Acclimation of nano-polymersome self-assembly to high osmotic stress environments

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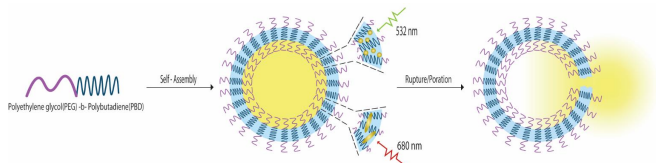
Abstract

Polymersomes hold great promise as carrier vesicles for the delivery of various types of cargo. Self-assembly of the amphiphilic diblock copolymer into spherical vesicles results in two compartments for encapsulation: an aqueous lumen and hydrophobic bilayer membrane. Recently, our laboratory developed methods for rendering these polymersomes light-responsive by including plasmonic gold nanoparticles within the hydrophobic membrane of the vesicle which would allow for high spatiotemporal control over cargo delivery. The work presented herein seeks to develop methods for delivery in sea urchin embryos, an important biological model organism for developmental biology studies. Most typically, material is introduced into sea urchin embryos via a microinjection technique, which is both a time consuming and challenging skill. This work seeks to develop methods to circumvent the need for microinjection by acclimating nano-scale polymersomes to an appropriate osmotic pressure solution (seawater) such that they can be incubated and uptaken by the developing sea urchin embryo. This is a difficult problem as polymersomes are extremely sensitive to the osmolarity of their environment. Various methods of self-assembly in seawater were developed and tested and it was determined that self-assembly in deionized water followed by a gentle, step-wise dialysis into seawater results in stable nano-vesicles. Finally, preliminary studies show promising results for uptake by sea urchin embryos.

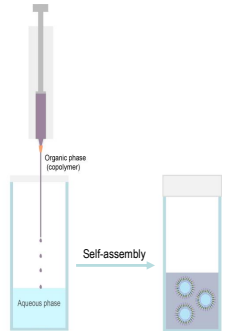
Polymersome-nanoparticle systems



Polymersomes hold great promise for future use in developmental biology studies:

- Polymersomes are self-assembled from diblock copolymer, PEG_{20}-b-PBD_{35}. Hydrophobic and hydrophilic constituents can be encapsulated into the membrane and core, respectively. **This process is highly sensitive to its environment (e.g., temperature, osmotic pressure).**
- Metal nanoparticles can be incorporated into the hydrophobic membrane as photosensitizers to facilitate rupture and cargo delivery with high spatiotemporal control.
- The ability to deliver cargo with high spatiotemporal control would be transformative for developmental biology research, where the time and location of processes (i.e., gene expression) is often difficult to ascertain.
- Sea urchin embryos are an important model organism for developmental biologists, however, often require challenging microinjection techniques to introduce materials into the developing embryo.
- By acclimating polymersomes into seawater environments, embryos can be incubated with vesicle samples for potential uptake without microinjection.

Standard self-assembly process for low salt environments



Self-assembly method:

- Nano-polymersomes are generally formed via a drip method whereby the organic phase (containing the diblock copolymer, dodecanethiol functionalized gold nanoparticles, and nile red fluorophore, where indicated) are injected into an aqueous phase of distilled water while undergoing vigorous mixing (e.g., stirring, vortexing, sonicating, or sonifying).

Characterization method:

- Post self-assembly, vesicles are characterized using dynamic light scattering (DLS). DLS reports number and intensity distributions, and the polydispersity index (PDI).
- The intensity distribution is indicative of the overall average size of the vesicles in the sample, however, the number distribution often highlights the presence of smaller structures (i.e., micelles) which are undesirable. These data must be analyzed in combination to get an accurate representation of sample composition.

Criteria for determining self-assembly success

- **Cloudy appearance** is indicative of scattering which occurs when molecular self-assembly has taken place.
- **DLS Data:**
  - **Intensity distribution < 250 nm** indicates the presence of nano-polymersomes
  - **Number distribution > 60 nm** indicates that the self-assembly process did not form undesirable micelles
  - **Polydispersity index (PDI) <0.3** indicates that self-assembled structures are uniform in size

Various methods of acclimation of nano-polymersome self-assembly into high salt environments

	Pre-Dialysis			Post-Dialysis		
Assembly method	Size distribution by number (nm)	Size distribution by intensity (nm)	PDI	Size distribution by number (nm)	Size distribution by intensity (nm)	PDI
Method 1 (n = 3) Average	349.67	467.07	1.00	N/A	N/A	N/A
Standard deviation	75.83	35.54				
Method 2 (n = 7) Average	143.35	301.37	0.34	N/A	N/A	N/A
Standard deviation	95.87	24.63	0.17			
Method 3 (n = 6) Average	115.29	211.25	0.22	110.89	201.13	0.22
Standard deviation	22.21	17.27	0.03	13.74	15.80	0.04
Method 4 (n = 3) Average	31.92	111.62	0.19	N/A	N/A	N/A
Standard deviation	6.86	55.79	0.10			
Method 5 (n = 4) Average	22.52	105.34	0.25	N/A	N/A	N/A
Standard deviation	13.02	32.38	0.01			
Method 6 (n = 5) Average	81.21	119.68	0.22	76.64	133.26	0.19
Standard deviation	7.35	24.88	0.06	8.47	8.56	0.03

Self-assembly methods

With AuNPs:

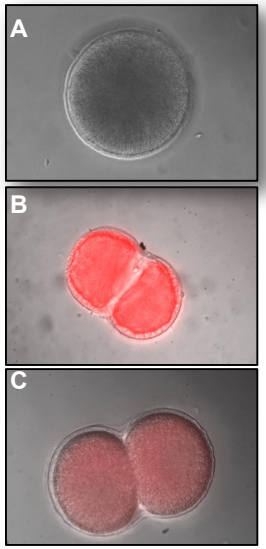
- Method 1: Slow drip injection of organic THF containing polymer and AuNPs into aqueous sea water (30 ppt) with stirring.
- Method 2: Slow drip injection of organic THF containing polymer and AuNPs into aqueous sea water (30 ppt) with sonification.
- Method 3: Slow drip injection of organic THF containing polymer and AuNPs into DI water while stirring, followed by dialysis into sea water (30 ppt).

Without AuNPs:

- Method 4: Slow drip injection of organic THF containing polymer (without AuNPs) into aqueous sea water (30 ppt) with stirring.
- Method 5: Slow drip injection of organic THF containing polymer (without AuNPs) into DI water while stirring.
- Method 6: Quick injection of organic DMSO containing polymer (without AuNPs) into DI water with while vortexting, followed by dialysis into sea water (30 ppt)

All methods of self-assembling nano-polymersomes directly in seawater yielded samples of micelles or large aggregates; thus, it was determined that samples must be assembled in low-salt environments and transitioned gently to a higher salt solution via dialysis.

Uptake studies in sea urchin embryos



Fertilized and de-jellied sea urchin embryos were stripped of their fertilization envelope, incubated with varying dilutions of a nanopolymersome sample, and imaged for approximately 2 hours.

The nano-polymersome sample used contained AuNPs and nile red fluorophore in the membrane, had an average size of 214 nm, and a PDI of 0.18 after gentle dialysis into 30 ppt sea water solution.

Representative images are shown:

A. Control sample incubated in sea water only.
B. Embryos incubated with the nanopolymersome sample (5-fold dilution)
C. Embryos incubated with the nanopolymersome sample (10-fold dilution).

Red fluorescence within the embryos is suggestive of uptake, as the nile red dye is contained within the polymersome membrane.

Note: Approximately 10 embryos were incubated per dilution. This preliminary experiment was performed once with two technical replicates. Additional trials will be performed to yield statistically relevant data.

Conclusions

- Direct self-assembly of nano-polymersomes into high salt solutions yields undesirable structure formation (micelles and large aggregates).
- Polymersomes must be transitioned into solutions of higher osmolarity via a gentle, step-wise dialysis procedure.
- Polymersomes self-assembled with gold nanoparticles successfully form via a slow drip method of organic tetrahydrofuran into aqueous distilled water followed by dialysis into saltwater.
- Polymersomes absent of gold nanoparticles successfully form via quick injection method of organic dimethyl sulfoxide into aqueous distilled water followed by dialysis into saltwater.
- Preliminary studies show promising uptake by sea urchin embryos at various dilutions

Future directions

- Study effects of polymersome uptake (with and without encapsulated AuNPs) on sea urchin embryos viability
- Demonstrate the ability to release polymersome cargo in a single cell with spatially and temportally controlled 532 nm femtosecond laser irradiation
- Concurrent studies with mammalian cell lines are also underway (not shown).

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