TECH SESSION 4: GENE DELIVERY

Stretch-based cell stimulation for non-viral gene delivery

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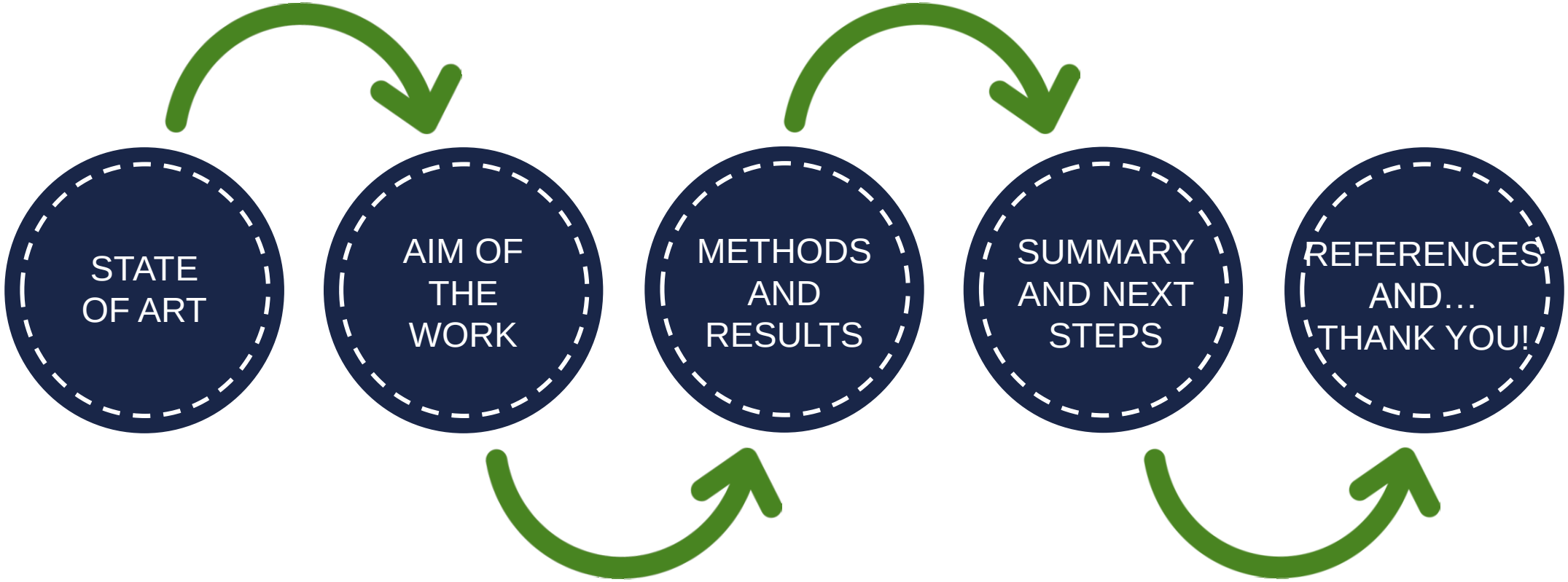
OUTLINE



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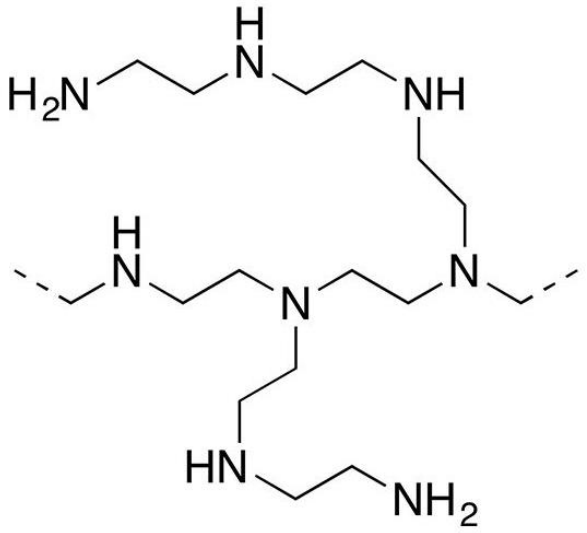
STATE OF ART:
Non-viral vectors

- Non-viral vectors are cationic lipids or polymers that drive exogenous genetic material into cells



branched-Polyethyleneimine (bPEI)

PROS

- Safe
- Simple to use
- Unlimited carrying capacity
- Tunable properties

CONS

- Low transfection efficiency
- Rarely used *in vivo*

Bono N, Ponti F, Mantovani D, Candiani G., Non-Viral in Vitro Gene Delivery: It is Now Time to Set the Bar!, Pharmaceutics, 2020; 12(2):183. https://doi.org/10.3390/pharmaceutics12020183

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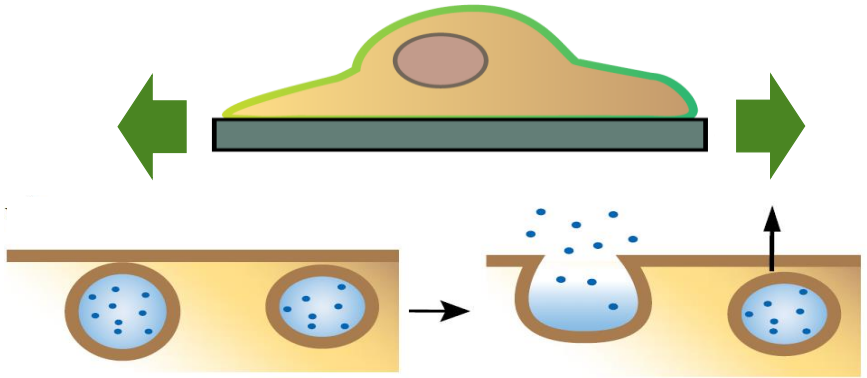
STATE OF ART:
Mechanotransduction and cell membrane tension

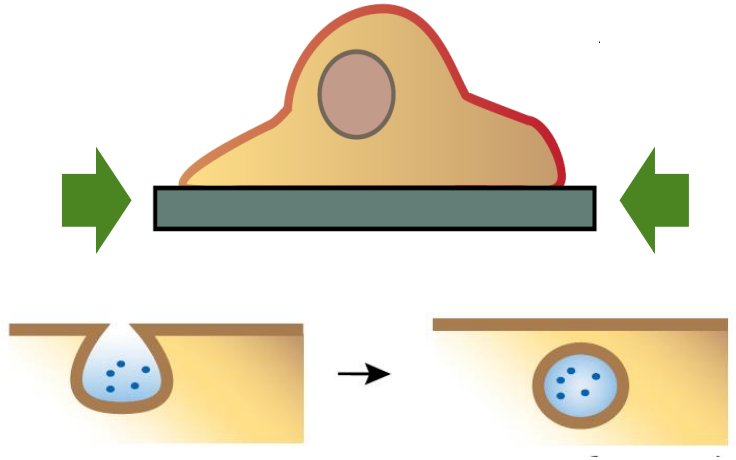
- Mechanotransduction converts mechanical stimuli to biochemical signals
- In literature, changes in cell membrane tension induce the activation of exocytic/endocytic processes.

HIGH MEMBRANE TENSION



EXOCYTOSIS

LOW MEMBRANE TENSION



ENDOCYTOSIS

Le Roux A-L, Quiroga X, Walani N, Arroyo M, Roca-Cusachs P, 2019, The plasma membrane as a mechanochemical transducer, Phil. Trans. R. Soc., B 374: 20180221, http://dx.doi.org/10.1098/rstb.2018.0221

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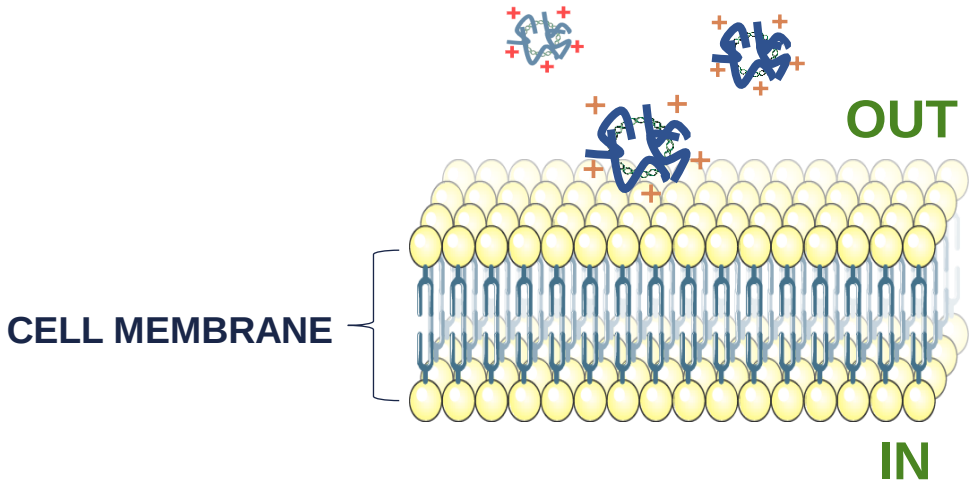
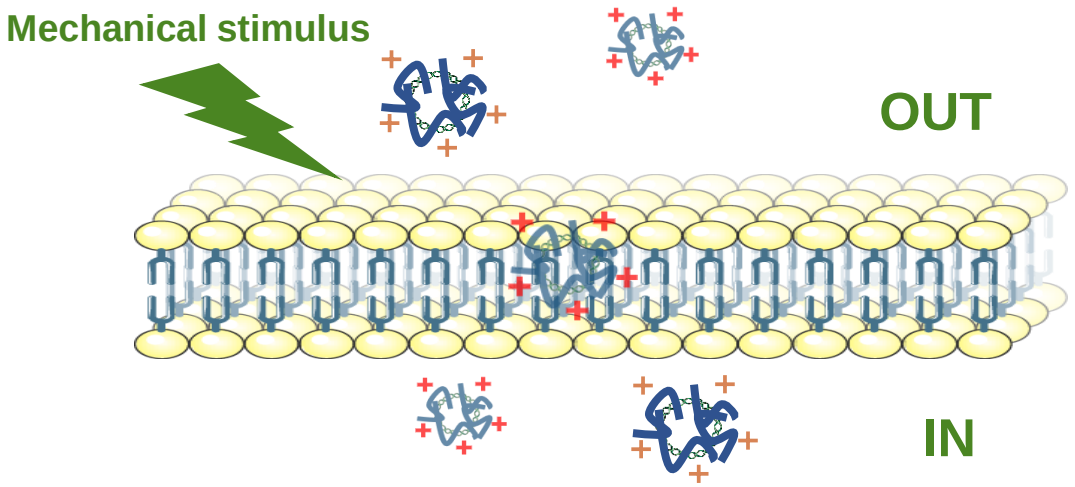
AIM OF THE WORK

- Find a new in vitro technology to boost the transfection efficiency (TE) of bPEI through cyclic stretch-based stimulation



STATIC CONDITIONS



UNDER CELL CYCLIC STRETCH

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METHODS AND RESULTS:
Design of the stretch system

1. Wide range of strain amplitude
2. Wide range of stimulation frequency
3. Constant and characterized equibiaxial strain profile

METHODS AND RESULTS:
Design of the stretch system

WORK RANGE:

Strain Amplitude (NE)= 0-20%

Strain Frequency (f)= 0-2Hz

Compatible for use in incubator

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METHODS AND RESULTS:
Experimental evaluation of strain

- ✓ Characterization of strain (NE_{Exp}) amplitude *vs.* puncher displacement
- ✓ Equibiaxial deformation of culture substrates

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METHODS AND RESULTS:
Finite Element Analysis (FEA)

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METHODS AND RESULTS:
Finite Element Analysis (FEA)

A

B

- ✓ Consistency of FEA analysis (NE_{Comp}) with experimental data (NE_{Exp})
- ✓ Uniformity of NE distribution in the middle region of culture substrates (1/3 of the total culture chamber)

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METHODS AND RESULTS:
Cyclic stretch-based transfection

TE of bPEI/pGL3 complexes on stimulated C2C12 cells

A

B

✓4-fold increase in TE for cyclically stretched cells with respect to unstimulated cells

✓Influence of strain frequency and amplitude in TE increase

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SUMMARY AND NEXT STEPS

- **Develop** a smart cyclic stretch-based culture system
- **Characterize** the mechanical-cues perceived by cells
- **Test** a novel in vitro non-viral gene delivery strategy boosting TE through cell-cyclic stretch

- **Shed light** on biological mechanisms behind stretch-based increase in TE
- **Optimize** the stimulation condition to maximize the results

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REFERENCES

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THANKS TO THE TEAM

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THANK YOU FOR YOUR KIND ATTENTION!

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