CARBON NANOTUBES AS SYNTHETIC ION CHANNELS
Luke Drnach, Michelle Guaragno, Steven Little
Swanson School of Engineering, McGowan Institute for Regenerative Medicine

INTRODUCTION
Cystic fibrosis is an ion channel disorder that affects the lives of over 30,000 Americans [1] with an additional 2,500 infants diagnosed every year in the U.S. alone. Caused by a genetic mutation that results in a loss-of-function of a chloride channel, cystic fibrosis is often characterized by a build-up of mucus in the linings of the lungs and the digestive tract. Symptoms include persistent coughing, malnutrition, and shortness of breath [2]. Severe lung infections are often comorbid with cystic fibrosis and, together with lung damage, are the main cause of death in patients with the disorder. Current treatments for cystic fibrosis include antibiotics for infections, diet and exercise for malnutrition, and mechanical loosening and clearing of the mucus in the lungs [3]; however, no current therapy effectively treats the underlying genetic mutation or the defect in the ion channel.

Carbon nanotubes have already gained much attention for their exceptional electrical, mechanical, and thermal properties [4] and additional efforts have been made to investigate the use of carbon nanotubes for biological applications, such as tissue engineering [5] and drug delivery [6]. Work in our lab focuses on applying carbon nanotubes (CNTs) to develop synthetic ion channels for treatment of ion channel disorders such as cystic fibrosis. Previous works in our lab have modeled the incorporation of short CNTs into liposomal membranes to form transmembrane channels [7], and other works have also modeled transport of specific ions through the interior of the nanotube [8].

We intend to build on these works to develop nanotubes that permit ion transport across biological membranes, thus acting as synthetic ion channels.

OBJECTIVE
The objective of this study was to demonstrate that carbon nanotubes can act as synthetic ion channels across model cell membranes by increasing the rate at which ions are transported across the membrane. Ion sensitive fluorescent dye were used to detect the presence of ions.

HYPOTHESIS/SUCCESS CRITERIA
In order for this study to be successful, three criteria must be met. First, the liposomes must encapsulate the fluorescent dye and any dye that remains outside the liposomes must be removed. Second, the carbon nanotubes must increase the rate at which ions move across the liposome membrane. Since the concentration of ions within the liposome is directly proportional to the fluorescence intensity of the dye, the carbon nanotubes must increase the rate at which the intensity of the dye changes (p<0.05 for initial difference). Finally, the liposomes must be scaled up to the size of a cell (>1 μm in diameter) to better model the interactions between the nanotubes and the cell.

METHODS
Liposome Preparation
Liposomes were prepared by dissolving phosphatidylcholine and cholesterol in a 7:3 mole ratio in 1mL diethyl ether and drying under nitrogen gas to form a lipid film. For liposomes with CNTs, the CNTs were added to the diethyl ether before adding the lipids. The films were frozen in liquid nitrogen and lyophilized for at least 12 hours. Liposomes were formed by adding buffer to the film to make a 10mg/mL stock solution and allowing the liposomes to swell on a shaker. For liposomes with calcein, the films were swelled in 50mM calcein in 1:4 (vol/vol) 1M NaOH:Hepes buffer (145mM NaCl, 2.5mM Hepes). For liposomes with HPTS, the films were swelled in 1mM HPTS in 100mM NaCl, 10mM Hepes, and 5mM Na2O3S2.

After swelling, 1mL of the liposome stock solution was subjected to 5 cycles of freezing in liquid nitrogen and thawing in 25°C water. The sample was then sonicated with a probe tip sonicator at 20% for 10 minutes. 500 μL of the sample were loaded onto a Sephacryl S-300 column and eluted with the additional buffer. Fractions were collected every 2 minutes and subjected to fluorescence spectrophotometry (λex= 470nm, λem=521nm for calcein; λex= 454nm, λem=512nm for HPTS).

Fluorescence Testing
For liposomal fractions with calcein, 50μL of a 100mM CoCl2 were added to 50μL of the liposomal fraction and the fluorescence intensity was recorded (λex= 470nm, λem=521nm). For fractions containing HPTS, 30μL of 0.5M NaOH and 30μL of buffer were added to 30μL of the liposomal fraction and the fluorescence intensity of the solution was recorded (λex= 454nm, λem=512nm).

Giant Liposomes
300μL of a 20mg/mL phosphatidylcholine in ether solution were poured down the side of a PDMS coated glass slide three times. The slide was open to the atmosphere and allowed to dry in a Petri dish for at least 24 hours. 3mL of deionized water
were added to the dish to cover the slide and the slide and water were heated to 50°C for 3 hours. 5μL of the resulting solution were imaged under a Nikon inverted light microscope.

**RESULTS**

![Fluorescence Intensity: HPTS Lipsomes](image)

Figure 1: Mean fluorescence intensity measurements of liposomes with CNT (red) and without CNT (blue) and HPTS dye under neutral and basic conditions. Measurements taken with a spectrophotometer with an excitation wavelength of 454nm and an emission wavelength of 512nm. 5 trials were conducted and standard deviations from the mean are shown.

In the case of both calcein and HPTS, the graphs of intensity against eluted volume displayed two peaks (data not shown), indicating that the liposomes encapsulating dye had been separated from the free dye. The results of the fluorescence intensity tests of HPTS are illustrated in Figure 1. In every trial, the intensity of the liposomes after addition of NaOH was greater than that of the liposomes without NaOH, regardless of the presence of CNTs. Averaged over five trials, the difference between groups was not statistically significant (p>0.05). Data for the fluorescence testing of liposomes with calcein is not shown.

Figure 2 shows the results of the formation of giant liposomes. The diameter of the liposome in the image was estimated as 100μm.

**DISCUSSION**

The objective of this project was to demonstrate that carbon nanotubes can increase the rate of ion transport across a model cell membrane using liposomes as the model membrane. To that end, liposomes encapsulating an ion-sensitive fluorescent dye – either HPTS or calcein – were prepared with or without carbon nanotubes present in the membrane. It was assumed that the dyes could not exit the liposome via the nanotube. The liposomes were successfully separated from any dye outside the liposome via chromatography to ensure that any changes in fluorescence corresponded to the net movement of ions across the membrane.

The separation was indicated by the presence of two distinct peaks in fluorescence intensity when measured against eluted volume and was successful for both dyes.

Transport of ions across the membrane was measured after the addition of the ions via fluorescence spectrophotometry. For liposomes encapsulating HPTS (Figure 1), intensity increases after the addition of NaOH; however, the intensity increases for liposomes with and without CNTs. This result suggests that equilibrium across the membrane is reached more rapidly in the absence of CNTs than is measurable by the instrument. Further studies into the precise time course of fluorescence intensity changes are required to demonstrate that CNTs increase the ion transport kinetics. Similar results also hold for liposomes encapsulating calcine (not shown).

Giants liposomes were successfully formed via a gentle swelling method at slightly elevated temperature. Images of the giant liposomes were compared to those in the literature to validate the formation of liposomes [9]. Circular structures with diameters greater than 1μm were observed, thus demonstrating successful formation of giant liposomes.

One major limitation in the study was the large variability in the fluorescence testing for ion transport. Large variations in the amount of dye encapsulated across trials resulted in high standard deviations in the control and test group fluorescence intensities, rendering the data statistically not significant (for comparisons between any two groups, p>0.05). However, in all trials with HPTS, an increase similar to that in Figure 1 was observed after the addition of NaOH, indicating that ion transport was occurring in every trial.

In order for this study to be considered successful, all three success criteria must have been met. In considering the results thus far, further studies into the precise kinetics of ion transport must be performed to meet the objective. Future studies can focus on meeting this requirement; after this criterion is validated, experiments can focus on studying the effects of CNTs in living cells.

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**REFERENCES**


