

PROTOTYPE CHAMBER FOR EVALUATING THREE-DIMENSIONAL INTESTINAL UPTAKE

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ABSTRACT

Biological systems are complex and highly intertwined, therefore, accurate quantification of information, such as membrane transport, is difficult. An important area of interest involves the transmembrane transport in the intestinal tract. In order to minimize variables and interferences, in vitro testing is used. An intestinal chamber was designed to collect data regarding the nutrient uptake through the epithelial cells of intact intestinal tissue of rainbow trout. In order to collect this data, a chamber prototype was designed to hold a cylindrical piece of living tissue. The outside of the intestine, the smooth muscle, is bathed in one media and an alternative media is delivered to the inside of the intestine, the epithelial cells. Thus, the transport of ions, nutrients, and drugs across various epithelial tissues is measured in a controlled environment.

INTRODUCTION

The intestinal tract of rainbow trout (Figure 1), and many other vertebrate organisms, includes an outer layer of smooth muscle cells and an inner layer of epithelial cells. Epithelial cells produce transporters which play a vital role in intestinal absorption. This is an essential process where nutrients such as amino acids, lipids, and carbohydrates are transported in and out of the epithelial cells (Figure 2). Organisms do this by building temporary molecular pumps which are implanted into the cell membrane to move large molecules from the intestinal tract into the bloodstream where they can be distributed throughout the body.

Ussing chambers are currently used to measure transmembrane transport. However, these chambers require the user to cut a small square of tissue from the organism. This causes significant damage to a large fraction of the cells and the tissue is viable for only 60-180 minutes. A chamber which houses a large piece of cylindrical tissue would allow cells to remain viable for an extended amount of time while achieving the same separation between cell types as an Ussing chamber. Additionally, novel research examining the relationship between the gut's microbiota and intestinal transport can be conducted by keeping these bacteria intact during experimentation.

In order to keep the cells viable and produce results comparable to an Ussing chamber, the smooth muscle must be bathed in one type of media at 18 °C with a turnover rate of approximately 15 minutes. An alternative media will be delivered to the epithelial cells. To further prolong the life of the cells, quick instillation of the tissue is vital. Finally, visibility of the attachment points will allow users to ensure the experiment was successful.

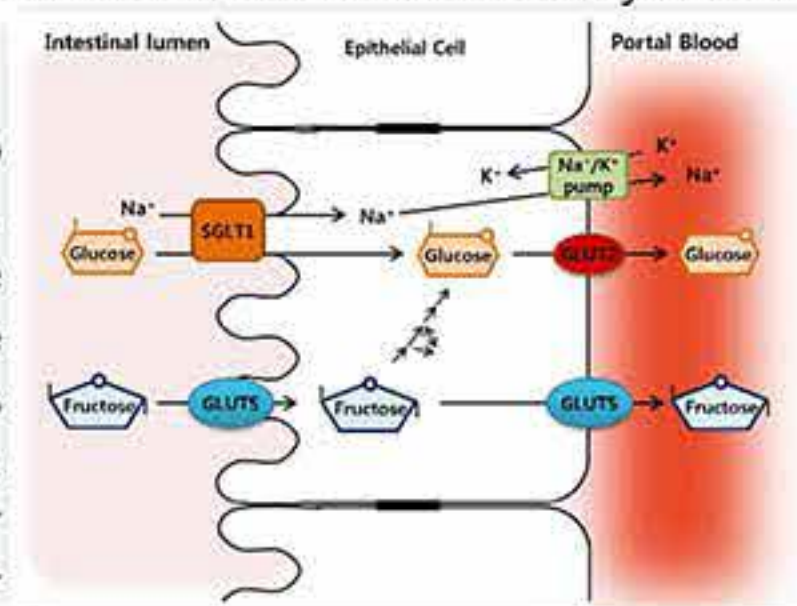


Figure 2: Intestinal transmembrane transport.

FLUID FLOW

To prolong the life of the tissue sample, it is vital that nutrients are delivered to the cells evenly. Additionally, any areas of stagnation may promote bacterial growth which would contaminate samples. Simulations were performed using SolidWorks 2019 to determine optimal placement of the inlet and outlet ports to maximize mixing around the tissue and minimize points of stagnation. Figure 3 shows simulation and port locations which provided the best results.

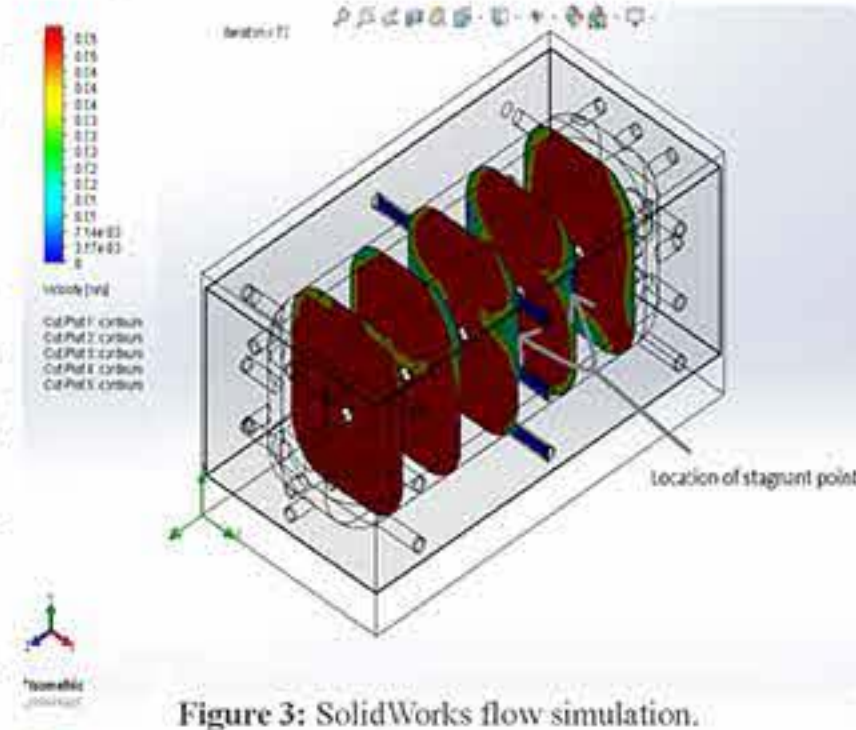


Figure 3: SolidWorks flow simulation.

MATERIAL AND METHODS

Polyoxymethylene (POM) was used to produce the chamber body for a variety of reasons. Most importantly, it is compatible with biological systems and will not negatively impact the tissue. It is also relatively inexpensive and easy to manufacture. Finally, POM produces a reusable chamber because it is durable and can be sterilized with steam or chemical treatments. Unfortunately, POM is opaque, therefore, a view port was designed to allow researchers to view the tissue and ensure it is properly attached throughout the duration of the experiment.

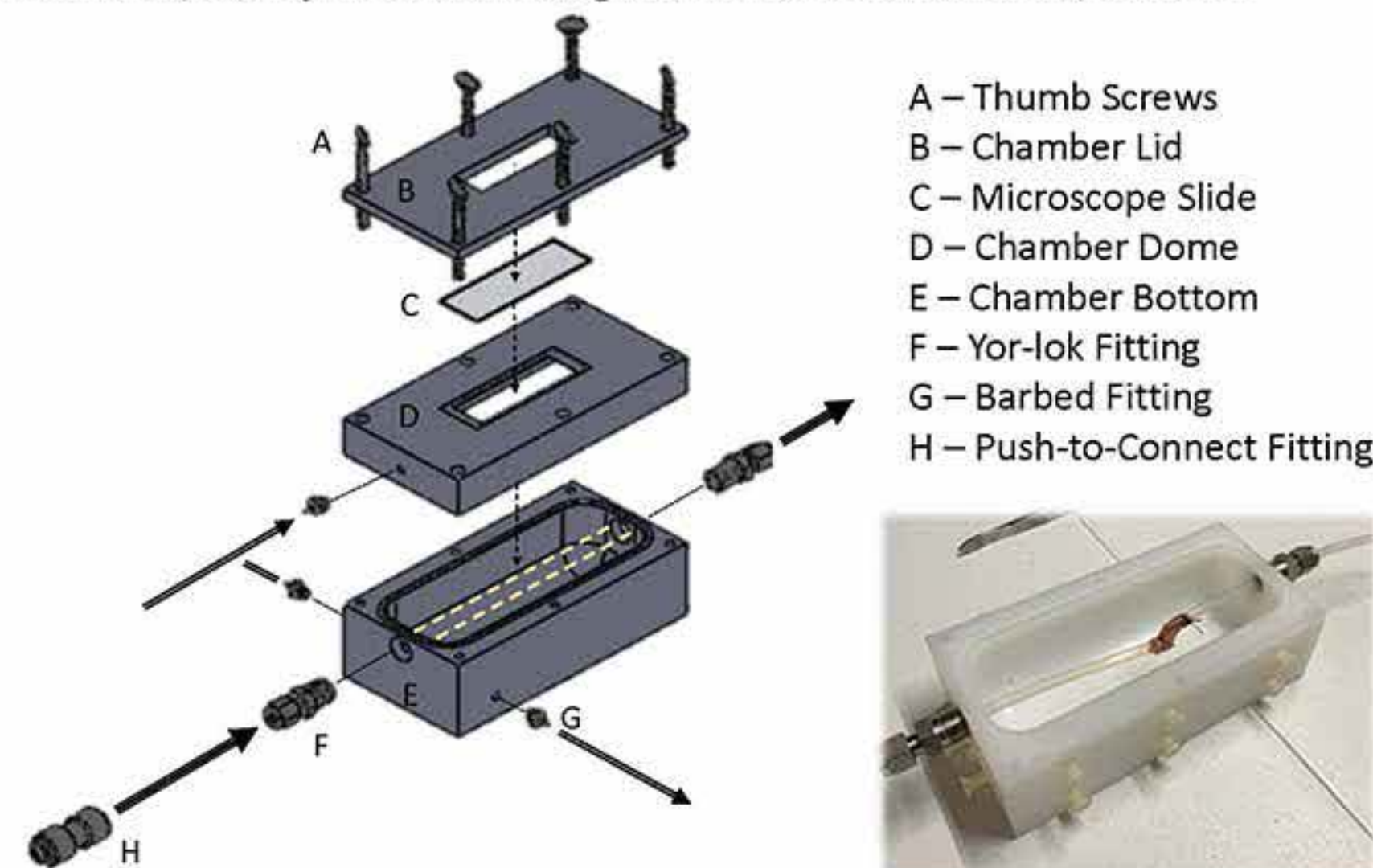


Figure 4: Chamber assembly pieces.

Three chamber components are made from POM. This configuration allows for easy access to the attachment points so that researchers can attach the tissue to the apparatus quickly to limit damage to the cells. Between each chamber component an O-ring is fitted during assembly to create a watertight seal.

Yor-lok fittings, which are mounted to the short sides of the chamber, allow nylon tubing to be passed through the wall of the chamber. This unique fitting produces a chamber which can accommodate various lengths of tissue.

Barbed fittings are used for the inlet and outlet ports for the outer media to quickly and easily press on silastic tubing.

Six thumb screws, installed vertically through the assembly, hold all the components together and allow the user to assemble and disassemble the chamber quickly without the need for tools.

ECONOMIC ANALYSIS

Ussing chambers sell for approximately \$1,200. In order to be competitive in this market, this chamber was priced at \$1,000. This chamber has a manufacturing cost of only \$423. At this price, assuming 30 units are sold per year, an expected pay-back-period is 3 years.

Table 1: Cost for product development.

Source	Hours	Price	Cost
Physical components	N/A	\$414.37	\$414.37
Manufacturing time	13.55	\$45/hr	\$609.75
Development team	1,250	\$40.73/hr	\$50,912.50
Total			\$51,936.62

Table 2: Cost for one full chamber.

Source	Hours	Price	Cost
Physical components	N/A	\$150.10	\$150.10
Manufacturing time	3.84	\$45/hr	\$172.80
Marketing and Distribution	10% of Revenue	\$100	\$100
Total			\$422.90

CONCLUSION

Successful implementation of this chamber will allow a deeper understanding in the biomedical field regarding irritable bowel syndrome, diabetes, and Crohn's disease, among others. Polyoxymethylene (POM) was used to manufacture the chamber components so it is compatible with steam and chemical sterilization. SolidWorks flow simulation were performed to determine the locations for the inlet and outlet ports and proper fittings were designed. All of these factors produced a chamber which will outperform Ussing chambers with regard to cell viability, experimental variability, and marketability.

FUTURE WORK

Design validation must be completed to ensure that the fully assembled chamber is watertight. Further testing to determine the duration of cell viability should also be done prior to marketing.

The chamber can be used to make measurements from tissue including stomach, large and small intestine, and gall and urinary bladder.

An amplifier and electrodes can be added to the chamber for diffusion- or electrophysiology-based measurements. Systems that consist of data acquisition hardware and software to record current, voltage and conductance/resistance can also be installed.

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