Project Proposal

Project Title: Analysis of the Taxis of *Escherichia coli* and *Chlamydomonas reinhardtii* Hybrids

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Project Description:

The aim of this project is to optimize the movement of the drug-carrying nanovehicle in a targeted drug delivery system using biohybrid microswimmers. These structures will be driven by a single encapsulated *Escherichia coli* cell coated with microalgae *Chlamydomonas reinhardtii*. This project aims to explore chemotaxis and phototaxis as a method of directional movement without the use of external machinery or electromagnets.

Researchable Question:

How is the movement of Escherichia coli and Chlamydomonas reinhardtii hybrids affected by the use of both a light source and chemical attractants bicarbonate and d-galactose?

Hypothesis:

If C. reinhardtii is used to form a biohybrid structure which is currently only motile through the natural fine movement of E.coli, the rate of movement would increase and will have a more directed path if using phototaxis and chemotaxis as a transport mechanism than if using chemotaxis alone.

Background:

Many medications, including chemotherapeutic drugs, are either taken orally or through intravenous therapy (IV). However, drugs such as these are to be taken over a long period of time and often cannot be transported directly to the target site, thereby reducing the drug's efficiency. This is because, if taken orally, the drug has to go through the digestive system before being absorbed into the bloodstream. If taken through an IV, the drug can bypass the metabolic and digestive system, but it still has to be circulated through the bloodstream before reaching the target site. Cancer therapeutics in particular have an infusion time of up to 24 hours. Furthermore, some therapeutics can inflict severe adverse effects, such as blood clotting and hypertension. These adverse effects are due to the drug or therapeutic's contact with healthy cells while in the bloodstream.

To address these problems with standard drug delivery, scientists have developed motile structures called "biohybrid microswimmers", which are microorganism-driven capsules that are being studied as cargo-carriers in a targeted drug delivery system. These structures are able to both mimic natural microorganism functions and be physiologically, chemically, and genetically modified to suit a particular need, such as locating and destroying cells (Wang et al., 2022). For this reason, biohybrids are studied in the biomedical field as nano-vehicles that can transport cargo, such as a chemotherapeutic, directly to the target site to avoid harming healthy cells and to achieve a faster infusion rate (Shao et al., 2016).

Biohybrids have been developed and modified in many studies to achieve biased movement towards a substance. This was done by exploring different types of microorganism movement.

Biohybrids utilize the natural properties and movement of microorganisms to move from one place to another. Microorganisms, the cells used as “engines” in biohybrids, can move in two ways. Fine movement is achieved by the natural motility of the cell, and coarse movement is achieved through an external stimulus that the organism is responsive to. For example, bacteria naturally move in a breaststroke-like movement called *ultrasonic propulsion*, pulling liquid from the front of the cell to its side. However, this movement is oscillating and uncontrolled. For this reason studies have been done on the coarse movement of microorganisms; that is, the movement of microorganisms towards or away from an environmental stimulus. For example, bacteria tend to exhibit a biased movement in the direction of a favorable chemical. (Shchelik, 2021). Biohybrids that utilize natural cell motility methods, such as the tendency of bacteria to move towards a chemoattractant, are capable of movement without the aid of an external mechanism, so pairing medications with microswimmers could result in a more efficient, economically friendly, and less invasive drug delivery. Targeted drug delivery has also been proven to reduce adverse side effects because of the specific nature of the delivery system, which prevents the drug from harming healthy cells outside of the target site (Shao, 2016).

Several methods have been used to create biohybrids. Stanton et. al. (2017) designed a structure consisting a conical microtube fabricated with a biopolymer and lined with a bacteria-attractant polydopamine layer (Stanton, 2017). The polydopamine layer traps *E. Coli*, the single-cell “driver” of the microswimmer, and restricts its flagella. This results in a more unidirectional movement when compared to other biohybrid designs, in which the microorganism engine is placed outside of the nonliving material. The biohybrid was then coated in magnetic particles and placed in a magnetic field which controlled its movement. This greatly increased the directionality of the biohybrid and eliminated any oscillating movement. One of the biggest drawbacks of this study is that attaching magnetic particles to this biohybrid system results in the production of large dense microparticles whose movement is impaired. Due to the density of the microtube, further research will have to be done on how to pair the microbots with a medicinal drug, which will further add to its mass.

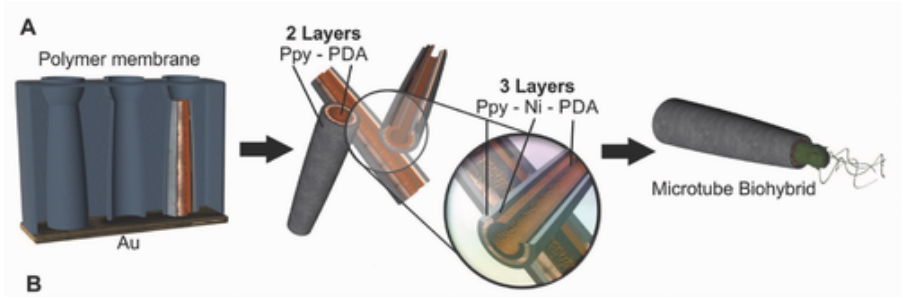


Figure 1: Assembly of *E. coli* biohybrid

The construction of the biohybrid- a nanotube lined with polypyrrole traps the *E coli* bacterium and restricts its flagella (Stanton, 2017)

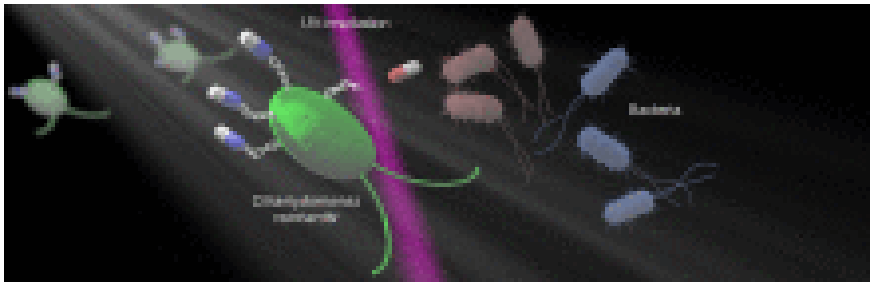
While *E. coli* is a useful tool to provide motility to cargo-carrying biohybrids, the toxicity of this bacteria to mammalian cells and tendency to multiply is not favorable in a living mammal. An alternative “engine” that scientists have studied is microalgae.

The property of *Chlamydomonas reinhardtii,* a biflagellate algae, to respond to environmental conditions has been studied in many cases. Microalgae has the ability to be motile in response to chemical gradients (a process called chemotaxis) or a light source (a process called phototaxis) (Choi, 2016) (Ueki,

2016). *C. reinhardtii* also has an autofluorescence property and a natural propulsion, which is achieved by pulling fluid from the cell's front around the cell. Microalgae, unlike bacteria, are also less likely to multiply when not wanted or harm mammalian cells (Yasa, 2018). There have been multiple studies exploring the application of *C. reinhardtii* in the context of targeted drug delivery.

In a study by Shchelik et. al (2021), antibiotics were attached through covalent bonding to *C. reinhardtii* and could be released on demand upon detection of a pH gradient. The algae also responded to chemical gradients and other external stimuli (Shchelik, 2021).

Figure 2: Attachment of Antibiotics to *Chlamydomonas reinhardtii* (Shchelik, 2021)



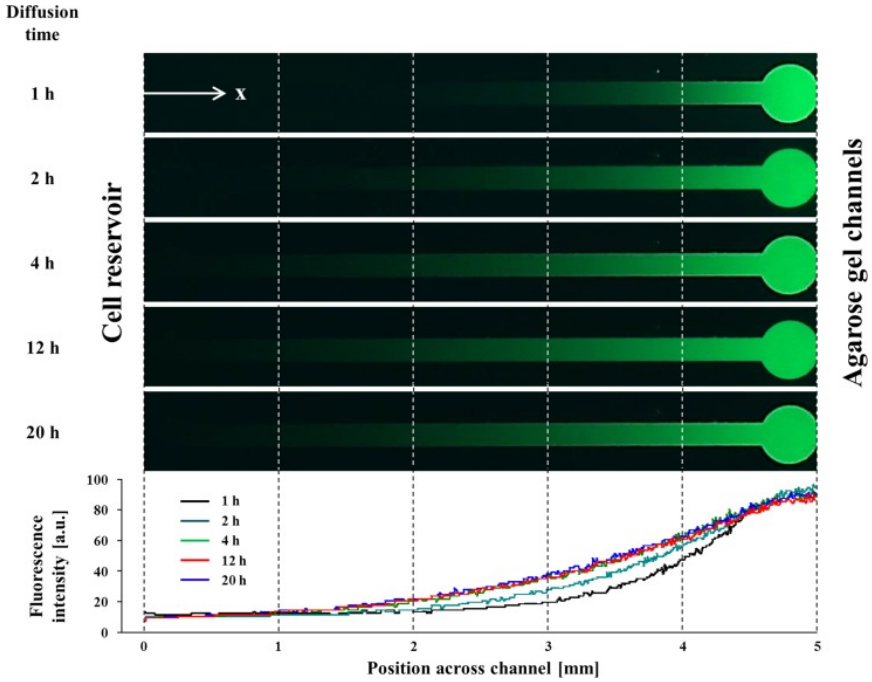
Antibiotics are attached to the microalgae cell surface through covalent bonding, and are released upon detection of a pH gradient (Shchelik, 2017)

One study by Yasa et. al. (2018) developed an algal biohybrid layered with multiple biopolymers: polystyrene and polyelectrolytes poly allylamine hydrochloride (PAH) and polysodium styrenesulfonate (PSS) in order to secure the algae and create a net charge that would attract magnetic particles. After magnetic particles were added to the algae, the biohybrid's coarse movement was measured in both a 2D and a 3D liquid environment subject to a 26mT magnetic field. This allowed the biohybrid to move much faster than it would have and with less oscillation that it would have without the presence of external forces. More research needs to be done on whether a biohybrid carrying magnetic particles is also able to carry cargo such as a therapeutic (Yasa, 2018).

A study by Shao et. al. (2016) involved the targeted drug delivery of chemotherapeutic drugs through a vehicle containing mesoporous nanoparticles that absorbed and released the drug when triggered by a chemical (pH) change. This study built on the structure of the biohybrid itself and successfully achieved the goal of reducing adverse effects in mammalian cells and attacking liver cancer cells in laboratory mice (Shao, 2016).

Some studies used cell migration assays to quantify the movement of *C. reinhardtii*. One study by Choi et. al. (2016) demonstrated the chemotaxis of *C. reinhardtii* to bicarbonate through a polydimethylsiloxane (PDMS) filter with agarose channels. The algae was placed into a reservoir containing a Tris-phosphate medium as a control solution that would not have an effect on the microalgal movement. At the end of the reservoir was bicarbonate, a chemical attractant of *C. reinhardtii*. Algal cells moved through five agarose channels held in place by a single-layer PDMS. This movement was quantified using a fluorescent microscope. The results showed that bicarbonate is a strong chemoattractant of *C. reinhardtii* and can contribute to the construction of a new type of biohybrid (Choi, 2016).

Figure 3: Movement of *C. reinhardtii* to bicarbonate through agarose channel



The images above show the migration of *C. reinhardtii* over multiple time stamps. After 12 hours, almost 80 microliters migrated through the channels (Choi, 2016).

This project will attempt to optimize the directional movement of a biohybrid structure by exploring its movement when exposed to both a chemical attractant and light source compared with only a chemical attractant. This will be modeled through a cell migration assay that contains two channels; one with a chemoattractant diffused through the channel and the other with both a chemoattractant and an LED, thus making the LED the independent variable. The hypothesized result is that more bacteria/algae hybrids will move towards the channel with both a chemoattractant and a light source.

Experimental Design/Research Plan Goals:

Cells under a magnetic field can move at a rapid rate and retain their motility in solutions at different viscosities, but motility is only possible in a magnetic field and if the biohybrid has magnetic particles attached. Another way to achieve cell motility is through microalgae's natural phototaxis. Algae is less dense than nickel, the material used to achieve movement in a magnetic field, as algae is usually less than 1g per centimeter cubed and nickel has a density of around 8g per centimeter cubed. Thus, the biohybrid's movement would not be as impaired by extra magnetic particles.

By coating *E. coli* bacteria with *C. reinhardtii* cells, the biohybrid is hypothesized to increase efficiency due to the bacteria's natural movement and the algae's phototaxis, yet avoid adverse effects to organic solutions.

Material List:

- Freshwater *Chlamydomonas reinhardtii* and culture kit
    - Culture salts and nutrient solution
    - 12 hour timed lamp
- *Escherichia coli* K12 + GFP (Green fluorescent protein) and culture kit
    - LB nutrient broth / ampicillin
    - Incubator
    - Hot water bath
- 4mm scalpel
- stir/heat pad
- water-based 3% agar gel solution, injected into the channels
- Water-based 10% agar gel solution
- Small plates
- D-galactose
- Bicarbonate
- LED
- poly(allylamine) solution
- poly(sodium 4-styrenesulfonate) solution

Testing Strategy:

The biohybrid will be created by coating *E. coli* K12 + GFP plasmid bacteria in 6 alternating layers of polyelectrolytes, poly(allylamine) and poly(sodium 4-styrenesulfonate), in order to achieve a net positive charge coating the bacterium. This will attract and trap the microalgae and restrict the flagella of *E. coli*. The bacteria will then be coated in *C. reinhardtii* solution.

The phototaxis of the biohybrid will be tested using a cell migration assay. The system will contain a 3% agarose channel surrounded by agarose at a higher percent. Arabinose will be added to one side of the reservoir and left to diffuse throughout the system. A chemoattractant will be added to one end of the channel. From the linear agarose channel will branch an alternate channel at a distance from the bacteria's starting point. At the end of this branch will be a chemoattractant and an LED stimulus. Arabinose will be added in small quantities throughout the assay.

Step by step:

Culture *E coli*

Transform the bacteria- add the GFP plasmid with ampicillin resistance

Culture *C reinhardtii*

Grow *C. reinhardtii* under optimal conditions

Positive control: d-galactose solution (will guarantee movement) - *E. coli*
Positive control: LED light source - *C. reinhardtii*
Positive control: HCO3 - *C. reinhardtii*

Biohybrid synthesis:
Coat *E coli* with six layers of polyelectrolytes (PAH and PSS) alternatively
wash with NaCl between each step
Incubate in *C reinhardtii* solution between each step

Cell migration assay:
The plate will be filled with 10% agar. A 4mm channel and a 4mm branching channel will be carved out of the plate and filled with 3% agar.
6mg/mL of arabinose will be diffused throughout the assay, along with 10^-3 M D-galactose at the end of each channel.
The biohybrids will be placed at the end of the channel and left to migrate for 1 hour with no external light other than the LED.

Data collection:
A video will be taken for the length of the cell migration assay. After a video is taken, ImageJ software will quantify the pixel brightness of the biohybrids.

Risk/Safety Concerns:

Will be handling *E Coli* and *C. Reinhardtii*. Personal safety equipment, such as a lab coat, goggles, and gloves will be worn when handling the microorganisms. Any waste will be bleached and autoclaved at 121 degrees celsius and 15psi.
I will be working in a bio safety hood and wear personal protective gear such as goggles, a lab coat, and gloves.
I will be wearing safety glasses and gloves when handling Poly(allylamine) solution and Poly(sodium 4-styrenesulfonate).
I will dispose of any materials and clean any surfaces contaminated with Poly(allylamine) solution and Poly(sodium 4-styrenesulfonate) by wiping them with 70% alcohol.

Data Analysis:

The closer the biohybrids get to the source of the arabinose diffusion, the brighter they will glow. I can measure the targeted motion of the biohybrids by comparing its fluorescence with its position on the graph. If the slope of the graph is steeper when the biohybrids are moving towards the branching channel, it supports my hypothesis that multiple attractants increase the directionality of the biohybrids- ImageJ software and pixel intensity versus position on the cell plate

References: (In APA Format with in-text citations):

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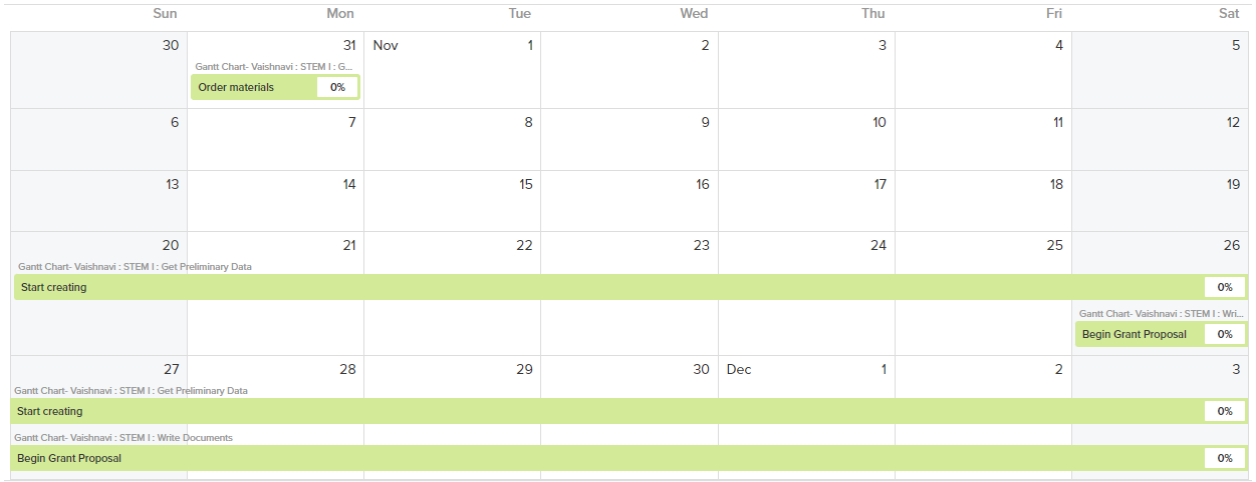
Timeline: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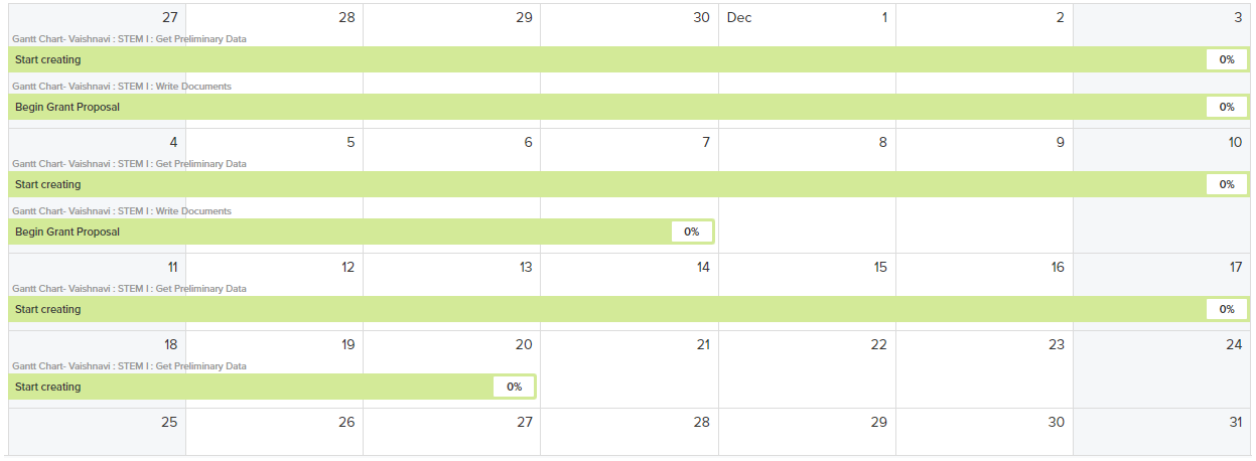
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