Analytical Methods to Evaluate Antibody Conjugation onto Polymeric Nanoparticles

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INTRODUCTION

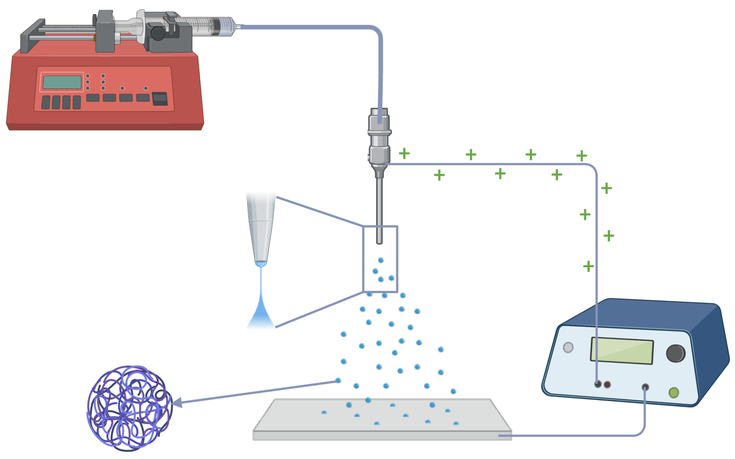
- Polymeric nanoparticles (PNPs) are promising drug delivery systems for cancer[1], however passive targeting via the **Enhanced Permeability and Retention** (EPR) effect is **unreliable** due to tumour heterogeneity and variable vascular permeability[2].
- Antibody-conjugated nanoparticles (ACNPs) **overcome this** through **active, tumour-specific receptor targeting**, improving cellular uptake, selectivity and therapeutic efficacy[3].
- However, ACNPs **face a key translational barrier** – the **lack of standardised conjugation protocols and robust characterisation methods** to confirm successful antibody attachment and preserved antigen-binding functionality[4].

AIMS & OBJECTIVES

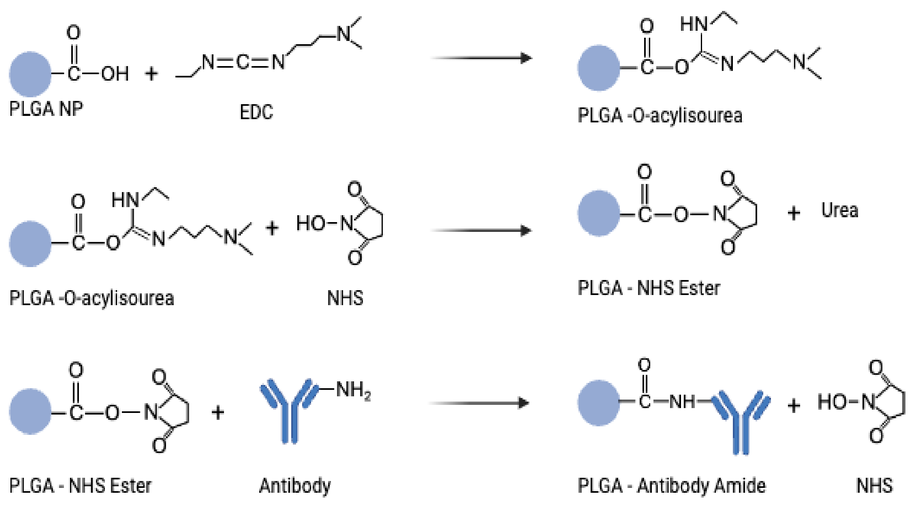
1. Develop a reproducible NHS/EDC carbodiimide conjugation protocol to attach Bevacizumab (model antibody) onto PLGA nanoparticles.
2. Validate conjugation using a multi-technique analytical panel to:
   - ➢ Confirm antibody attachment
   - ➢ Quantify conjugation efficiency
   - ➢ Confirm preserved antigen-binding activity – critical for active tumour targeting

METHODOLOGY

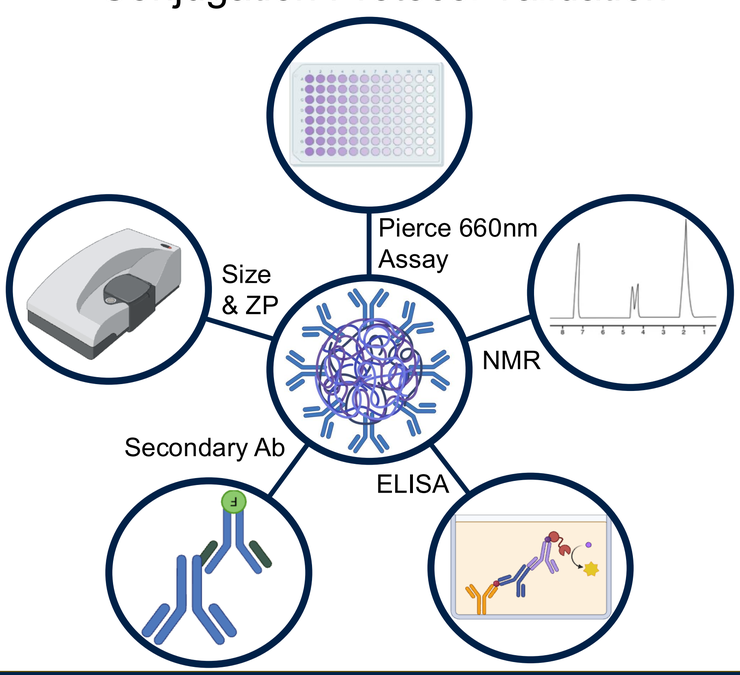
Nanoparticle Fabrication → Antibody Conjugation → Conjugation Protocol Validation



Electrospraying

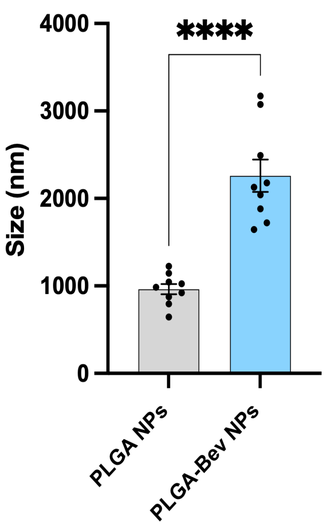


NHS/EDC carbodiimide coupling

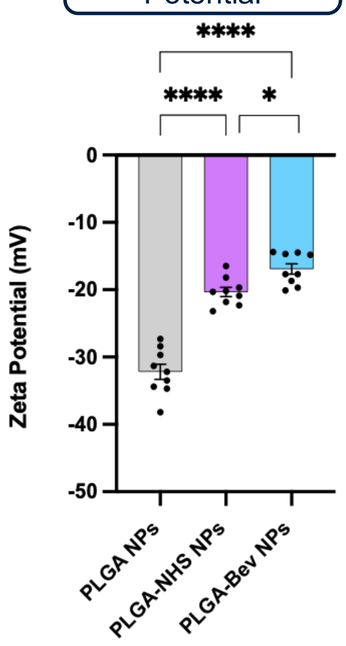


RESULTS:

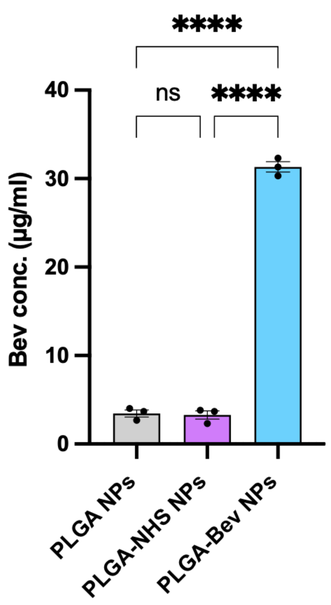
Hydrodynamic Diameter



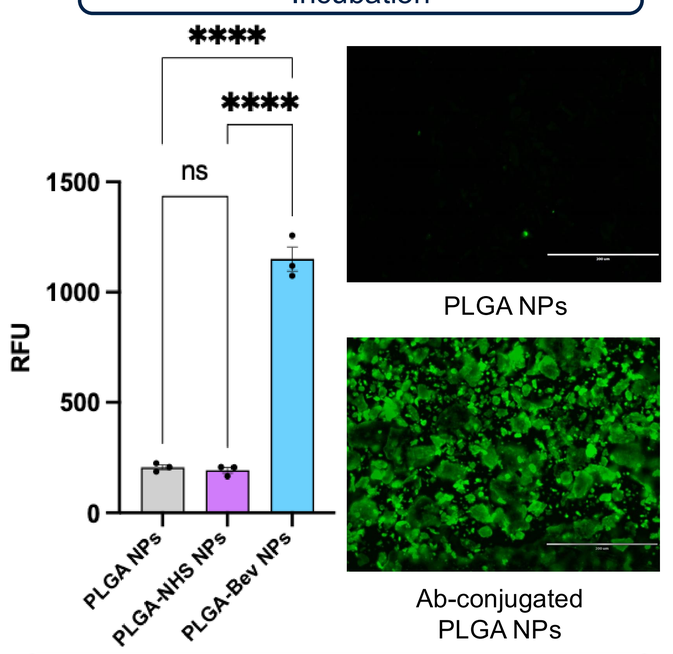
Zeta Potential



Pierce 660nm Assay



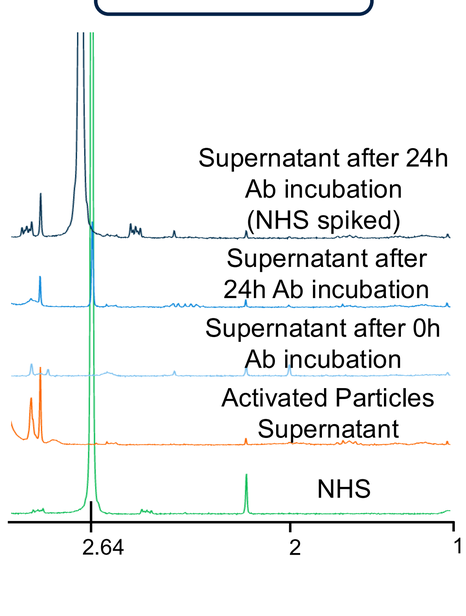
AlexaFluor-Secondary Antibody Incubation



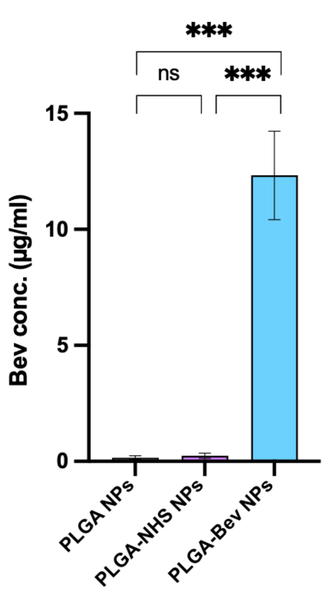
PLGA NPs

Ab-conjugated PLGA NPs

^{1}H NMR



Sandwich ELISA



Size increase following Ab conjugation – consistent with successful attachment	Reduction in surface charge seen as negative COOH groups form amide bonds with the Ab	Ab conc significantly higher in conjugated NPs vs activated or plain NPs	Significantly higher levels of fluorescent secondary antibody attached to conjugated NPs compared to unconjugated NPs	NHS (end-product of conjugation reaction) detected only in conjugated supernatant, not in activated	Conjugated Ab retained high antigen-binding activity, confirming functional integrity post-conjugation

CONCLUSION:

- A **robust antibody conjugation protocol was successfully developed** and applied to fabricate Bevacizumab-conjugated PLGA nanoparticles.
- Conjugation was validated using multiple analytical techniques, **confirming and quantifying antibody attachment** as well as **proving preserved binding activity post-conjugation**, critical for active targeting, **addressing key translational barriers in ACNP development.**

REFERENCES:

1)Kumari M et al. doi: 10.3762/bjnano.14.75
2) Fan D et al. https://doi.org/10.1038/s41392-023-01536-y
3) Beach MA et al. DOI: 10.1021/acs.chemrev.3c00705
4) Zielińska A et al. 10.3390/molecules25163731

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