Cationic methacrylate copolymers for gene delivery

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Introduction & Aim

Ease of synthesis and property tunability of polymers have drawn attention to their use as delivery systems. Cationic polymers interact and bind with negatively charged molecules, making them attractive candidates to transport genetic material across cell membranes. While these have been reported as good transfection agents[1], a balance between high transfection efficiency and low cytotoxicity remains hard to achieve. Key design criteria such as architecture has been suggested to influence toxicity, polyplex formation and transfection efficiency[2,3].

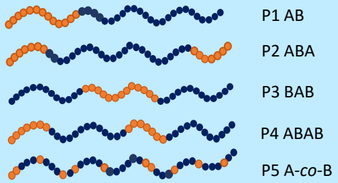


Figure 1: Illustration of architectures designed. PEGMA (A) and DMAEMA (B) units represented in orange and dark blue, respectively.

The **aim of this project** was to synthesise copolymers where monomer position in the polymer chain varies (architecture) and study the effect such variation plays on cytotoxicity.

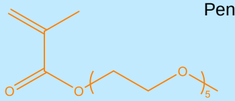
Experimental

Polymer Synthesis: five linear copolymers of different architectures and constant molar mass (10 kg mol^{-1}) and composition (50-50 w/w%)

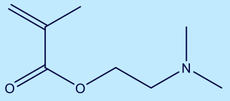
- Synthesised through **Group Transfer Polymerisation (GTP):**
  - Fast synthesis time (~15min. per block)
  - 100% monomer conversion
  - High yield (20 to 30g of polymer per synthesis)

Polymer Characterisation & *in-vitro:*

- Gel Permeation Chromatography (**GPC**): MM & molar mass distribution;
- H^1 Nuclear Magnetic Resonance (**NMR**): composition;
- H^+ Titrations: dissociation constant (**pK_a value**) of DMAEMA
- U.V-Vis: cloud point temperature.
- MTT assay: metabolic activity (cell viability)



Penta(ethylene glycol) methyl ether methacrylate
PEGMA
non-ionic & hydrophilic



2-(dimethylamino)ethyl methacrylate
DMAEMA
Ionic & hydrophilic
pH & thermo-responsive

Figure 2: Chemical structure and key properties of monomers used.

Results

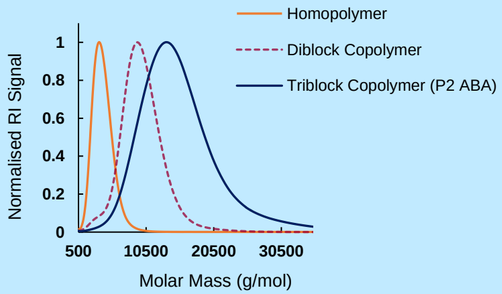


Figure 3: GPC traces of the P2 copolymer in dark blue and its linear precursors (Đ 1.14). This is a representative result of the polymer series synthesised.

Table 1: Experimental molar mass, dispersity and compositions of copolymers synthesised.

Polymer	M_n (g/mol)	Đ	Composition (H^1)
P1	10 100	1.10	49-51
P2	11 800	1.14	56-44
P3	9400	1.16	50-50
P4	11 500	1.20	49-51
P5	11 200	1.19	51-49

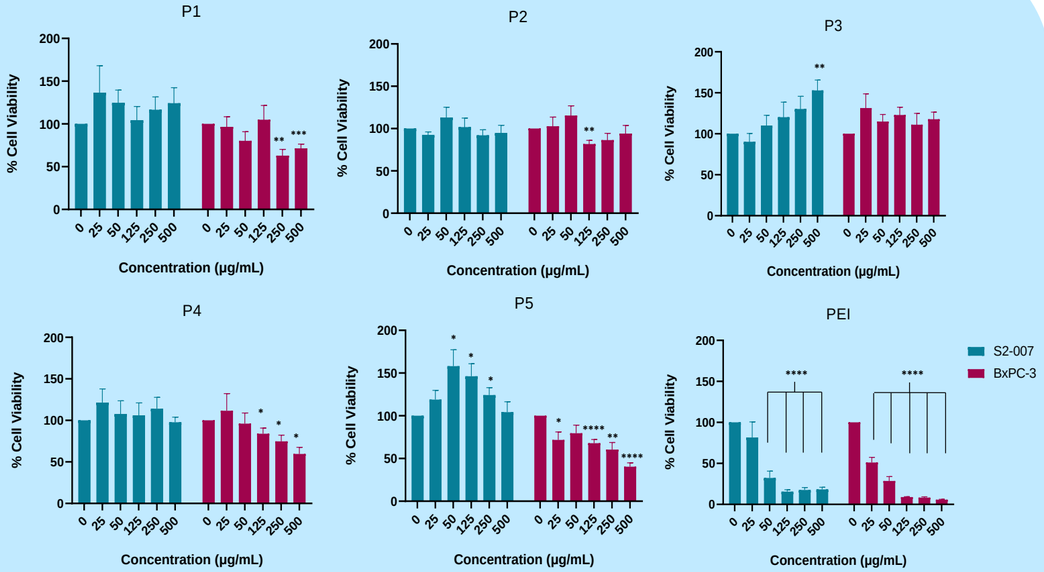


Figure 4. **Cell viability data for five polymers (P1-P5).** Cell viability was assessed by MTT assay after exposure to increasing concentrations of the polymers or control (PEI, polyethylenimine) for 24 hours.

Conclusion & Future Work

Triblock and tetrablock copolymers showed less toxicity than the statistical copolymer, in BxPC-3 cells, while no decrease in cell viability was observed for S2-007 cells. Overall, all copolymers were significantly less toxic than PEI, the gold standard in polymer vectors. These results suggest **a)** an architectural effect on toxicity, and **b)** cell line dependent toxicity.

Future work aims to further understand the effect of polymer architecture on polyplex formation, transfection and cellular uptake to highlight suitable candidates for gene delivery.

References

1. Georgiou *et al.* Biomacromolecules, 2005 (6): 2990–2997
2. Le Bohec *et al.* Polym. Chem., 2019 (10): 1968-1977
3. Rinkenauer *et al.* J.Mater.Chem.B, 2015 (3)

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