Development of a Dual-Responsive Hydrogel Platform for Tumor-Targeted Drug Delivery

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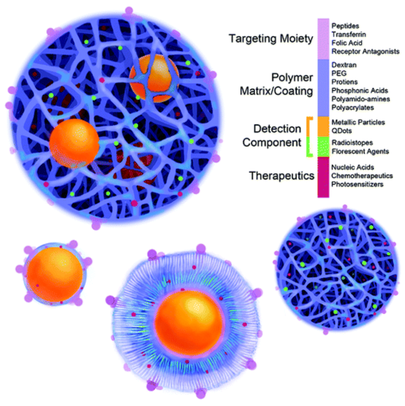
INTRODUCTION

Current Cancer Therapy

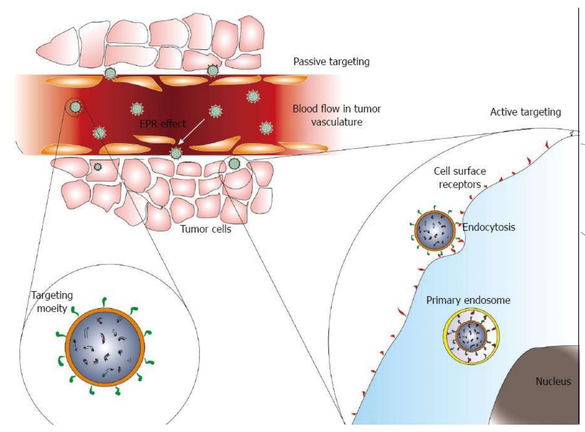
- Non-specific biodistribution
- Side effects
- Time between cycles gives cancer cells an opportunity to recover
- Cancer cells exposed to but not killed may become drug resistant

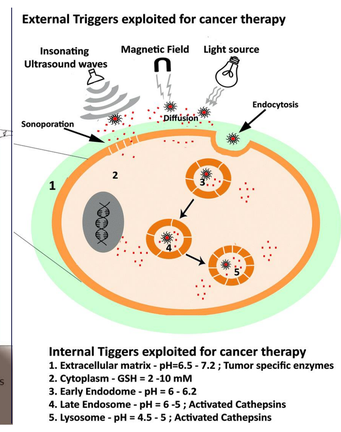
Nanoparticles as a Delivery Vehicle

- Potential to:
  - Overcome dose limiting toxicities
  - Improve the therapeutic margin
  - Improve patient quality of life
- Ability to deliver multiple therapeutic and / or diagnostic agents



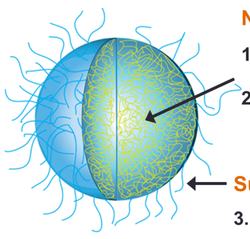
- Polymeric nanoparticles are an attractive option for cancer therapy due to their:[1]
  - favorable size distribution
  - high drug carrying capacity
  - tunable properties
  - ease of surface functionalization
- Intelligent polymers that respond to biological cues are of great interest because of their ability to provide controlled release at a specific site.[1,2]





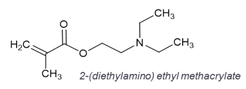
NANOGEL SYNTHESIS

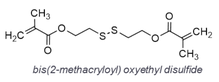
We have developed cationic nanoscale hydrogels (nanogels) that respond to the acidic intracellular environment for controlled, simultaneous delivery of multiple therapeutic agents



Nanogel Core

1. **Cationic Monomer**
2. **Crosslinking Agent**


2-(diethylamino) ethyl methacrylate


bis(2-methacryloyl) oxyethyl disulfide

Surface Grafts

3. **Hydrophilic Stealth Agent**
4. **Targeting Moiety**
   - Polymer backbone amenable to many conjugation strategies

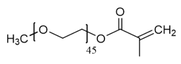
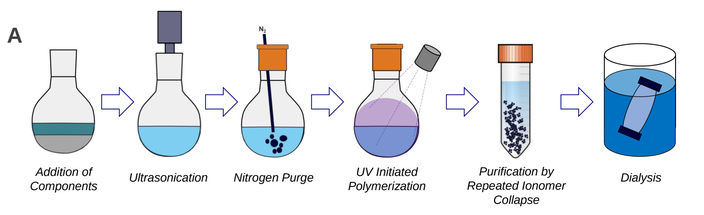

Poly(ethylene glycol) methyl ether methacrylate

Figure 1. The NGs are comprised of: (i) a hydrophilic, cationic monomer that imparts the pH-response by ionization of amine pendant groups, (ii) an enzyme-responsive, biodegradable crosslinker, (iii) a hydrophilic graft to impart serum stability, and (iv) can be functionalized with a variety of targeting moieties.



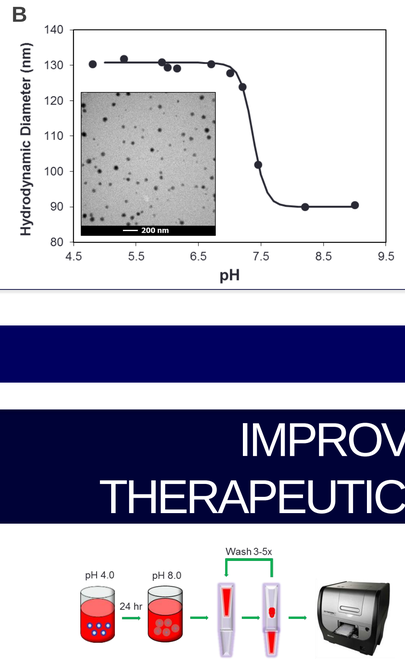


Figure 2. Synthesis of stimuli-responsive NGs.

(A) NGs were synthesized using an aqueous one-pot, UV-initiated emulsion polymerization technique[3].

(B) By DLS, NGs had a suitable size for tumor accumulation and showed a rapid transition to the swollen state with a large volume swelling ratio (VSR). Inset: Image of dry NGs by TEM (scale bar 200 nm).

INFLUENCE OF MOLECULAR ARCHITECTURE

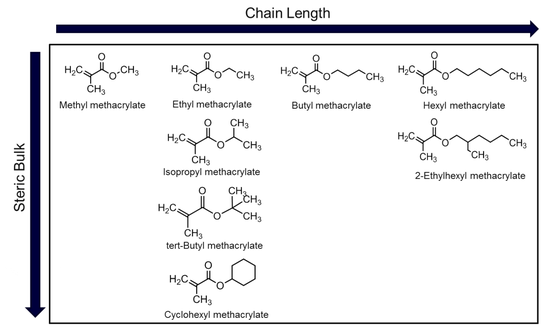


Figure 3. Understanding the effect of copolymer composition will improve the delivery potential. Hydrogel thermodynamic response (relative swelling ratio) and dynamic behavior (NG pKa and membrane disruption potential) were investigated through systematic variation of monomer functionality and chain length.

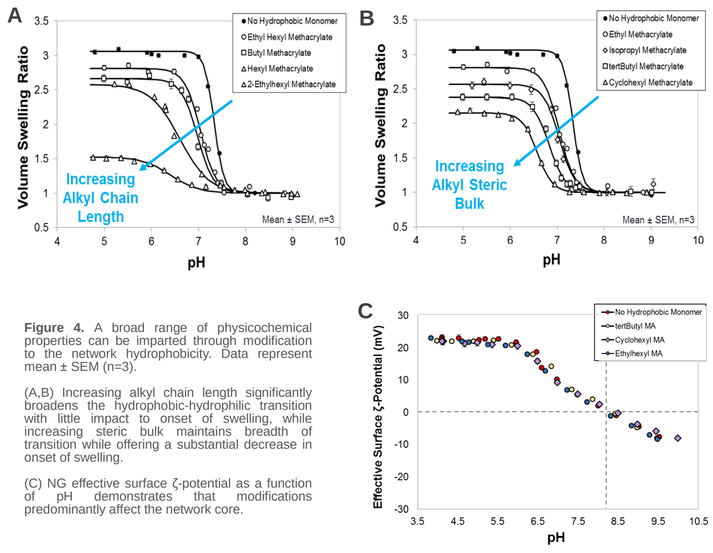


Figure 4. A broad range of physicochemical properties can be imparted through modification to the network hydrophobicity. Data represent mean ± SEM (n=3).

(A,B) Increasing alkyl chain length significantly broadens the hydrophobic-hydrophilic transition with little impact to onset of swelling, while increasing steric bulk maintains breadth of transition while offering a substantial decrease in onset of swelling.

(C) NG effective surface ζ-potential as a function of pH demonstrates that modifications predominantly affect the network core.

INFLUENCE OF MOLECULAR ARCHITECTURE

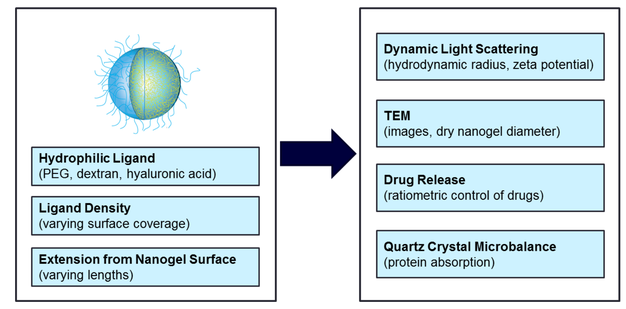


Figure 5. The rational design and characterization of P(DEAEMA)-g-PEGMA networks is necessary for tailoring the NG system to allow for effective transport to the tumor site.

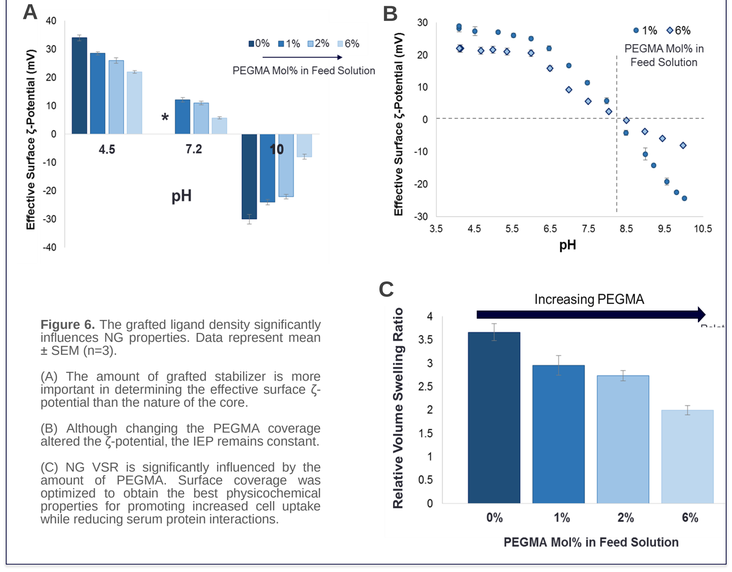


Figure 6. The grafted ligand density significantly influences NG properties. Data represent mean ± SEM (n=3).

(A) The amount of grafted stabilizer is more important in determining the effective surface ζ-potential than the nature of the core.

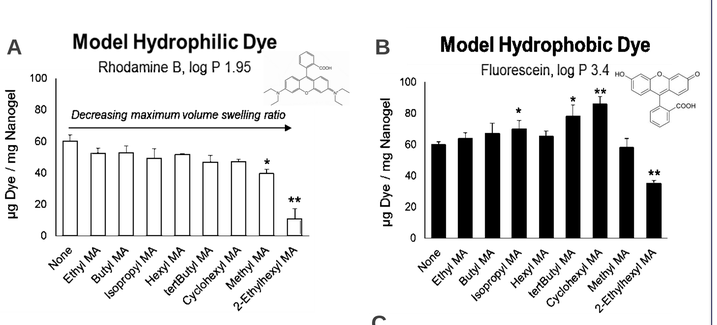
(B) Although changing the PEGMA coverage altered the ζ-potential, the IEP remains constant.

(C) NG VSR is significantly influenced by the amount of PEGMA. Surface coverage was optimized to obtain the best physicochemical properties for promoting increased cell uptake while reducing serum protein interactions.

IMPROVED THERAPEUTIC LOADING

$$\frac{\mu g\ Dye}{mg\ Nanogel} = \frac{m_o - m_f}{m_{nanogel}}$$

m_o is the initial mass of dye in solution
m_f is the final dye mass in in the filtrate + washes
$m_{nanogel}$ is the initial mass of nanogel in solution



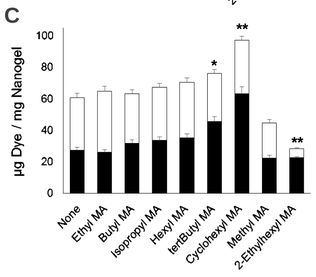


Figure 7. Dyes with similar MW and charge to common chemotherapeutic agents were used to enable rapid screening and selection of NGs with improved delivery potential. Data represent mean ± SEM (n=3, *p<0.05, **p<0.01 from none).

(A,B) Single agent loading showed that hydrophilic compound loading is minorly affected by variation in NG hydrophobicity, and - as expected - trends with VSR. Hydrophobic loading is influenced by improved interactions between drug and hydrogel, and is able to overcome the decrease in VSR.

(C) Co-loading studies demonstrated similar trends.

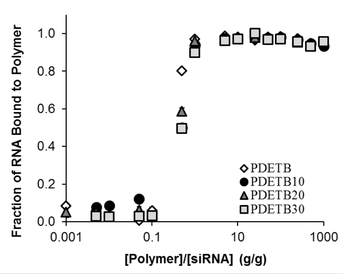


Figure 8. NGs are also highly suited for protecting and delivering biomacromolecular therapeutic agents. siRNA loading and is unaffected by variations in network hydrophobicity, as shown with formulations varying in tert-butyl methacrylate (0, 10, 20, 30 mol%). [4] Data represent mean ± SEM (n=3).

CELLULAR UPTAKE AND ENDOSOMAL ESCAPE

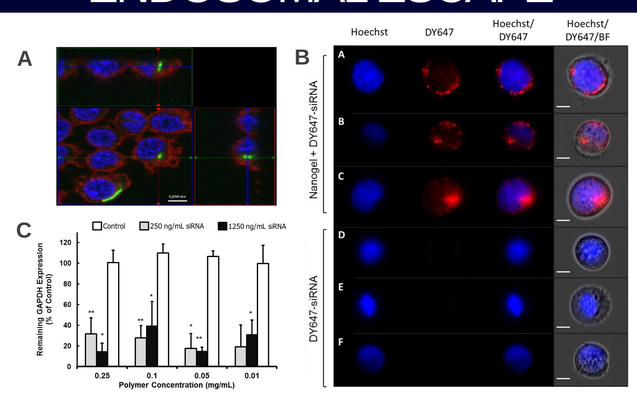


Figure 9. NGs are capable of enhancing cellular uptake of therapeutics and inducing a robust gene silencing effect.

(A) RAW264.7 cells showing internalization of fluorescently-labeled NGs via confocal microscopy.[5] Cells were incubated with 0.05 mg/ml NPs for 2 hrs prior to rinsing, fixing, staining, and mounting. Red: cell membrane stained with WGA Alexa Fluor 594 conjugate; blue: nuclei stained with DAPI; green: fluorescently-labeled NGs.

(B) siRNA delivery to Caco-2 cells.[4] Nuclear stain (Hoechst 33342) shown in blue and DY647-siRNA (DyLight 647) shown in red. Three representative images are shown, and scale bar represents 7 µm.

(C) GAPDH knockdown in Caco-2 cells following exposure to siRNA-loaded NGs.[4] Expression levels measured 48 hrs after transfection using KDalert™ GAPDH assay. Data represent mean of % remaining GAPDH expression ± SEM (n=3, *p<0.05, **p<0.01).

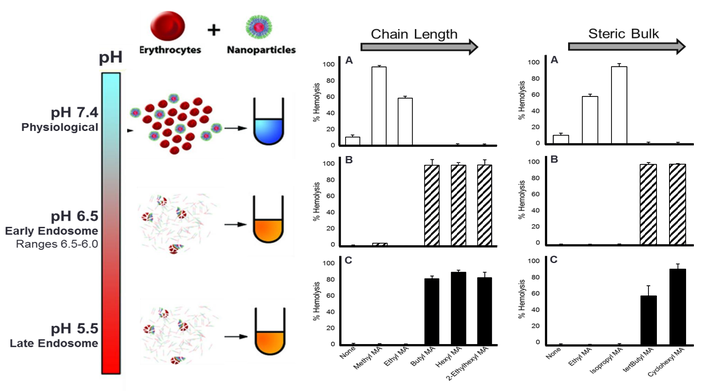


Figure 10. In order to effectively deliver drugs, the NG must be able to enable endosomal escape once uptaken by tumor cells. To estimate their ability to enable escape, NGs were incubated with whole red blood cells from sheep in phosphate buffers at (A) pH 7.4, (B) pH 6.5, and (C) pH 5.5.[6] Data are represented as mean ± SEM (n=3).

CONCLUSIONS

- Intelligent nanoscale hydrogels (nanogels) can be designed to take advantage of unique phenotypic features of diseased tissues in order to deliver a drug or imaging agent.
- NGs comprised of P(DEAEMA)-g-PEGMA show promise as a platform for controlled delivery of multiple therapeutic agents.
- A broad range of physicochemical properties can be imparted by modifying the network hydrophobicity.
- The thermodynamic response and dynamic behavior must be balanced with the ability to entrap therapeutics and release with the desired biological cue.
- Loading studies showed NGs can entrap therapeutic agents with a variety of physicochemical properties, and increased NG hydrophobicity has potential for increasing drug-polymer interactions.
- Copolymers made from butyl, tert-butyl, and cyclohexyl methacrylate monomers display enhanced ability to facilitate endosomal escape.
- Adjusting the PEGMA ligand density leads to varying NG surface characteristics that can be exploited to enable long-circulation and effective transport of the nanoparticles a tumor.

REFERENCES

[1] Moore, M.E.; Liechty, W.B.; Peppas, N.A. *Responsive Theranostic Systems: Integration of Diagnostic Imaging Agents and Responsive Controlled Release Drug Delivery Carriers*. PNAS. 2011, 44 (10), p. 1061-1070.

[2] Romberg, B.; et al. Pharm Research. *Nanoparticle delivery of cancer drugs*. (2008) Vol. 25, No. 1., p. 55-71.

[3] Liechty, W. B.; Scheuerle, R. L.; Peppas, N. A. *Stimuli-responsive nanogels containing t-butyl methacrylate and 2-(t-butylamino)ethyl methacrylate*. Polymer. 2013 (54), p. 3784-3795.

[4] Liechty, W. B.; Peppas, N. A. *Tunable, responsive nanoscale hydrogels for intracellular delivery of small interfering RNA*. The University of Texas at Austin, Dissertation. 2013.

[5] Forbes, D.C.; Creixell, M.; Frizzell, H.; Peppas, N.A. *Polycationic nanoparticles synthesized using ARGET ATRP for drug delivery*. EJPB. 2013 (84), p. 472-478.

[6] Evans, B. C.; et al. *Ex Vivo Red Blood Cell Hemolysis Assay for the Evaluation of pH-responsive Endosomolytic Agents for Cytosolic Delivery of Biomacromolecular Drugs*. JOVE. 2013 (73).

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