Preparation of Nanogels via Strain-Promoted Alkyne-Azide Cycloaddition

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Introduction

- The delivery of cell and drug-based chemotherapeutics to tumors have presented major challenges in cancer therapies.
- Current small molecule chemotherapeutics can be improved by increasing overall therapeutic efficacy and decreasing harmful off-target effects.
- The DeForest Group has recently developed a chemical framework for degrading user-programmable hydrogels based on multiple environmental cues following Boolean logic.
- To translate these materials for chemotherapeutics *in vivo*, we have established nanoparticle formulation strategies that enable degradable nanogels to circulate in the bloodstream to the desired target site.

Methods

- Using cyclohexane, water, and a nonionic detergent, we first optimized ultrasonication conditions for micelle preparation. The variables tested were sonication power, run time, and concentration of Span 80 surfactant and water relative to cyclohexane.
- With the ability to generate micelles via water-in-oil reverse emulsion, we then incorporated the materials used to produce our recently developed degradable hydrogels.
- The two main ingredients for hydrogel formation, four-arm poly(ethyleneglycol) tetrabicyclononyne and two-arm poly(ethyleneglycol) diazide, were ultrasonicated separately under the conditions we optimized and remained in the aqueous phase.
- All of the components were ultrasonicated together and allowed to crosslink in the aqueous phase via strain-promoted alkyne-azide cycloaddition.
- The resulting sample was purified by centrifugation, rotary evaporation and dialysis against ethanol and water.

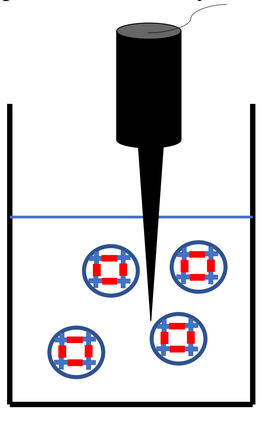
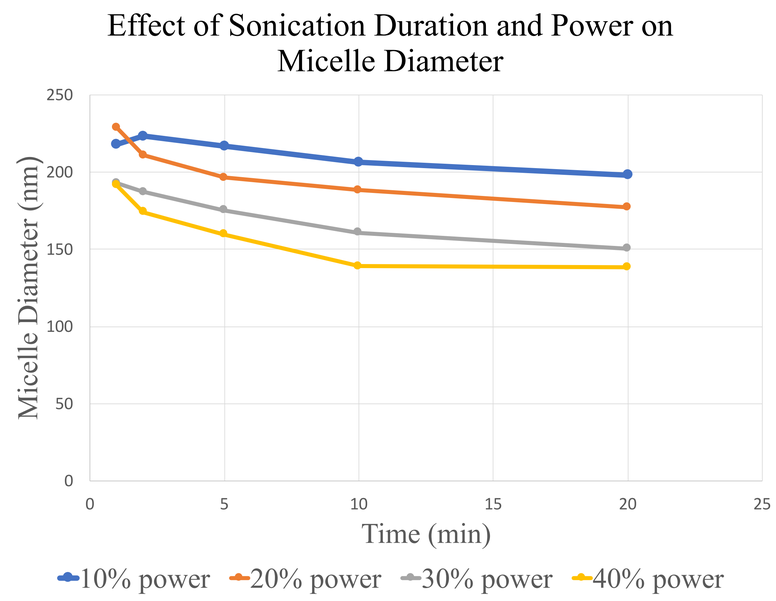


Figure 1. Scheme of nanogel synthesis. Poly(ethyleneglycol) tetrabicyclononyne and poly(ethyleneglycol) diazide were ultrasonicated together in water-in-oil emulsions on ice. Nanogels were crosslinked in the aqueous phase and surface-protected by surfactants and azide-acid molecules.

Results



Surfactant Wt %	Avg. Micelle Diameter (nm)
0	3255
1	158.7
2.5	**149.2**
4	157.3
5	164.3

Figure 2. Optimization of sonication conditions using cyclohexane, DI water and surfactant. Sonicator power and duration were first varied to scale micelle diameter. The results obtained by dynamic light scattering suggested that by tuning these two variables, desired micelle size between **140** nm to **230** nm can be finely controlled.

Table 1. Effect of Span 80 surfactant weight percentage on micelle diameter. All other variables were held constant. Samples were ultrasonicated for 10 minutes at 40% amplitude. Micelle diameters were characterized with dynamic light scattering.

- Through these micelle optimization experiments, it was clear that multiple variables contribute to micelle diameter and a combination of different parameters allowed for production of different micelles.
- The conditions that produced the smallest micelles were 2.5 surfactant weight percentage, 10 minutes of sonication duration, and 40% sonication amplitude. These conditions were used to prepare nanogels.
- Preliminary testing of the designed nanogel synthesis protocol suggested that under these conditions, nanogels as small as **77** nm can be created before purification, and stable over time.
- Dialysis and centrifugation have been promising methods for purification.

Strain-Promoted Alkyne-Azide Cycloaddition

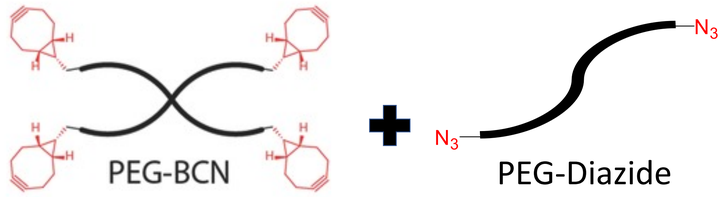
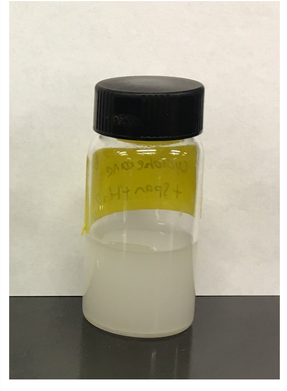


Figure 3. Four-arm poly(ethyleneglycol) tetrabicyclononyne and two-arm poly(ethyleneglycol) diazide were reacted in the aqueous phase under ultrasonication. The crosslinking between the functional groups gave rise to the nanogels.

Conclusion

- Preliminary data showed that nanogels of the desired size can be synthesized under ultrasonication and strain-promoted alkyne-azide cycloaddition.
- The protocol is quick in production, stable over time and highly tunable in terms of size.
- The system is versatile and other materials can be incorporated to allow for desired degradation.

Future Work



- Optimize synthesis and purification protocol.
- Incorporate "smart" materials such as synthetic peptide crosslinkers in order to achieve specific degradation effects.
- Load nanogels with cell and drug-based chemotherapeutics and use biological models to determine nanogel efficacy.

Figure 4. Image of nanogels emulsified in organic solvent prior to purification

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