# Enabling Biofuels From Microalgae:

## Widening the Bottleneck in Research by Using a Gene Delivery Platform with Superior Efficiency and Throughput

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## Background and Motivation

### Why microalgae?

$CO_2 + H_2O$ + nutrients + ☀ → **Biofuels**, **Pharmaceuticals**

Microalgae can be used to produce carbon-neutral biofuels (diesel, gasoline, jet fuel, butanol, ethanol, methane, hydrogen) and pharmaceuticals (proteins, drugs) without competing with food resources for arable farmland.

### What is the holdup?

Researchers need to *genetically engineer* superior strains of microalgae, but gene delivery is a bottleneck in the research effort. Microalgae have a **cell wall** that impedes delivery.

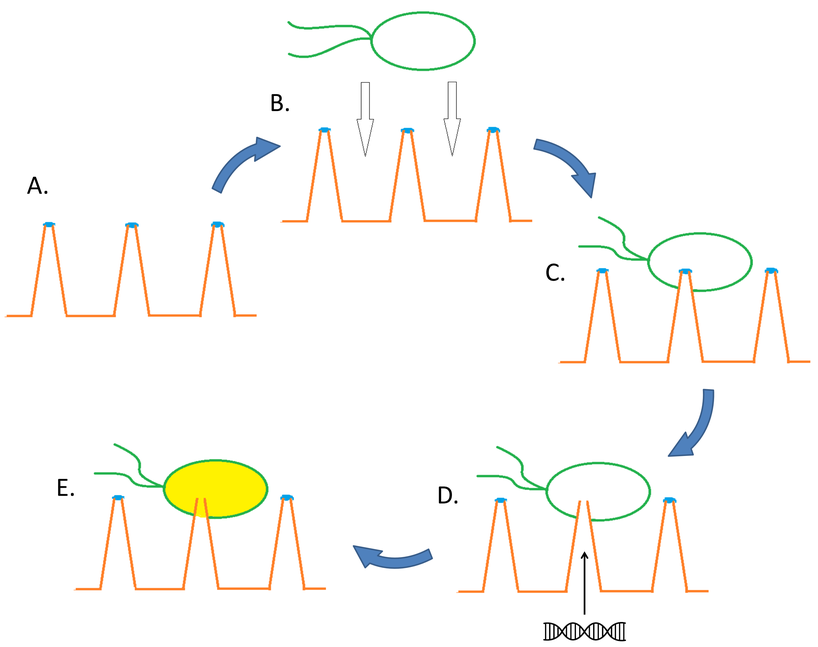
### Current technology

- Difficult setup
- Time consuming
- Low-throughput
- Requires algae pretreatment
- Specialized equipment

### Our unique approach

- Simple setup
- Quick
- Hundreds of samples per experiment
- No pretreatment
- No specialized equipment

## The Novel Gene Delivery Approach for Microalgae



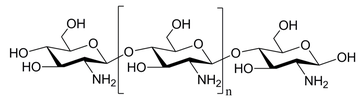

**Scheme 1. Gene Delivery Using Hollow Microneedle Arrays.**

A) Gold microneedle array with degradable cap.
B) Microalgae are pierced by centrifugal force.
C) Intracellular molecules degrade the cap.
D) Genetic material diffuses through the needles and into the cells.
E) Exogenes are incorporated into the cells' genomes and expressed.

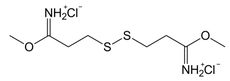
## Experimental: Degradable Film Development

The focus of this presentation is our study to develop a biocompatible cap or plug for the microneedles that will selectively degrade within the microalgae.

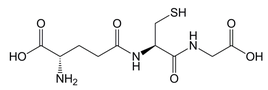
Chitosan
a biocompatible polymer



Dimethyl 3,3´-dithiobispropionimidate•2HCl (DTBP)
cross-linker that reacts with primary amine groups at pH 8-9



Glutathione (GSH)
intracellular molecule that reduces (cleaves) the disulphur bond of DTBP





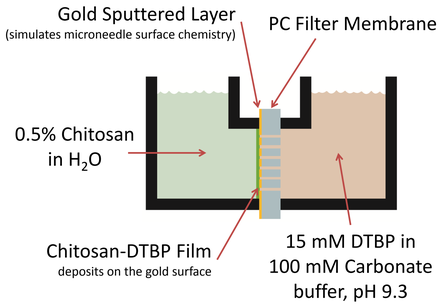
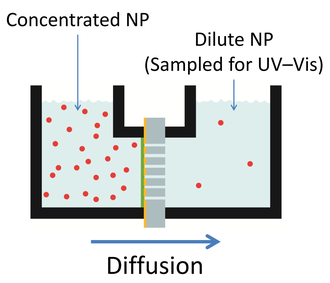



**Figure 1. Chitosan-DTBP Film Formation on Polycarbonate Filter Membranes.** After sputtering gold onto one side of the membrane, the polycarbonate (PC) filter membrane is placed in a custom-built U-cell. A solution of 0.5% (w/v) chitosan is added to the half-cell on the gold-sputtered side, and a solution of 15 mM DTBP in a pH 9.3 buffer is added to the other half-cell. After 12 hours, a cross-linked gel is found on the sputtered surface of the membrane (the chitosan-DTBP film).

## Results: Nanoparticle Diffusion Across Membrane



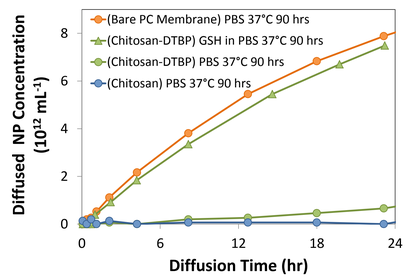




**Figure 2. Diffusion of Gold Nanoparticles (NP) Across Membranes.** Nanoparticle diffusion was used to demonstrate the ability of the chitosan film to plug micro-channels, and its degradation by GSH molecules. Diffusion of 5 nm gold nanoparticles was monitored in time using UV-Vis spectrometry at a peak absorbance of $\lambda_p$ = 515 nm. Both chitosan and chitosan-DTBP films were effective at inhibiting nanoparticle diffusion. After a chitosan-DTBP film was treated with 10 mM GSH in 10 mM phosphate buffered saline (PBS) at 37°C for 90 hours, the rate of diffusion was nearly identical to that of a membrane that had no film, indicating successful decomposition of the membrane by GSH.

## Results: Effect of GSH exposure to Chitosan-DTBP Films

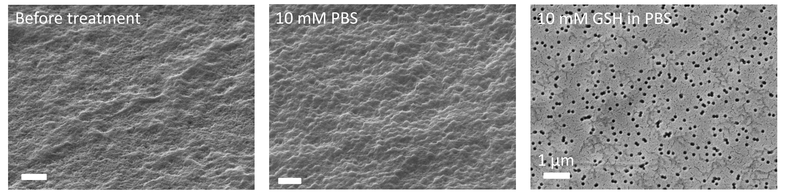



**Figure 3. FE-SEM Images of Chitosan-DTBP Films After Solution Treatment.** Field-emission scanning electron micrographs (FE-SEM) of chitosan-DTBP film deposited on gold sputtered PC filter membranes with 100 nm diameter pores. There was no noticeable change in the film coverage or morphology after a 90-hour treatment in PBS at 37°C. After treatment with glutathione in PBS at 37°C for 90 hours, FE-SEM revealed the porous feature of the underlying PC filter membrane, indicating the GSH-induced total dissolution of the Chitosan-DTBP film. All scale bars represent 1 µm.

## Results: Microneedle Arrays and Microalgae

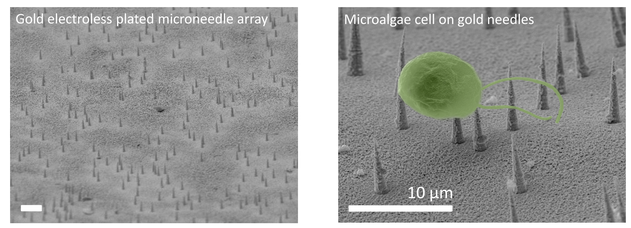



**Figure 4. FE-SEM Images of Gold Needle Arrays, Microalgae.** Field-emission scanning electron micrographs of gold electroless plated microneedle arrays (left) prepared in our lab, and microalgae centrifuged onto a needle array (right). These needles successfully interfaced with the microalgae without breakage. Currently, the prepared microneedles have closed, solid tips. Both scale bars represent 10 µm.

## Conclusions

- Chitosan-DTBP gels can cover PC membrane pores and successfully reduce the diffusion of 5 nm nanoparticles
- Treatment by GSH effectively degrades the chitosan-DTBP film

## Future Work

- Develop hollow micro-needle arrays
- Incorporate degradable chitosan plug on hollow microneedle arrays
- Optimize efficiency of gene delivery

## Acknowledgements

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