Stimuli Responsive Dual Nanogel System for Dendritic Cell Modulation and Immune Checkpoint Blockade

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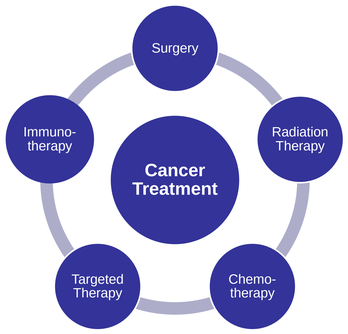
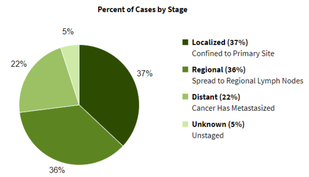
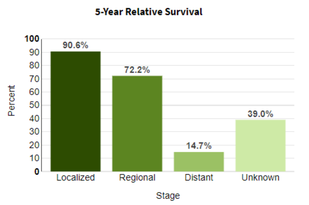
BACKGROUND AND MOTIVATION

- American Cancer Society estimates 149,500 new cases of colorectal cancer (CRC) along with 52,980 CRC related deaths in 2021, ranking 4th and 2nd respectively[1].







Immunotherapy Advantages
- Generic cancer treatment
- Multiple target pathways
- High specificity, Few side effects
- Immunologic memory

Immunotherapy Disadvantages
- Tumor microenvironment immunosuppression[2]
- Complex system of signaling pathways
- Potential autoimmune side effects

- Recent growing trend investigating combination treatments that work in synergy to produce a more robust therapeutic response, while reducing dosage and toxicity levels[3].

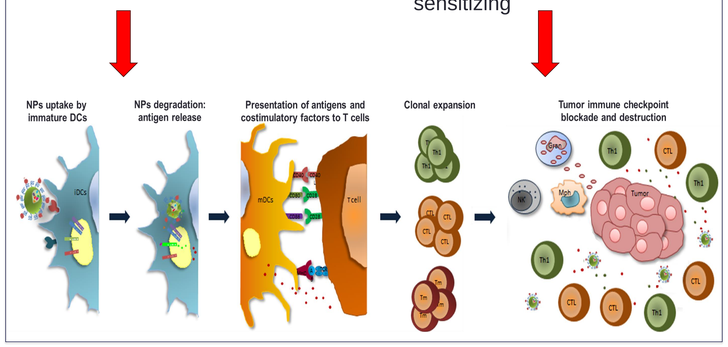
PROJECT GOAL

In this work, we present a dual delivery platform for more effective immunotherapeutic treatments by combining intelligent pH responsive biomaterials for DC activation with targeted immune checkpoint blockade treatment to achieve a synergistic effect between multiple immune response phases.

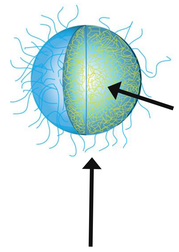
Approach: Develop a dual nanoparticle system to achieve a synergistic effect at the priming and effector phases

(1) Priming Phase
- DC-targeted nanogel
- Activate DCs and recruit T cells

(2) Effector Phase
- Tumor-targeted nanogel
- Enhance T cell response by sensitizing

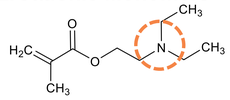


MATERIALS AND METHODS

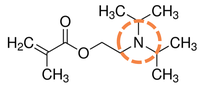


Nanogel Core Design

1. Cationic Monomers

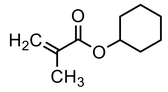


2-(diethylamino)ethyl methacrylate (DEAEMA)



2-(diidopropylamino)ethyl methacrylate (DPAEMA)

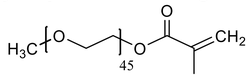
2. Hydrophobic Monomer



Cyclohexyl methacrylate (CHMA)

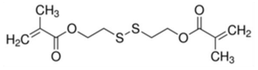
Surface Grafts

3. Hydrophilic Stealth Agent



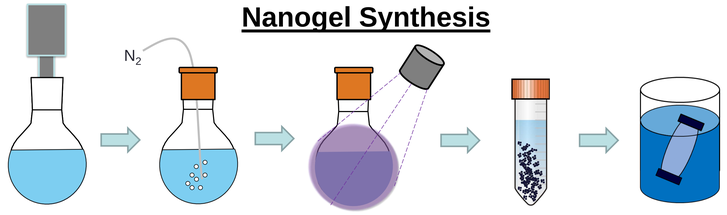
Poly(ethylene glycol) methyl ether methacrylate (PEGMA)

4. Crosslinking Agent



Bis(2-methacryloyl)oxyethyl disulfide (Disulfide XL)

Nanogel Synthesis



Ultrasonication (form oil-in-water emulsion)

Nitrogen Purge (eliminate free radical scavengers)

UV-initiated Photopolymerization (2.5 hr, 140 mW/cm^2)

Purification (4 ionomer collapse cycles)

Dialysis (10-14 changes, distilled water)

Nanogel Functionalization and Loading

Priming Phase Nanogel

Targeting Ligand

Anti-DEC205 Antibody

Cargo

Cytosine phosphorothioate-guanine (CpG) oligodeoxynucleotide (TLR9 ligand)

Polyinosinic:polycytidylic acid (poly(I:C)) (TLR3 ligand)

Tumor specific antigen for MC-38 cell line

Effector Phase Nanogel

Targeting Ligand

Carcinoembryonic Antigen (CEA) Antibody

Cargo

PD-L1 siRNA

Doxorubicin

RESULTS

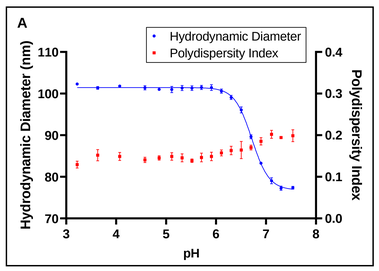


Figure 1. (A) Dynamic Light Scattering (DLS) hydrodynamic diameter swelling curve and polydispersity index for nanogel formulation. Samples were measured at 0.5 mg/mL in 1X PBS at 25°C. Data represents mean ± SEM (n=3).

Equation 1. Best Fit Curve for pH Swelling

A – Maximum Volume Swelling Ratio (VSR)

B – Slope of the Swelling Transition

C – Critical Swelling pH

D – Minimum Volume Swelling Ratio

$$VSR = \frac{A-D}{1+\exp(B*(pH-C))} + D$$

RESULTS

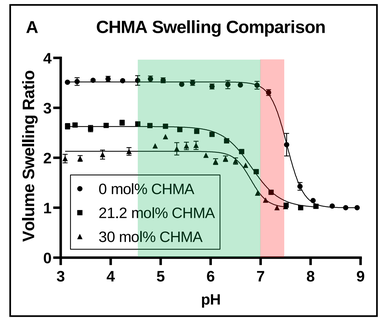


Figure 2. (A) Volume swelling curves for nanogels with varying amounts of hydrophobic monomer, CHMA. Samples were measured at 0.5 mg/mL in 1X PBS at 25°C. Best fit curve was determined based on Equation 1 using the variables in Table 1. Data represents mean ± SEM (n=3).

Critical swelling pH shifts from physiological environment pH (red region in Figure 2A) to tumor extracellular and intracellular environment pH (green region in Figure 2A) as highlighted in Table 1.

Table 1. Best Fit Swelling Curve Variables

	0 mol% CHMA	21.2 mol% CHMA	30 mol% CHMA
A	3.520	2.627	2.130
B	6.042	3.509	6.031
C	7.527	6.816	6.805
D	1.000	1.000	1.000

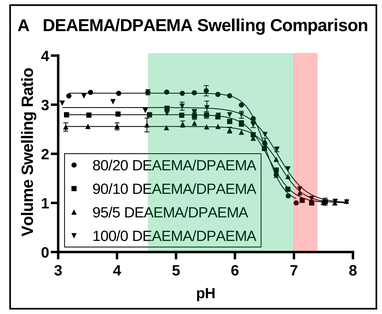


Figure 3. (A) Volume swelling curves for nanogels with varying mol ratios of cationic monomers, DEAEMA and DPAEMA. Samples were measured at 0.5 mg/mL in 1X PBS at 25°C. Best fit curve was determined based on Equation 1 using the variables in Table 2. Data represents mean ± SEM (n=3).

Critical swelling pH shifts further into the relevant pH range of the tumor microenvironment and the early endosome (pH ≈ 6.5), while maintaining stability and VSR as highlighted in Table 2.

Table 2. Best Fit Swelling Curve Variables

	80/20 DEAEMA/DPAEMA	90/10 DEAEMA/DPAEMA	95/5 DEAEMA/DPAEMA	100/0 DEAEMA/DPAEMA
A	3.236	2.797	2.557	2.943
B	6.100	4.916	4.930	4.156
C	6.518	6.579	6.754	6.732
D	1.000	1.000	1.000	1.000

CONCLUSIONS

- Successfully synthesized and characterized cationic nanoscale hydrogels with varied composition to improve relevant characteristics.
- Fine tunable critical swelling pH to reach relevant endosomal conditions.
- Further *in vitro* and *in vivo* studies will be required to confirm therapeutic efficacy of the dual delivery platform to synergistically stimulate an antitumor immune response for complete tumor eradication.

ACKNOWLEDGEMENTS

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