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Building Robust Protocells from Giant Polymersomes Made with Gel-Assisted Rehydration

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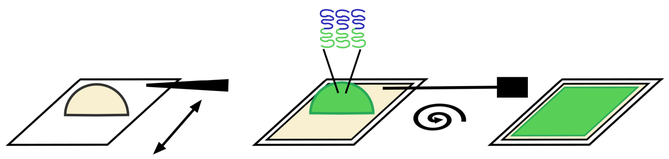
ABSTRACT

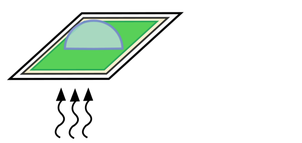
Polymer vesicles, or polymersomes, are being widely explored as synthetic analogs of lipid vesicles based on their stability, robustness, barrier properties, chemical versatility and tunable physical characteristics. Traditional methods to prepare giant polymersomes (>4 μm) are both time and labor intensive, yielding low numbers of intact polymersomes. Here, we present the rapid and high-yielding formation of giant unilamellar polymersomes using gel-assisted rehydration, and describe a mechanism of how formation and size distribution of polymersomes may be achieved. Using this method, polymersomes were formed from an array of polymer compositions, including a pH sensitive polymer, rendering polymersome formation reversible. Furthermore, polymersomes were successfully formed in a variety of biological rehydration solutions, including mammalian cell culture media. Likewise, polymersomes were able to successfully encapsulate biological materials, indicating that gel-assisted rehydration is a versatile method for building polymersome-based protocells. Polymersome size was easily tunable by altering temperature during rehydration or adding fluidizers to the polymer membrane, generating giant-sized polymersomes (>100 μm). The correlation between size and membrane fluidization suggests a unique mechanism from that proposed for giant lipid vesicle formation in which both polymer diffusivity and osmotic potential drive the formation and size distribution of the polymersomes. Overall, this technique is capable of reliably producing polymersomes from different polymer compositions and charges with far better yields and much less difficulty than traditional methods. Furthermore, vesicles formed in biological buffers and media and encapsulating biological materials make them readily useful for biomimicry studies.

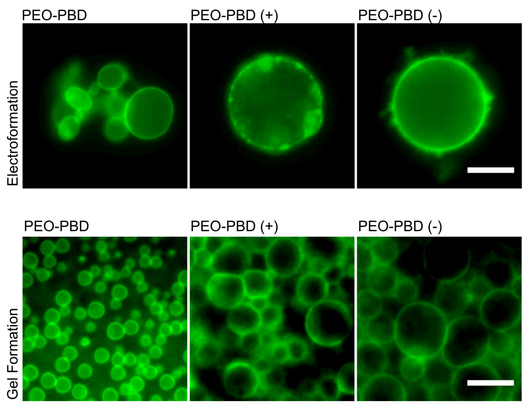
NEW METHOD TO FORM POLYMERSOMES

1. Spread dissolved agarose on coverslip with a pipette tip
2. Spread polymer solution in circular motion on agarose films with needle
3. Dehydrate polymers on agarose films



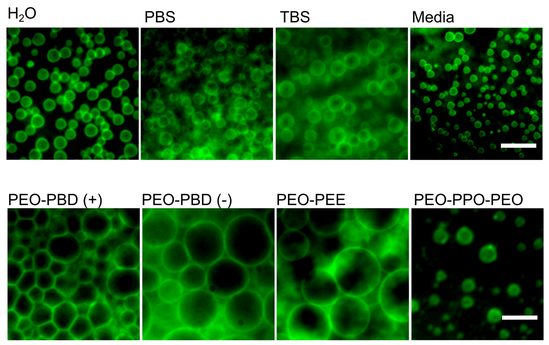
4. Rehydrate polymers with desired solution and heat
5. Giant polymersomes form after 25 minutes





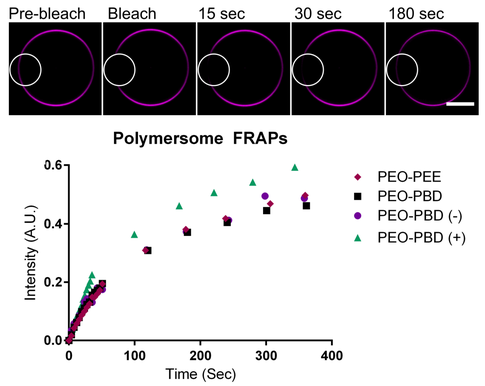
RESULTS

Gel-Assisted rehydration robustly forms giant polymersomes



- PEO-PBD polymersomes were formed in a variety of rehydration solutions
- Polymersomes were formed from a variety of polymer compositions, including charged polymers

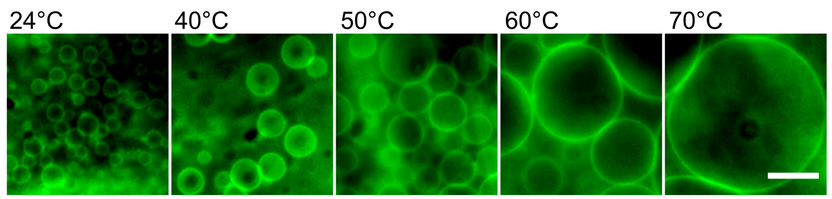
Polymersome membranes are fluid as characterized by FRAP

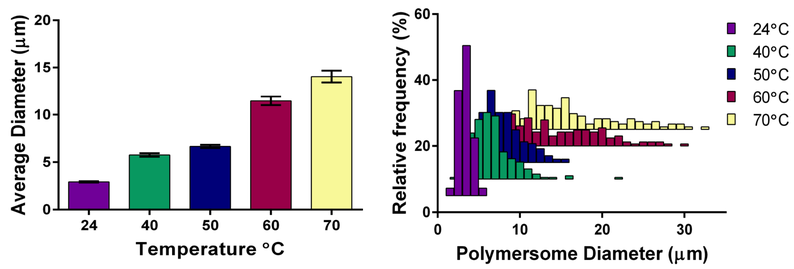


- Fluorescence recovery after photobleaching (FRAP) was used to measure polymersome membrane fluidity
- Calculated diffusion coefficients fall within expected ranges for polymersome membranes

Polymer	Diffusion Coefficient (μm²/s)
PEO-PEE	0.0287 ± 0.009
PEO-PBD	0.0144 ± 0.006
PEO-PBD (-)	0.0244 ± 0.003
PEO-PBD (+)	0.0142 ± 0.007

Temperature regulates size of polymersomes

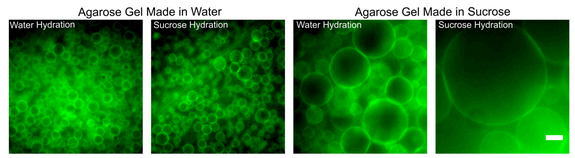


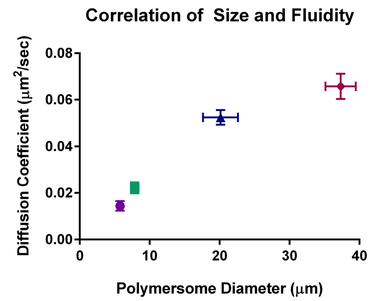


- Increasing the temperature during rehydration increases the average size of the polymersomes as well as the dispersity of the size distribution

RESULTS

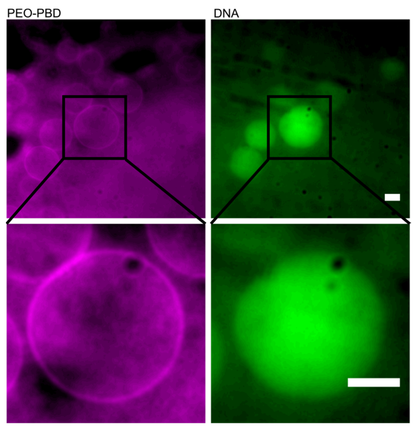
Membrane fluidization drives polymersome formation





- The addition of sucrose acts as a small molecule membrane fluidizing agent
- Sucrose was added in the agarose gel, in the rehydration solution or in a combination of both and polymersome size and diffusion was characterized
- Sucrose increases polymersome size and membrane diffusion coefficients indicating that membrane fluidization drives polymersome formation

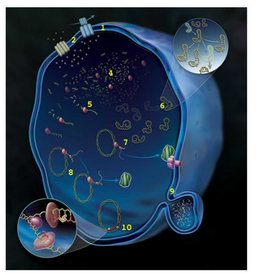
DNA can be encapsulated in polymersomes using this method



- Fluorescently-labeled ssDNA oligos were efficiently encapsulated in giant polymersomes using gel-assisted rehydration
- DNA was added in between the agarose layer and the polymer layer
- Upon rehydration, DNA was found to be encapsulated within polymersomes

CONCLUSIONS

- Gel-assisted rehydration is a robust alternative for forming giant polymersomes



- Polymersomes can be formed in many rehydration solutions and with many compositions, unlike in electroformation
- Polymersome formation is aided by membrane fluidization
- Small molecules can be encapsulated in polymersomes
- This method can be used for building polymersome-based protocells

Deamer, D. *Trends in Biotechnol.* **23**(7): 336-338 (2005).
http://www.the-scientist.com/?articles.view/articleNo/19728/title/The-Final-Step-s--/

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