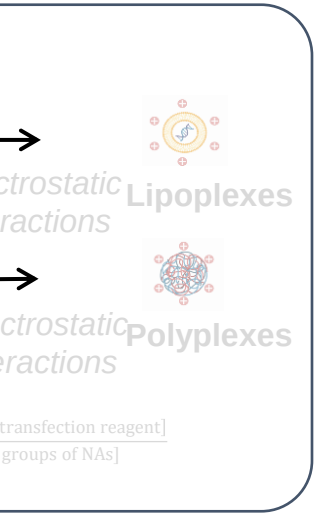
Background: Vectors



Advantages

- Low cytotoxicity
- Safety and ease preparation
- Tunable properties

Drawbacks

- Lower efficacy

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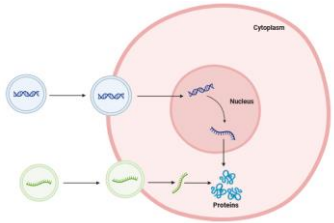
Background: Cellular barriers

DNA

RNA

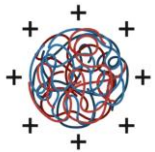


PLASMA MEMBRANE

SIZE

SURFACE CHARGE

CELL TYPE



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[2] Porello et al. *Polym Chem.*, 2024, 15, 2800-2826

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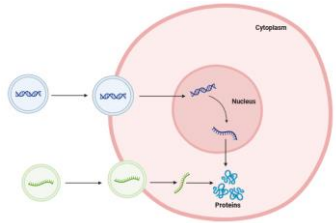
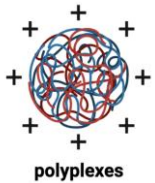
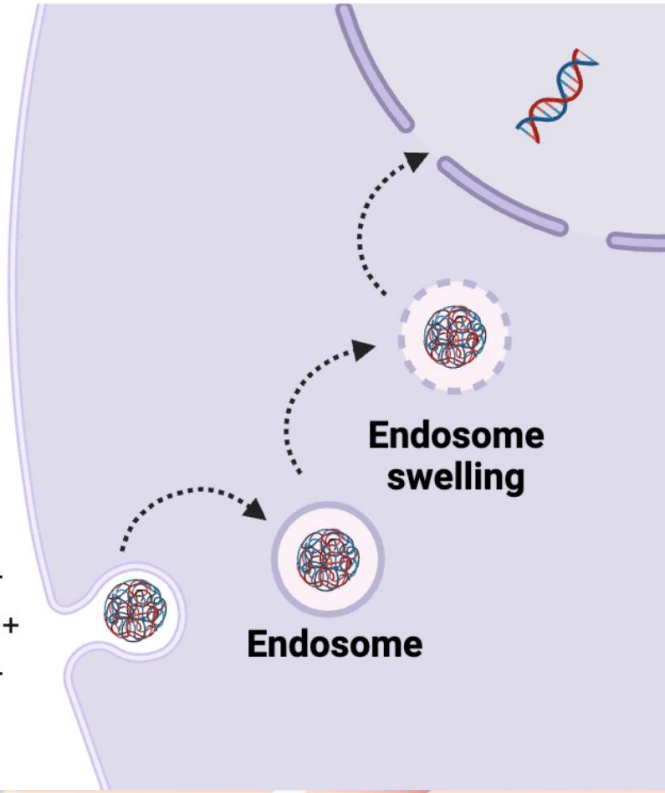


RELEASE INTO THE CYTOSOL

ENDO-LYSOSOMAL ESCAPE





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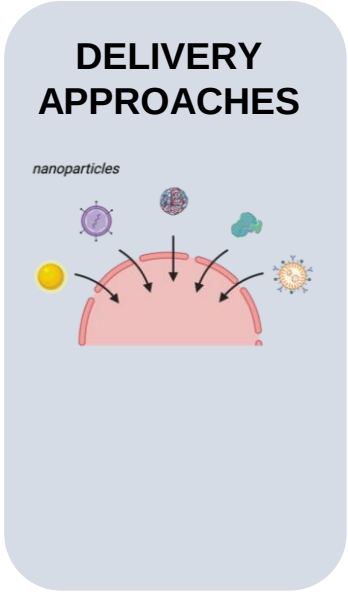

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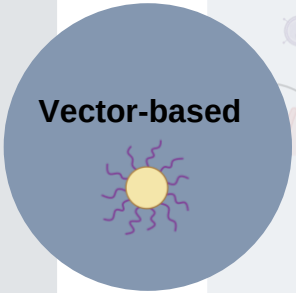
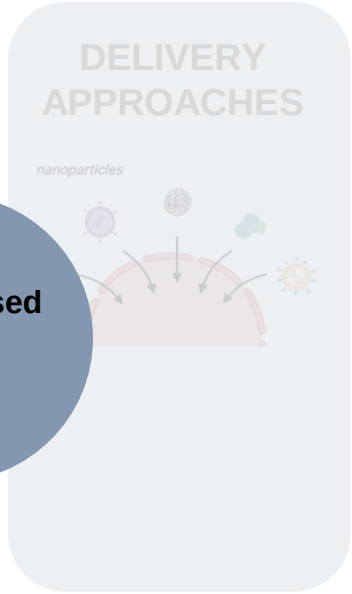
Background: Delivery approaches

1. conjugation of targeting moeties[3]
2. PEG-y-lation[4]
3. addition of bio-reducible and stimuli-responsive moieties[5]





[3] Varga et al., *Biotechnology and Bioengineering*, 2000, 70 (6), 593-605
[4] Hatakeyama, *Gene Therapy*, 2006, **14**, 68–77
[5] Chen et al., *Journal of Controlled Release*, 2014, 191, 123-130

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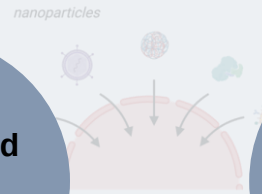
DELIVERY APPROACHES



Vector-based

Cell-based

1. **substrate stiffness[6]**, topography, chemical composition[7,8]
2. **mechanical[9,10,11], electrical stimulation[12]**

[3] Varga et al., *Biotechnology and Bioengineering*, 2000, 70 (6), 593-605
[4] Hatakeyama, *Gene Therapy*, 2006, **14**, 68–77
[5] Chen et al., *Journal of Controlled Release*, 2014, 191, 123-130

[6] Bono, Oldani, **Fruzzetti et al**., In preparation
[7] Martino et al., *Frontiers in Physiology*, 2018, 9, 824
[8] Stewart et al., *Chem. Rev*. 2018, 118, 16, 7409–7531
[9] Ponti et al., *Journal of Nanobiotechnology,* 2022, 20, 363.
[10] **Fruzzetti et al.;** *Submitted*
[11] Ruzzante**, Fruzzetti et al.,** *Int J Pharmaceutics*, 2025, 669.
[12] D'Apuzzo, **Fruzzetti et al.,** In preparation

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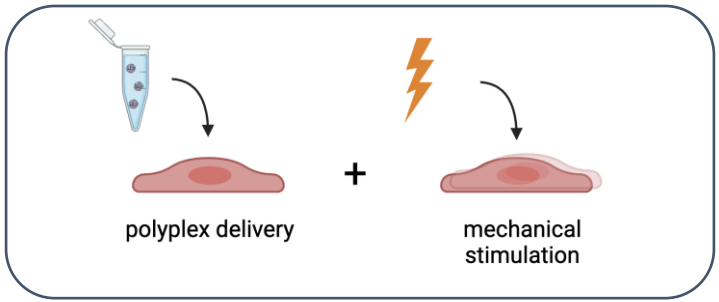
Aim of the work

DESIGN AND DEVELOPMENT OF A DEVICE TO APPLY A **CYCLIC STIMULATION** TO **ENHANCE THE TRANSFECTION EFFICIENCY** OF GOLD STANDARD POLYPLEXES

mechanofection



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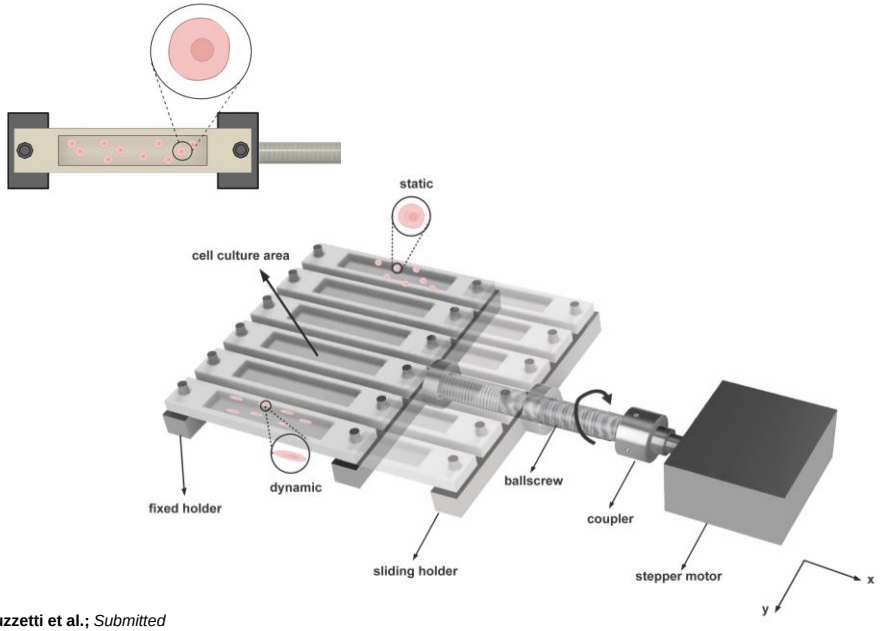
Programmable cell-stretching device

DESIGN



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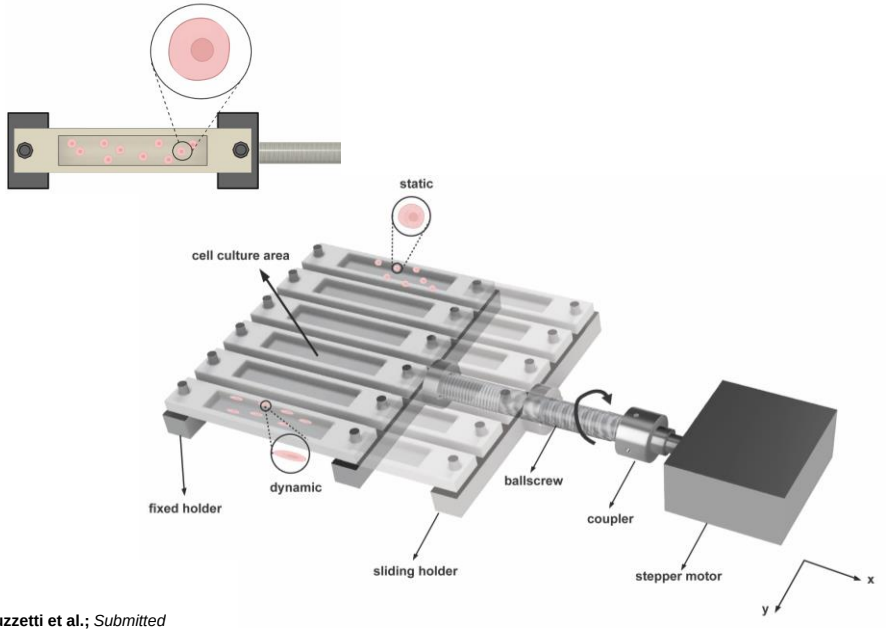

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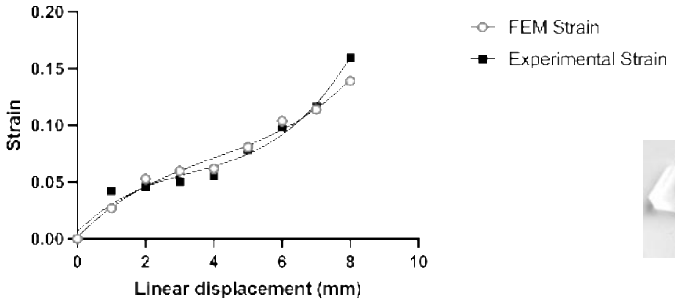
VALIDATION

Strain amplitude

Strain analysis: FEM vs experimental measurement



PDMS casting

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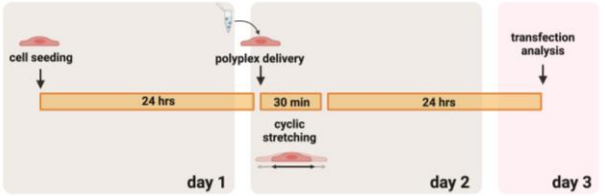
Cyclic stretching enhances transfection efficiency

Evaluation of the effects of cyclic stretching on the **TE** of **DNA/*b*PEI** and **mRNA/*b*PEI polyplexes** in **HeLa** cells and **hMyo**



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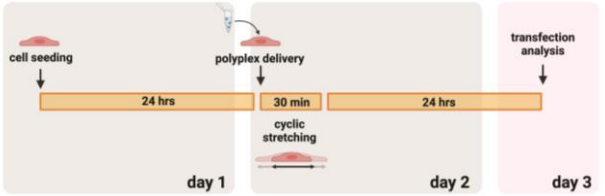
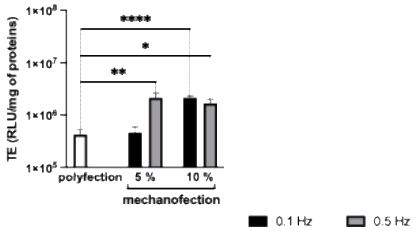
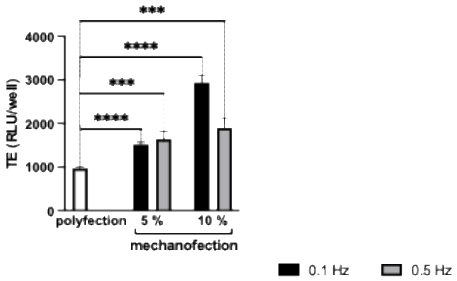


HeLa

pDNA



hMyo



✓ **Enhanced TE** of **pDNA/*b*PEI** polyplexes

✓ 0.1 Hz | 10 % | 30 min

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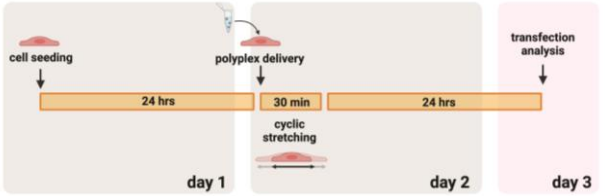
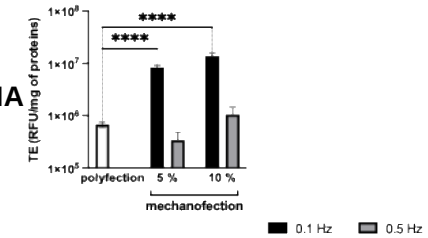
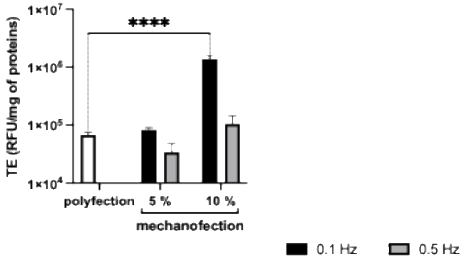


HeLa | **hMyo**

- Enhanced TE of pDNA/*b*PEI polyplexes
- 0.1 Hz, 10 %, 30 min
- **Enhanced uptake of mRNA/*b*PEI** polyplexes

mRNA





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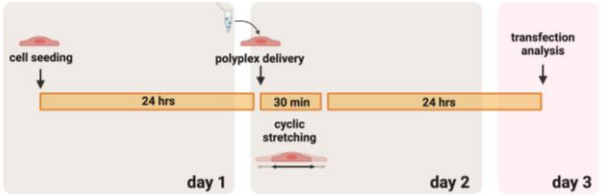
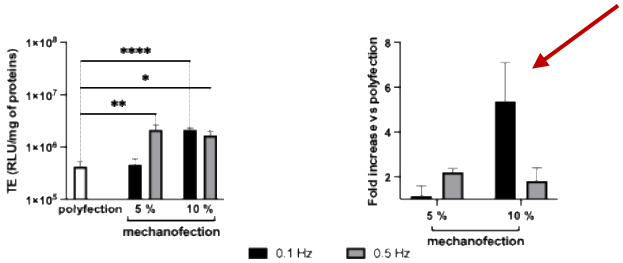
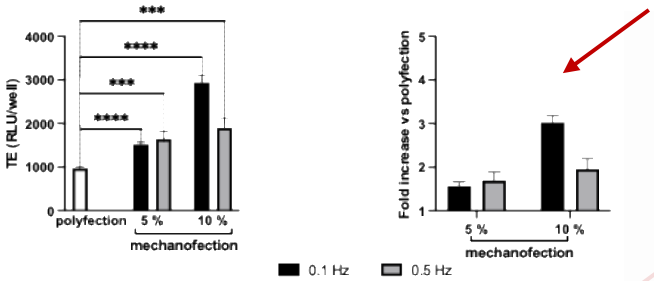


HeLa

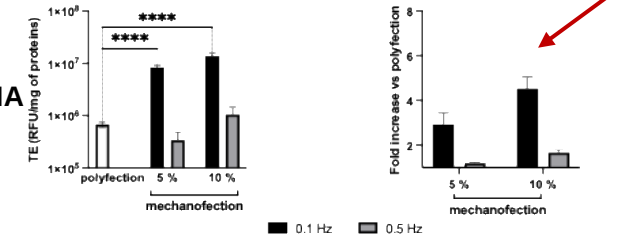
hMyo

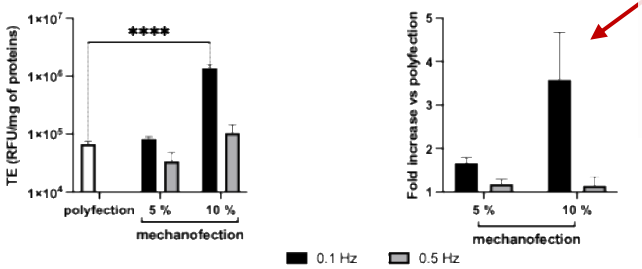
pDNA





mRNA





Fruzzetti et al.; *Submitted*

✓ Enhanced TE of pDNA/*b*PEI polyplexes

✓ 0.1 Hz 10 % 30 min

✓ Enhanced uptake of mRNA/*b*PEI polyplexes

✓ **HeLa: 5-fold increase**
hMyo: 3-fold increase

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Stretching enhances endocytosis

Quantitative evaluation of **endocytosis-related gene expression** patterns through rt-PCR

Fruzzetti et al.; *Submitted*

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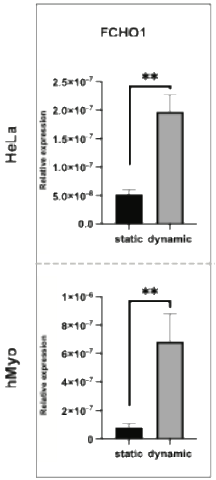

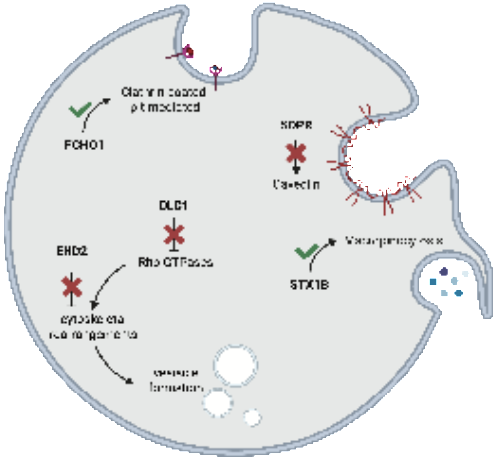

✓ Enhanced **clathrin-mediated endocytosis**

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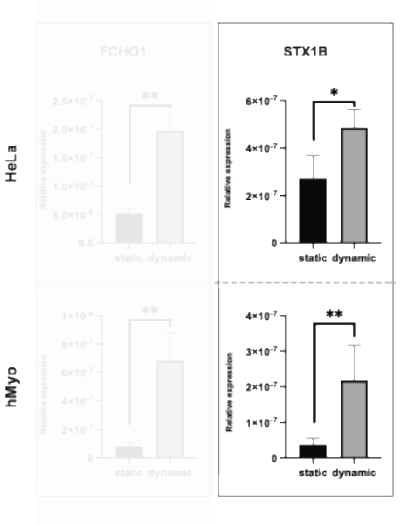
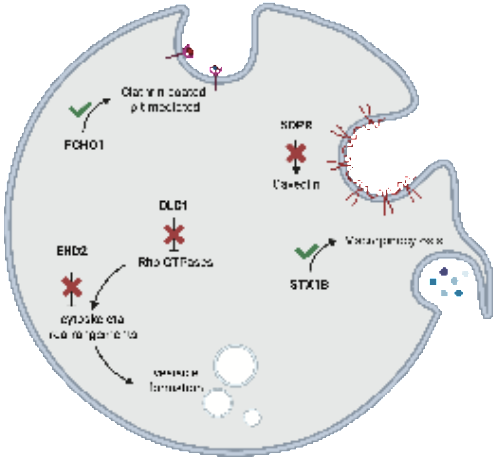
Stretching enhances endocytosis

Quantitative evaluation of **endocytosis-related gene expression** patterns through rt-PCR





- Enhanced **clathrin-mediated endocytosis**
- Enhanced **macropinocytosis**

Fruzzetti et al.; *Submitted*

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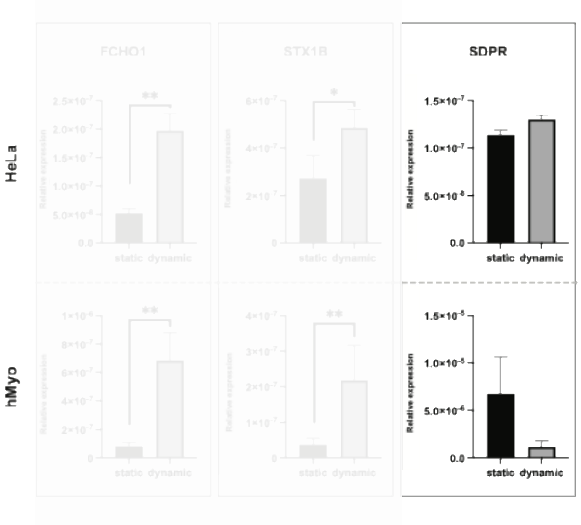
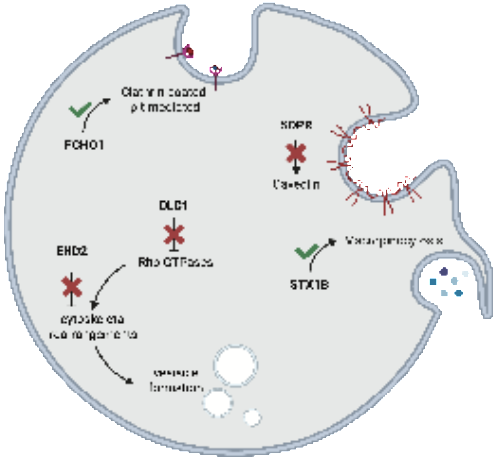
Stretching enhances endocytosis

Quantitative evaluation of **endocytosis-related gene expression** patterns through rt-PCR





- Enhanced **clathrin-mediated endocytosis**
- Enhanced **macropinocytosis**
- Comparable caveolae-mediated endocytosis

Fruzzetti et al.; *Submitted*

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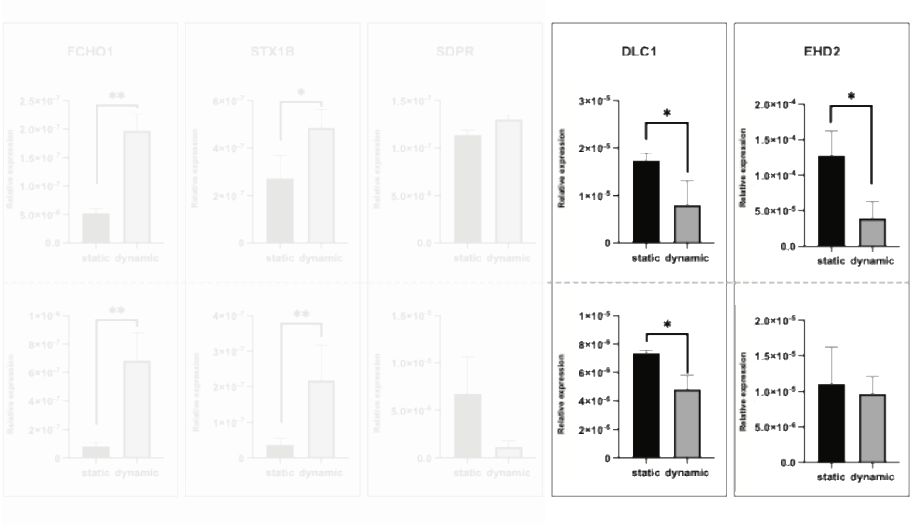
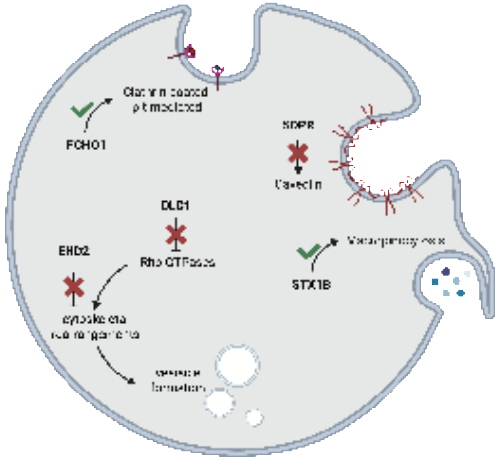
Stretching enhances endocytosis

Quantitative evaluation of **endocytosis-related gene expression** patterns through rt-PCR





- Enhanced **clathrin-mediated endocytosis**
- Enhanced **macropinocytosis**
- Comparable caveolae-mediated endocytosis
- Enabled **vesicle formation**

Fruzzetti et al.; *Submitted*

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Stretching enhances YAP nuclear translocation

YAP

mechanosensitive transcriptional activator [13]

Fruzzetti et al.; *Submitted*

[13] Elosegui-Artola et al., *Cell*, 2017, *171 (6)*, 1397-1410

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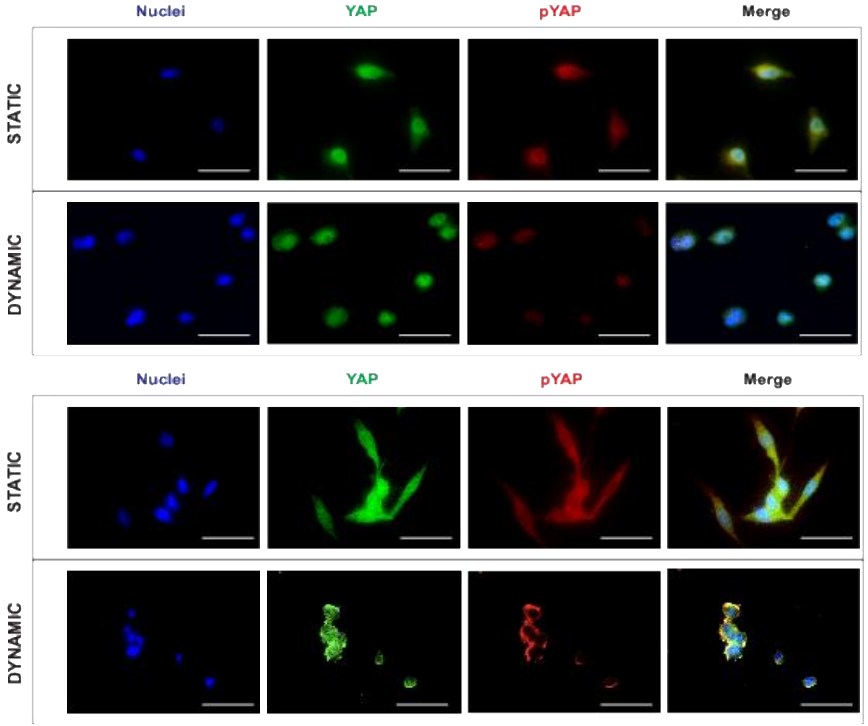
Stretching enhances YAP nuclear translocation

YAP

mechanosensitive transcriptional activator [13]

HeLa

hMyo



✓ **YAP nuclear translocation**

✓ **YAP nuclear translocation**

Fruzzetti et al.; *Submitted*

[13] Elosegui-Artola et al., *Cell*, 2017, *171 (6)*, 1397-1410

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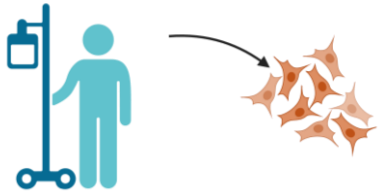
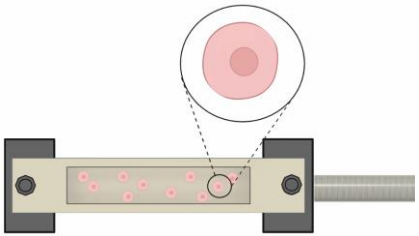
Conclusions and future perspectives

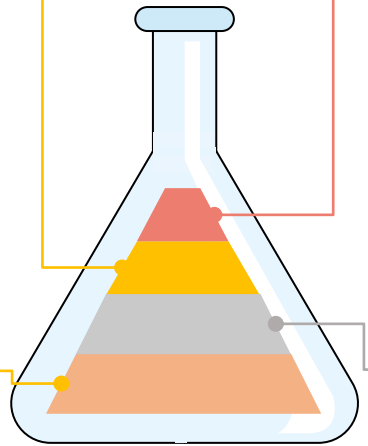
Clathrin-mediated endocytosis, macropinocytosis and **YAP** nuclear **translocation**

Amyotrophic Lateral Sclerosis **patients-derived hMyo**

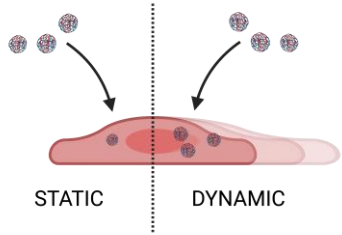


Programmable **cell-stretching device**





Cyclic cell **stimulation** enhances **transfection efficiency**



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Thank you for your kind attention!

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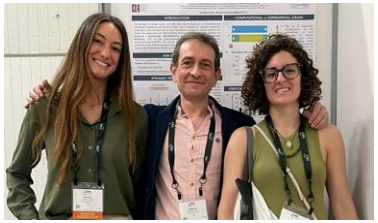

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+ 16 BSc students

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JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS