The *in vitro*, *ex vivo* and *in vivo* effect of polymer hydrophobicity on charge reversible vectors for self-amplifying RNA

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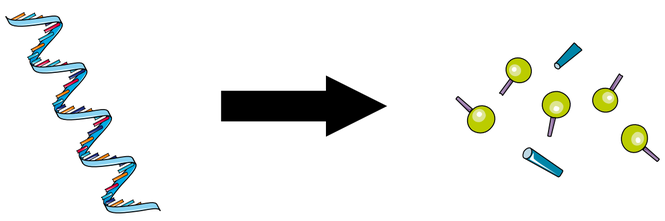
RNA vaccines

RNA (ribonucleic acid) is the code for proteins, and can be engineered to instruct muscle cells to produce antigens for vaccination..



Advantages:

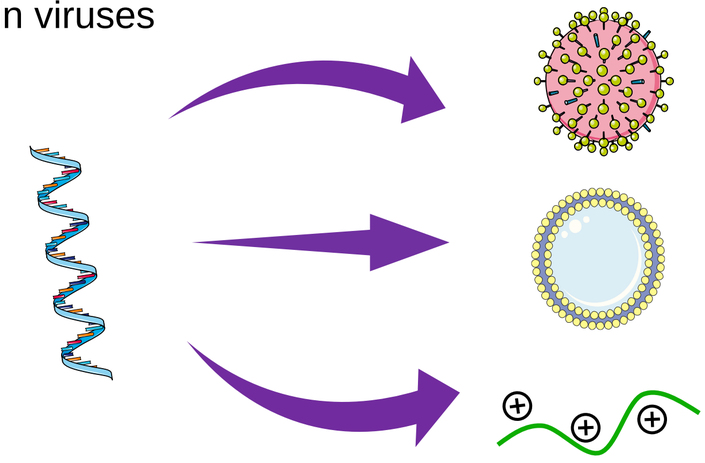
- ❑ RNA manufacture the same regardless of target pathogen.
- ❑ Only takes 2 weeks to produce doses
- ❑ No cells required
- ❑ Can be manufactured in poorer nations
- ❑ RNA automatically stimulates the immune system for better immunisation

Disadvantages:

- ❑ RNA is degraded by bodily enzymes due to our defence mechanisms

Formulation solutions:

- ❑ To protect RNA from degradation and improve cytosolic delivery, it is typically formulated with cationic polymers, lipids or in viruses

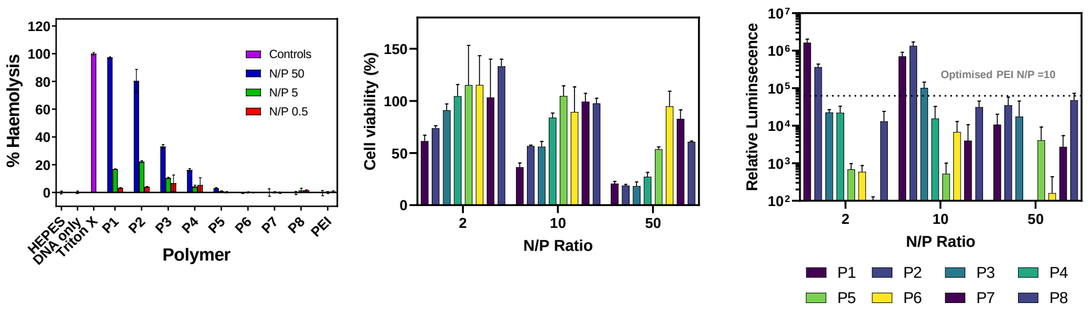


Aim

- ❑ Polymer hydrophobicity is key factor in polyplex (polymer-RNA complexes) gene delivery as it modulates cell membrane interaction.
- ❑ ***What role does hydrophobicity play in polymer vectors to deliver self-amplifying RNA in models specifically designed to test vaccination?***

In vitro - hydrophobic is best

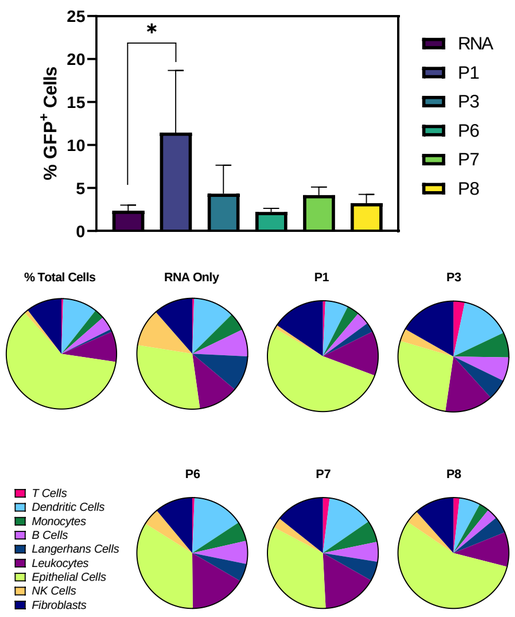
Membrane interaction and performance in HEK293T



- ❑ Hydrophobic polymers yield the highest transfection efficiency but also cause highest toxicity and membrane interaction

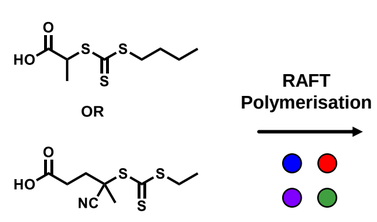
Ex vivo - hydrophobic is best

Transfection in human skin per cell type

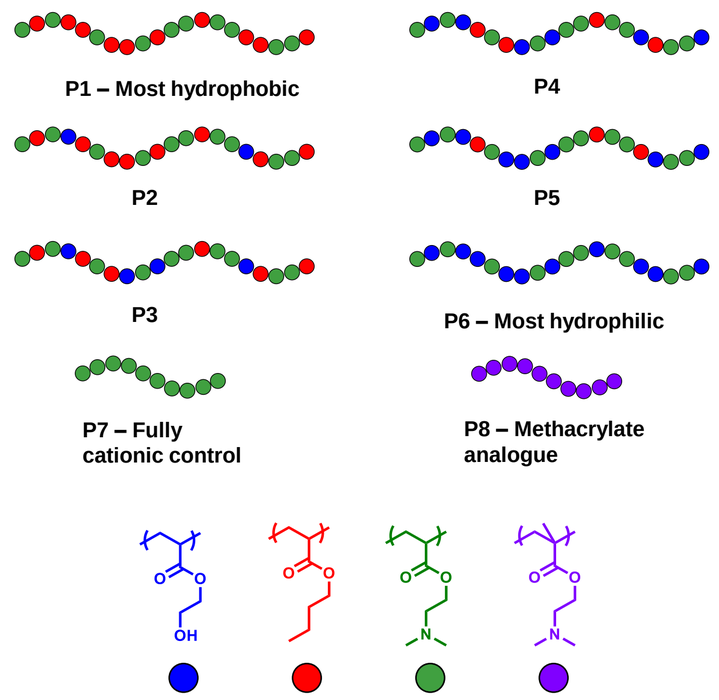


- ❑ Hydrophobic polymers again induce the highest transfection efficiency in human skin explants.
- ❑ Most hydrophobic polymers improve transfection by enhancing saRNA expression in epithelial cells and also some immune cell types

Polymer synthesis



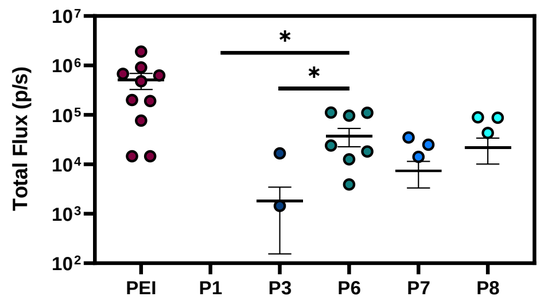
Polymer	$M_{n,SEC}$ (g mol^{-1})	$Đ$
P1	3300	1.16
P2	5400	1.22
P3	7000	1.18
P4	7300	1.21
P5	7800	1.24
P6	10300	1.15
P7	3100	1.21
P8	5000	1.22

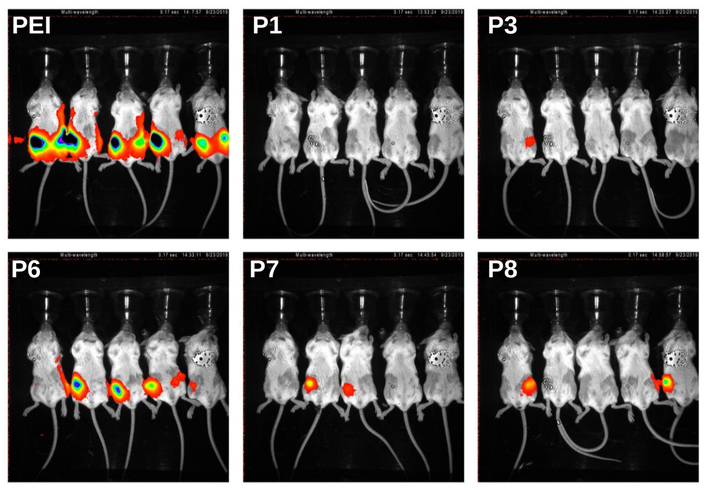


In vivo - hydrophilic is best

Transfection in mice

- ❑ None of the polymers outperformed PEI
- ❑ Interestingly the most hydrophilic polymers induced the highest saRNA expression.
- ❑ We hypothesise this is due to the toxicity of the hydrophobic polymers, possibly inducing inflammation type response, thus shutting down RNA expression pathways





Conclusions

- ❑ *In vitro*: hydrophobic best
- ❑ *Ex vivo*: hydrophobic best
- ❑ *In vivo*: hydrophilic best

Our data will help shape future polymer designs for the most efficient saRNA expression

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