Design of Stealth Polymeric Nanoparticles.

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Aims

- To understand the role of the complement system in nanoparticle destruction.
- To evaluate the selected polymers for nanoparticle coating and analyse their effectiveness of the methods used.
- To determine whether the polymers selected are an effective barrier against complement detection and discuss future advancements of this technology.

Introduction

Nanoparticles (NPs) as a drug delivery system (DDS) is a revolutionary prospect in delivering medications with the intention to reduce adverse side effect profiles, as well as drug cycles. An e.g., was doxorubicin (DOX) loaded into dextran-NPs: a chemotherapy used to target cancer cells **only** (1). It was not successful initially, as these NPs were detected by an innate branch of the immune system called the Complement System (**figure 1**) (2).

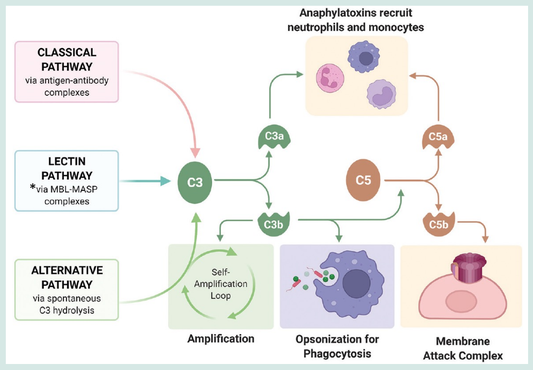

Figure 1 (left)- A simplified summary of the Complement System: the 'Classical', 'Lectin' and 'Alternate' pathways, triggered by different stimuli, all converging to C5 convertase (known as the 'Terminal Pathway'). (2)
***MBL-MASP:** mannose binding lectins- MBL associated serine proteases.

The three pathways are triggered by different stimuli, and lead to a series of bioactive fragments, all converging to **C3 Convertase**. This is split too, producing: anaphylatoxins contributing to inflammation, opsonization, leading to phagocytosis, and the formation of the membrane attack complex (MAC), where several serine proteases attach to the cell membrane, bursting and destroying the cell via osmotic movement. (3,4)

These immune responses apply to NPs too, and techniques were used to prevent destruction. E.g.: attaching polymers to NP surfaces to disguise them from the immune system. With DOX-NPs, a polymer called PEG was added via PEGylation, and although effective, there were drawbacks.

This project is an attempt to continue this, with a polymer called Polyethylenimine (PEI) (**figure 2**) as a primary coating, and a carboxylate (G4 family), as a secondary layer.

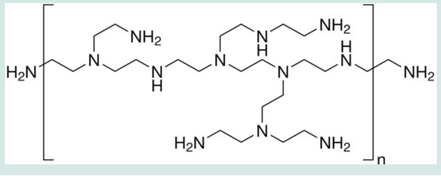

Figure 2- a single unit of branched PEI (right) used in experiments to coat sulfated polystyrene nanoparticles.

Methods

A set concentration of sulfated polystyrene nanoparticles (PS NPs) were mixed with varying concentrations of PEI dropwise via continuous vortexing. The total volume was made up to 1mL with filtered Milli Q water. The PEI-PS NPs were incubated overnight at room temperature in the dark.

They were then centrifuged twice (for 45 minutes at 13,000 rotations per minute at 4°C). After each centrifugation, 0.99ml (i.e.: supernatants) were removed (for analysis), and final PEI-PS pellets were made up to 0.5mL.

PS-PEI NPs were analysed via dynamic light scattering (DLS), Ultra-violet (UV) technology and NP tracking analysis (NTA). These techniques confirmed PEI coating via changes in charge, measuring free PEI in supernatants 1 and 2, and changes in size.

Two PEI concentrations were selected to add different concentrations of G4 carboxylates to PEI-PS. Supernatants were collected again, and analytics preformed: same as PEI.

Results

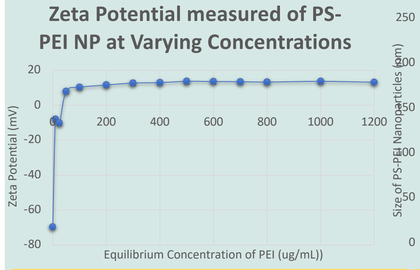

Figure 3 (above)- Zeta Potential of PSPEI NPs at varying concentrations of PEI (average of six results). Measured via DLS.

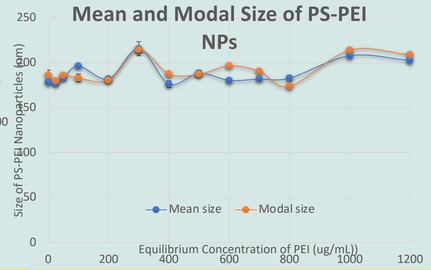

Figure 4 (above)- Mean and modal sizes of PSPEI at varying concentrations of PEI (average of five results). Measured via NTA.

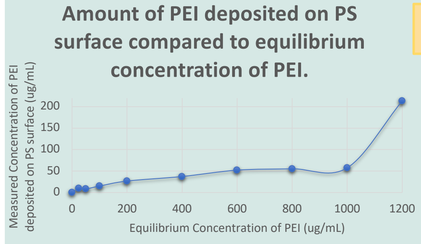

Figure 5 (left)- Actual PEI measured on PS surface Vs. starting PEI concentration.

These figures refer to the increase in nanoparticle size with proof by increase in charge (due to negative sulphate interactions on PS surface with NH_3^+ groups from PEI)(**figure 3**). However, NTA size fluctuates with increasing PEI (see discussion). PEI 800 and 1000 were chosen thereafter, due to higher deposition of PEI on surface. 1200 was not selected due to large PEI amount- it does not follow the gentle incline (**figure 5**). **Figure 6**: next stage.

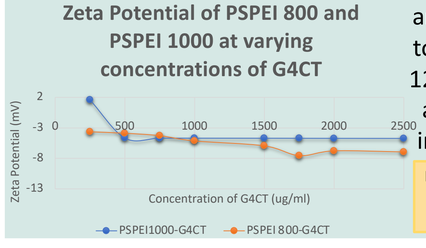

Figure 6 (left)- Zeta Potential of PSPEI 800 and 1000 at varying concentrations of G4CT (average of six results).

Discussion

The first stage of the experiment looked at how effectively PS NPs can be coated with PEI. The DLS (**figure 3**) indicated good coating, with a general increase in charge bar one concentration decreasing before the normal trend resumes. The NTA however, showed fluctuation in sizes (**figure 4**), when the expected trend would be an overall increase in size. PEI is branched, and so can compress, lay differently due to NP characteristics (e.g.: curvature, density, porosity (**figure 7**)) or entangle from concentration to concentration, potentially showing a smaller sized particle than expected at higher concentrations, even though there is more PEI. (5)

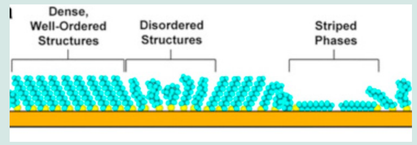

Figure 7 (left)- A schematic view detailing the potential arrangement of PEI chains, and how they can compress, explaining fluctuating NTA sizes. (5)

NTA can also analyse PS-PEI NPs at varying angles, depending on how they present themselves to the camera, affecting sizes too.

800 and 1000 ug/mL were chosen due to their reliability of results:

1) Their charge (**figure 3**)- within the plateaued range (i.e.: stable).
2) Sizes (**figure 4**)- more than the size of PS **only-** an increase in size. Trend: higher concentrations more suitable than lower concentrations due to incomplete coating of PS (i.e.: less reliable and stable as a DDS).
3) **Figure 5:** followed the increase in actual PEI deposition to PS surface (at most larger concentrations (i.e.: not concentration 1200 ug/mL).

The zeta potential measured with varying concentrations of carboxylates showed good coating and can be an additional barrier against the complement system: i.e.: destroying this barrier and leaving PS-PEI NP to deliver drug(s), whilst still disguising the NP.
Figure 6 indicates that more G4CT deposition occurred with the lower concentration (PSPEI 800), which may be more effective as an additional barrier method against the immune system, as discussed above.

Conclusions and Next Steps

The methods used have produced **repeatable, accurate** results. Most hypothesized trends were seen in the results, with exceptions to the sizing. However, to determine this (in vitro), I would conduct **ELISA** tests with PSPEI 800 and 1000, and with certain concentrations of G4CT, to determine if complement activity occurs. I would also have measured the sizes of PSPEI-G4CT, if the NTA camera had not broken, to confirm size increase and see patterns like **compression**: an unpredicted side effect. **Ultimately, PEI has great potential in being a part of the NP DDS, with the second layer of carboxylates increasing chances of successful targeted drug delivery.**

References

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