Engineering Artificial Mechanosensitive Cells by Combining Cell-Free Expression and Ultrathin Double Emulsion Template

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A major goal in the Liu lab is to build an artificial cell which can sense and respond to external stimuli. Bottom up *in vitro* reconstitution is a general approach to design and construct an artificial cell. Active biological ingredients encapsulated into a synthetic membrane would form an artificial cell which imitates one or more behaviors of a cell. A glass capillary microfluidic device is used to generate double emulsion droplets with an ability of forming lipid bilayer vesicles with encapsulation capability. Mechanosensitive channels (MscL) are bacterial membrane proteins that open with increased membrane tension and are reconstituted into vesicles using an in-house Hela-based cell free expression system. When the cell free expression components are encapsulated inside the lipid vesicles, we have shown promising results in insertion into the lipid bilayer membrane. When membrane tension is applied to the lipid vesicles by micropippette aspiration, MscL responds and allows influx of ions and small molecules, which can serve as second messengers for biochemical reactions. One potential application of our design is for building artificial platelets. When natural platelets are activated, they expose their lipid phosphatidylserine (PS) to the outer leaflet. Thus, coupling a mechanical input of fluid shear stress to a biochemical output of vesicle fusion to expose PS is an essential step towards building artificial platelets. Even though this project is at its beginning stage, the success of the ultimate goal to synthesize artificial platelets will have a paramount significance in medicine and biosensing.

Engineering Artificial Mechanosensitive Cells by Combining Cell Free Expression and Ulthrathin Double Emulsion Template

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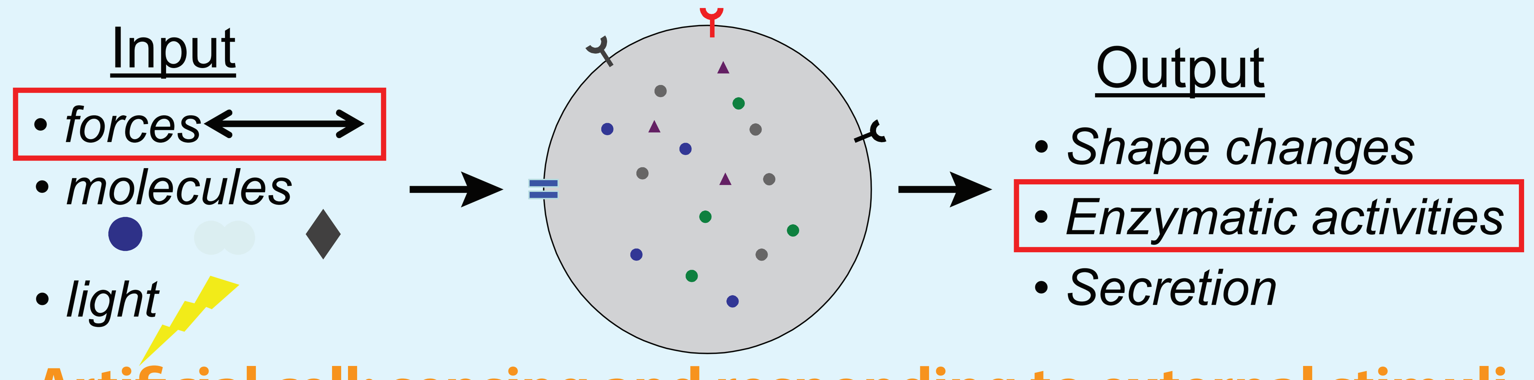
Abstract

Bottom up *in vitro* reconstitution is a general approach that provides the possibility to design and construct an artificial cell which can sense and respond to external stimuli. Active biological ingredients encapsulated into a synthethic vesicle would form an artificial cell which imitates one or more behaviors of a cell.

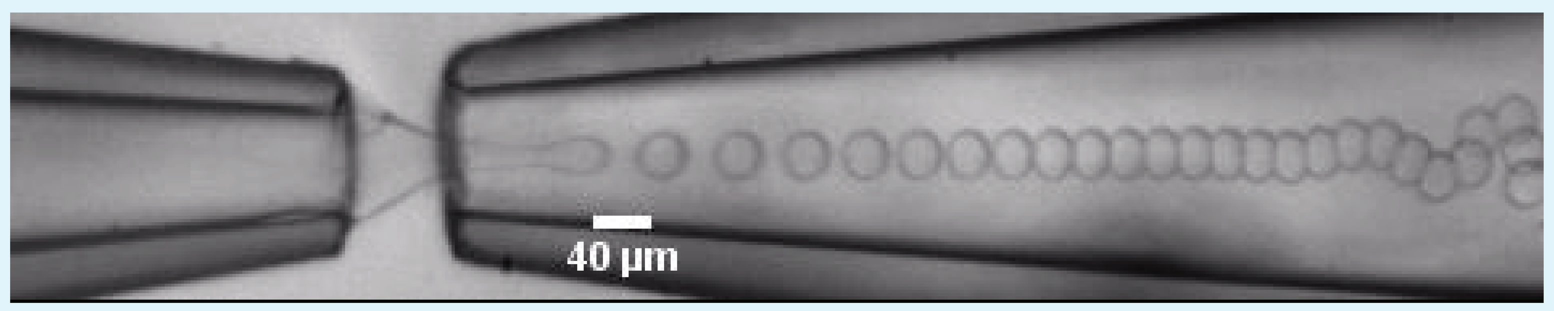


Artificial cell: sensing and responding to external stimuli

A glass capillary microfluidic device is used to generate double emulsion droplets with an ability of forming lipid bilayer with encapsulation capability. Mechanosensitive channels (MscL) are reconstituted into vesicles using an in-house Hela-based cell free expression system (CFE). When the CFE components are encapsulated inside the lipid vesicles, we have shown promising results in insertion into the lipid bilayer membrane. When membrane tension is applied to the lipid vesicles by micropippette aspiration, MscL responds and allows influx of ions and small molecules which can serve as second messengers for biochemical reactions. One potential application of our design is to build artificial platelets. Eventhough this project is at its beginning stage, the success of the ultimate goal will have a paramount significance in medicine and biosening.

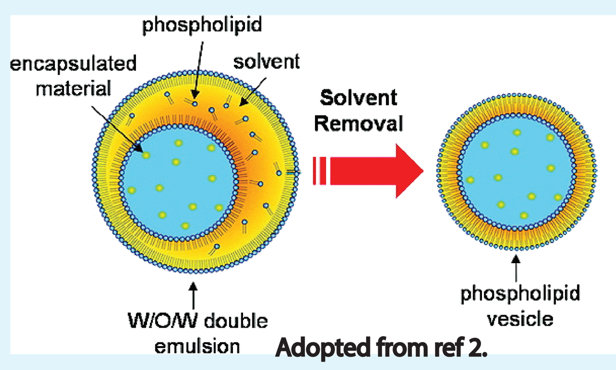
Double Emulsion Template

Double emulsions form when three phases flow into the device using syringe pumps. Aqueous-in-oil-in-aqueous double emulsions are generated using a glass micro-capillary microfluidic device that combines a co-flow and a flow.

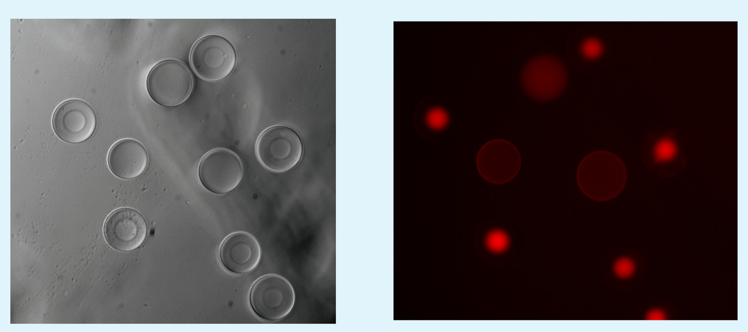


Ultra-thin double emulsions with size of 40 μm forming in the collection tube, captured by high speed camera.

The inner phase is an aqueous solution of model encapsulant, and the outer phase is aqueous phase of nonionic surfactant. The middle phase is a solution of phospholipids dissolved in a mixture of volatile organic solvents. Upon evaporation, or dewetting, lipid vesicles form.



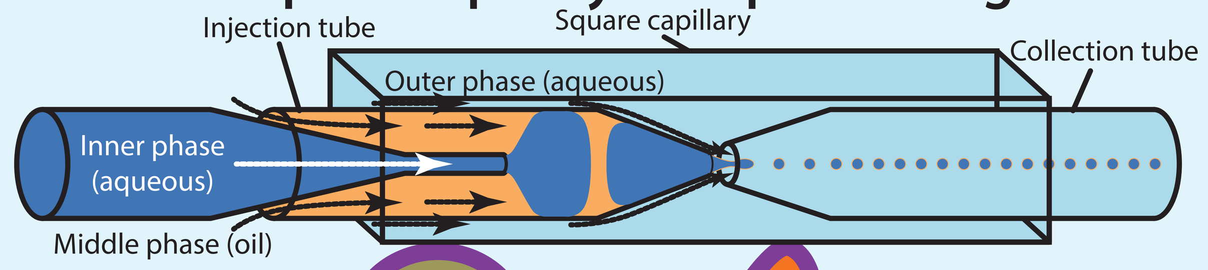
Double emulsions with lipids dissolved in middle volatile oil phase form lipid vesicles after oil evaporation.



Double emulsions with lipids dissolved in middle volatile oil phase form lipid vesicles after dewetting.

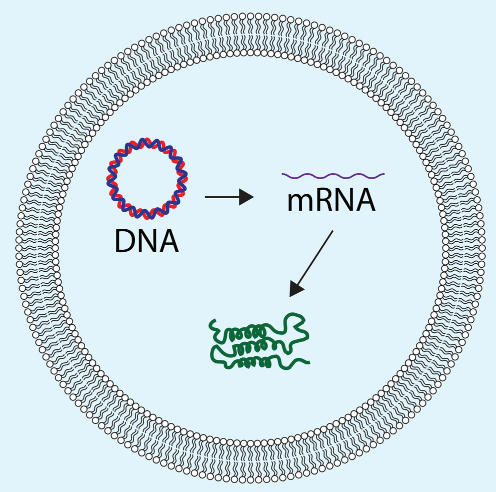
Microfluidic Device

Three round glass capillaries (inlet, injection and collection capillaries) are inserted in a square capillary with precise alignment and appropriate surface treatment.

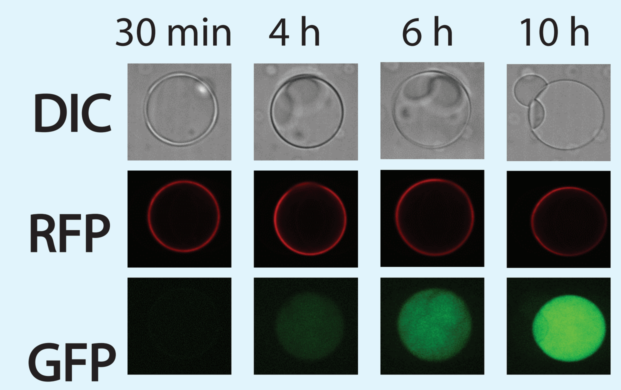


Cell-Free Expression System

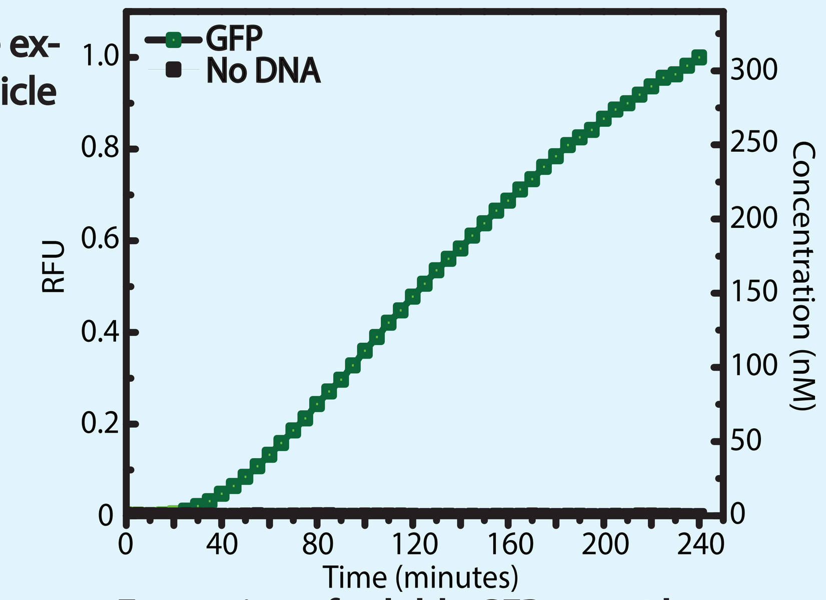
HeLa-based cell-free expression system can be used to express membrane proteins. The plasmid DNA is transcribed into mRNA and the mRNA is translated to form protein in a single reaction mix.



Encapsulation of the cell-free expression system in a lipid vesicle to express protein.

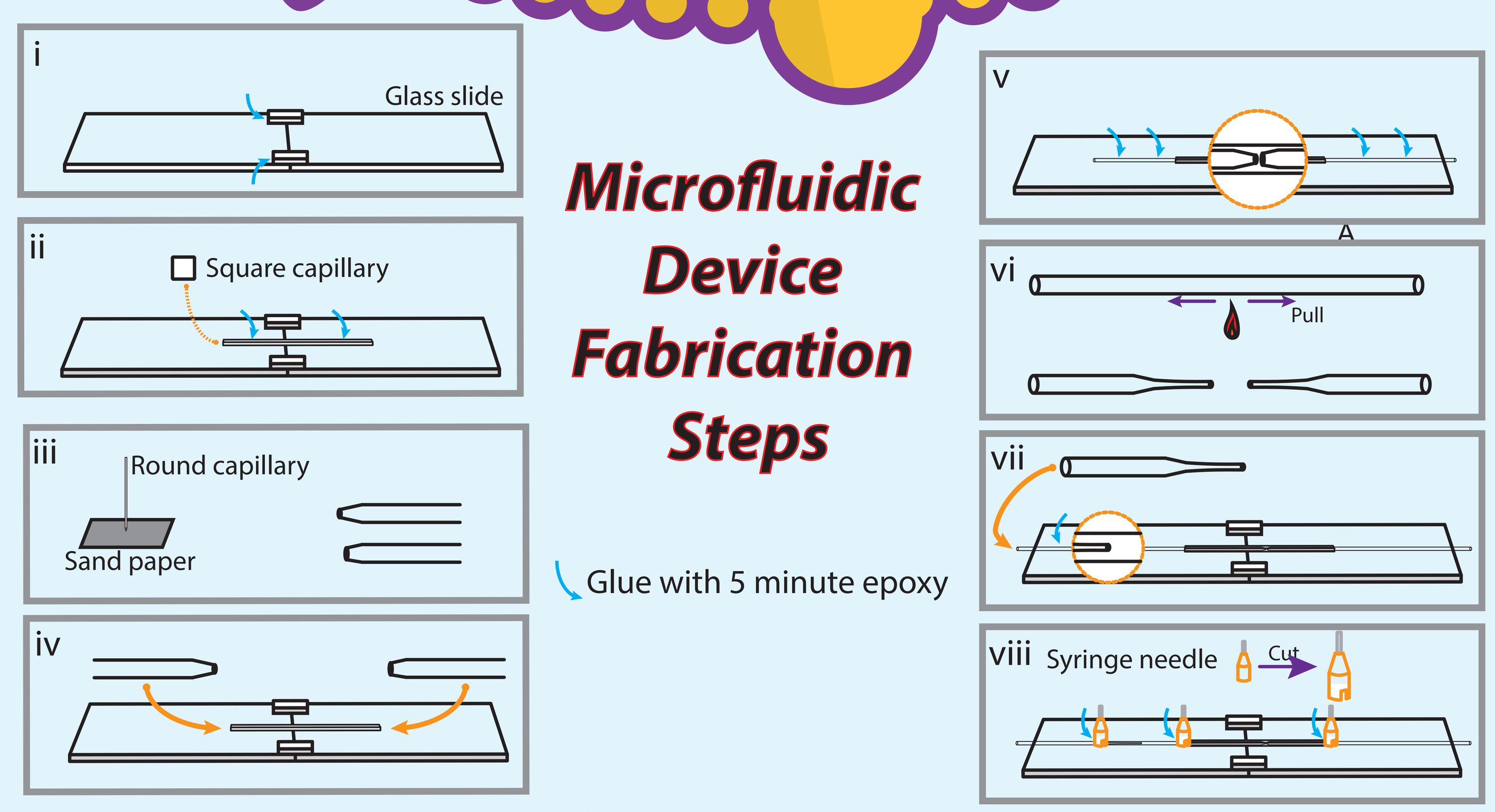


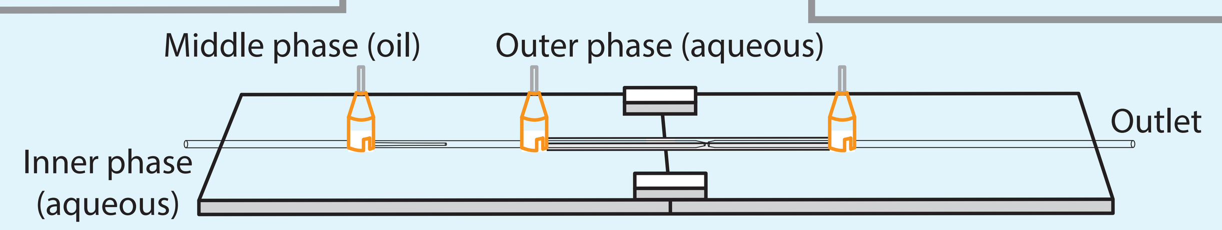
Generation of green fluorescent proteins observed, when CFE was encapsulated



Expression of soluble GFP over 4 hours at 32˚C measured by a plate reader

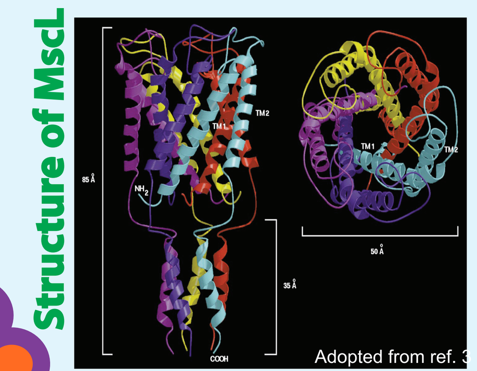
Microfluidic Device Fabrication Steps



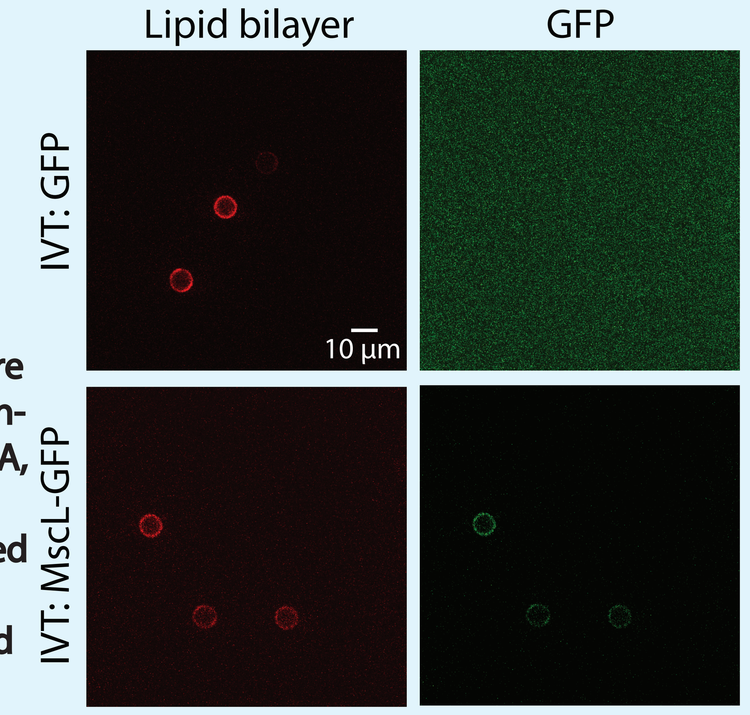


Reconstituting MscL

Mechanosensitive channels are bacterial membrane proteins that open with increased embrane tension and reconstituted into vesicles using an in-house Hela-based cell free expression system.

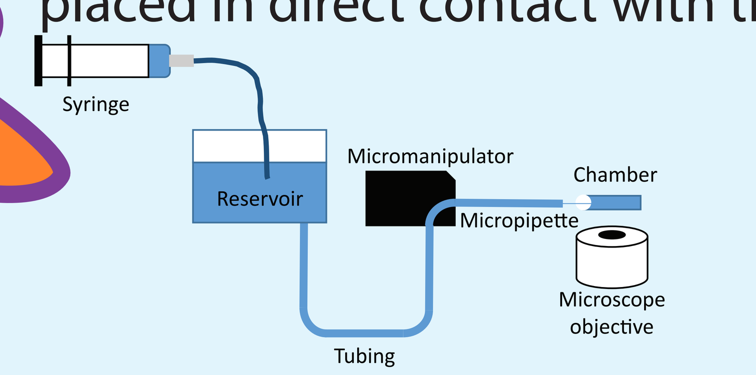


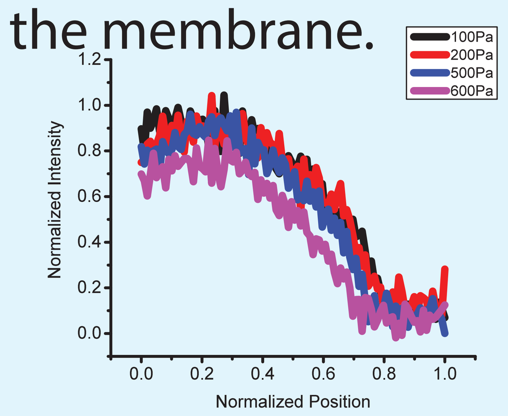
When lipid coated beads are incubated with CFE that contains MscL-eGFP plasmi DNA, a green fluorescence signal shows up on the lipid-coated beads which indicates that MscL is successfully inserted into the lipid membrane.

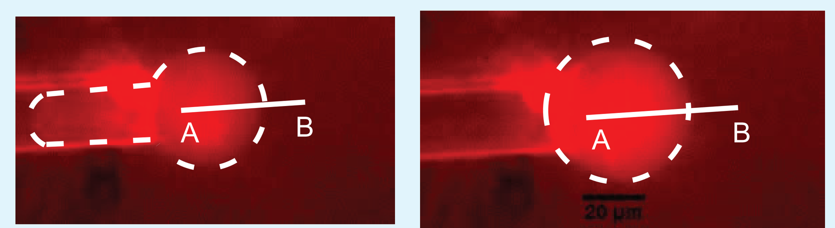


Applying Membrane Tension

Micropippette aspiration is used to apply membrane tension to lipid vesicles. This is done by precisely controlled suction through a glass capillary placed in direct contact with the membrane.







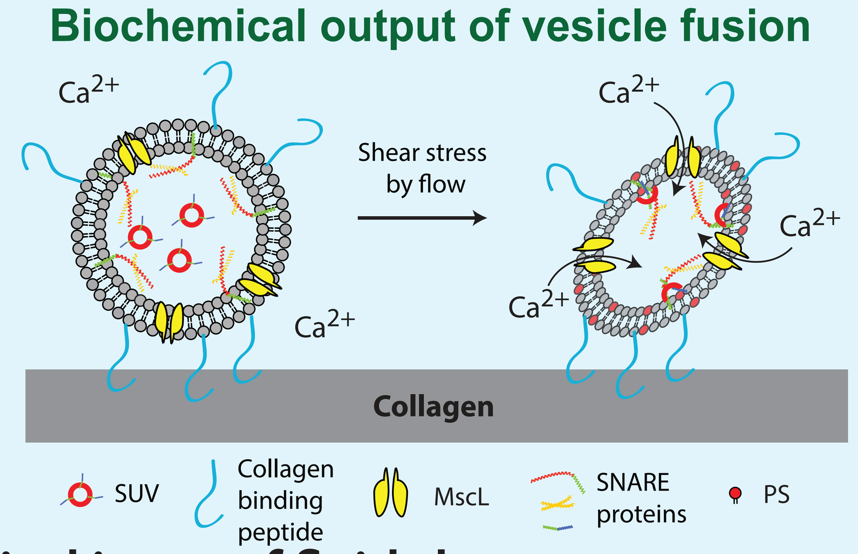
600 Pa,4.5 mN/m — 100 Pa, 0.75mN/m

We encapsulated 1mM calcium ions and 5uM calcium indicator (Rhod-2). With increased membrane tension, a decrease in fluoresence intensity is observed.

Future Plan

The ultimate goal of our design is to build artificial platelets. When natural platelets are activated, they expose their lipid phosphatidylserine (PS) to the outer leaflet. Thus, coupling a mechanical input of fluid shear stress to a biochemical output of vesicle fusion to expose PS is an essential step towards building artificial platelets.

Biochemical output of vesicle fusion



Reference

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