# The Cy5 labeled PEG-PLGA Nanoparticles have permeability through the Blood Brain Barrier

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# Abstract

We conducted an experiment using In Vitro Blood-Brain Barrier(BBB) model, and the Cells needed to generate the In Vitro BBB is human Cerebral Microvascular Endothelial Cells(hCMEC) and we used it to investigate the permeability of PEG-PLGA Nanoparticles through the Blood-Brain-Barrier. We developed a nanoparticle system for drug delivery across the blood–brain barrier (BBB). The nanoparticle consists of poly(lactide-co-glycolide)-poly(ethylene glycol)-poly(lactide-co-glycolide) (PLGA-PEG-PLGA). We tested the hypothesis "The intensity of the Nanoparticles in the lower chamber of the transwell will increase overtime which will indicate the permeability of Cy5-PEG-PLGA through the Blood-Brain-Barrier." To test the permeability, we seeded the HCMECs on the transwells with various pore sizes for last 4-5 weeks The calculation then didn’t significantly prove that the Cy5-PEG-PLGA can permeate through the BBB and their intensity increases overtime.

# Background

Blood vessels are critical to deliver oxygen and nutrients to all of the tissues and organs throughout the body. The blood vessels that vascularize the central nervous system (CNS) possess unique properties, termed the blood–brain barrier, which allows these vessels to tightly regulate the movement of ions, molecules, and cells between the blood and the brain. This precise control of CNS homeostasis allows for proper neuronal function and also protects the neural tissue from toxins and pathogens, and alterations of these barrier properties are an important component of the pathology and progression of different neurological diseases.

The physiological barrier is coordinated by a series of physical, transport, and metabolic properties possessed by the Endothelial Cells (ECs) that form the walls of the blood vessels, and these properties are regulated by interactions with different vascular, immune, and neural cells. Understanding how these different cell populations interact to regulate the barrier properties is essential for understanding how the brain functions during health and disease. The BBB is composed of a monolayer of hCMEC that line blood vessels which interact with neurons, microglia, pericytes, astrocytes, and the extracellular matrix (ECM) to form the Neurovascular Unit(NVU)(Pong et al., 2020). The NVU is dynamic and functions to regulate molecular and cellular trafficking between the bloodstream and brain parenchyma through physical, enzymatic, transport, and immunological processes that are vital for brain homeostasis.

HCMECs are a central element of the microvasculature that forms the blood-brain barrier (BBB) and shields the brain against toxins and immune cells via paracellular, transcellular, transporter, and extracellular matrix proteins. The BBB endothelial cells are characterized by high expression of tight junction proteins and selective pathways for controlled transport between circulating blood and the CNS. While BBB protects the brain against toxins and pathogens, it can prevent the supply of medication and necessary substances to treat various deadly brain diseases such as brain tumors, schizophrenia, etc. PLGA nanoparticles (NPs) have been reported to improve drug penetration across the BBB both in vitro and in vivo(Pong et al., 2020). PLGA NP-based drug products can be achieved with higher efficiency, larger quantity, and better quality(Zhi et al., 2021) In vitro blood-brain-barrier models have been widely used to simulate in vivo models due to their low cost, feasibility, and repeatability and to maintain substitution of the in vivo functions, we quantified the barrier functions by ensuring the permeability of the BBB to the water, ions, and other solutes while we generated the BBB on the Transwell filter.

# The Blood-Brain-Barrier

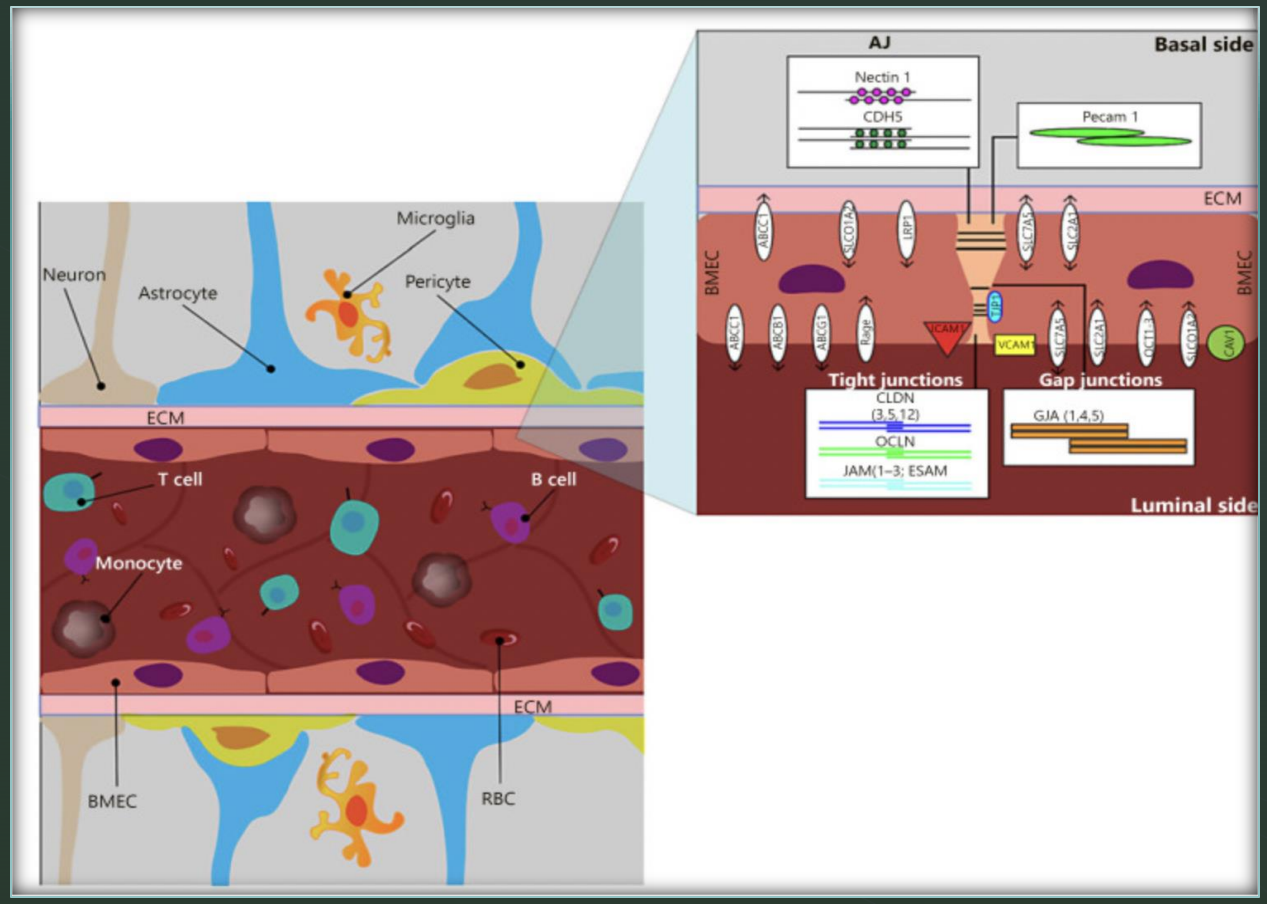



**Figure 1**: Sagittal section of the NVU depicting BMEC and the proteins associated with paracellular, transcellular, transporter, and extracellular matrix function. Paracellular proteins across the BBB

(Pong et al., 2020)

# Experimental Methods

The experiment was conducted using the Cy5 Labeled nanoparticles. The nanoparticles are made of PLGA-PEG, and the size was 350-400 nm, made of PLGA-PEG and encapsulating a Cy5 fluorescent dye (excitation/emission 640/670 nm). The MNP formulation has about 2 µg of dye per mg of MNP. The charge of the Nanoparticles was -30 MeV.

The concentration at which I found the linear range for the plate reader after calibration was 1 mg/mL. I put the fluorescently labeled solute Nanoparticles at a concentration of 1mg/mL at the top chamber of the Transwell filter and collected the solution(50 µL) from the bottom chamber every 5 minutes for 40 minutes and refilled 50 µL Ringer 1% Bovine-Serum-Albumin solution to the bottom chamber each time after taking 50 µL Cy5 mixed solution. Then, we put the collected fluorescent solutions from the bottom chamber into a Black 96 well plate and determined their intensity using a plate reader at Professor Williams's Lab.

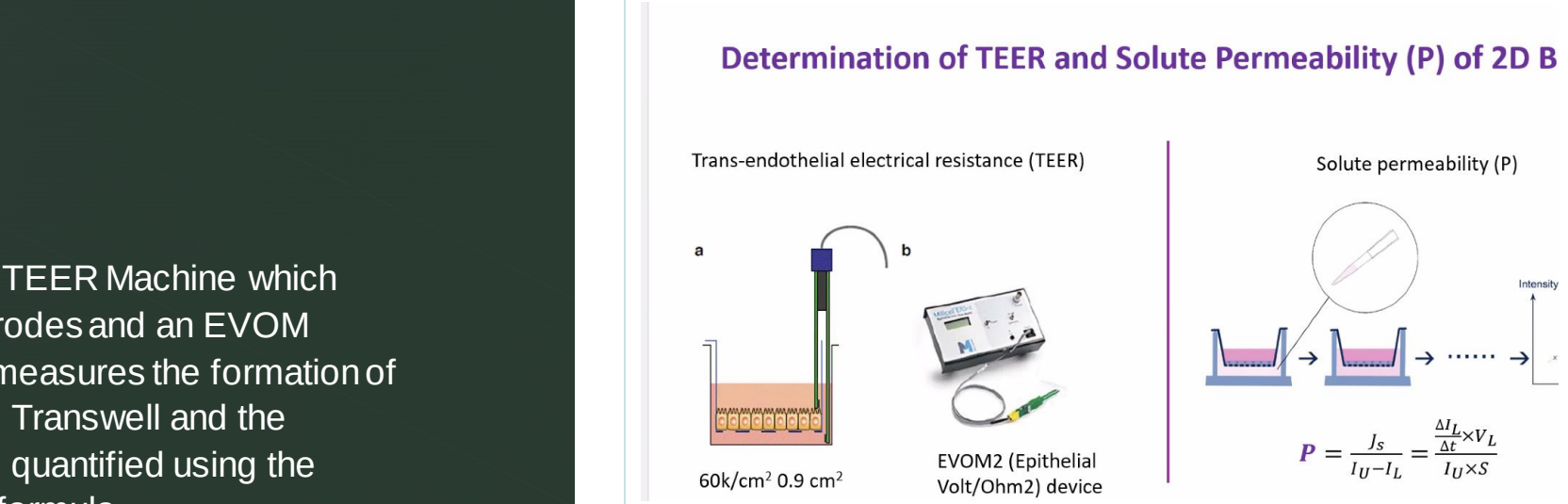
The Transwell with a 3.0 µm pore was used with an area of 1.12 cm^2 and the Transwell membranes were coated with rat tail type I collagen overnight at 37 °C in a dry incubator. The fluorescent intensity was tested on Wells B, C, D, E, and calibration was done on F and G well. The calibration curve expressed a linear relationship. We used the Transepithelial/Transendothelial electrical resistance(TEER) Machine to check if the Blood-Brain-Barrier was generated. placing the electrode in three places of the Transwell and making sure the electrodes are at the right location(shorter in the upper chamber and longer electrode in the lower chamber and finding the average in three different place

I took 1 mL of alcohol(70%), 1 mL of PBS, and 1 mL of EBM in three microtubes and used the electrodes to measure TEER before putting the electrodes in the Transwell. Then, I mixed the Ringer Solution 1(liquid) with Glucose, Herpes (salt and Acid), Sodium Hydrogen Carbonate($NaHCO_3$), 1 g BSA(Bovine Serum Albumin), 80 mL of Alcohol, and mixed. Removed the bubbles. So that there are in total 100 mL of the solution.

Then, I mixed 1mg/mL of Nanoparticles with Ringer solution in the dark room. Then, I added 0.5 mL of the diluted nanoparticles to each upper chamber of the Transwell, getting rid of the water that was already there previously. I added 1.5 Blank solutions to each lower chamber and set a timer for 30 minutes. I used the 96 well plates to transfer 50 microliters of solution from the lower chamber after mixing those for 30 minutes. And repeat this every 30 minutes for 6 hours. This experiment was continued for 3 weeks.

# The TEER Machine

**Figure 2**: The TEER Machine which includes electrodes and an EVOM device which measures the formation of the BBB in the Transwell and the permeability is quantified using the mathematical formula




(Feng et al., 2010)

# The Transwell System

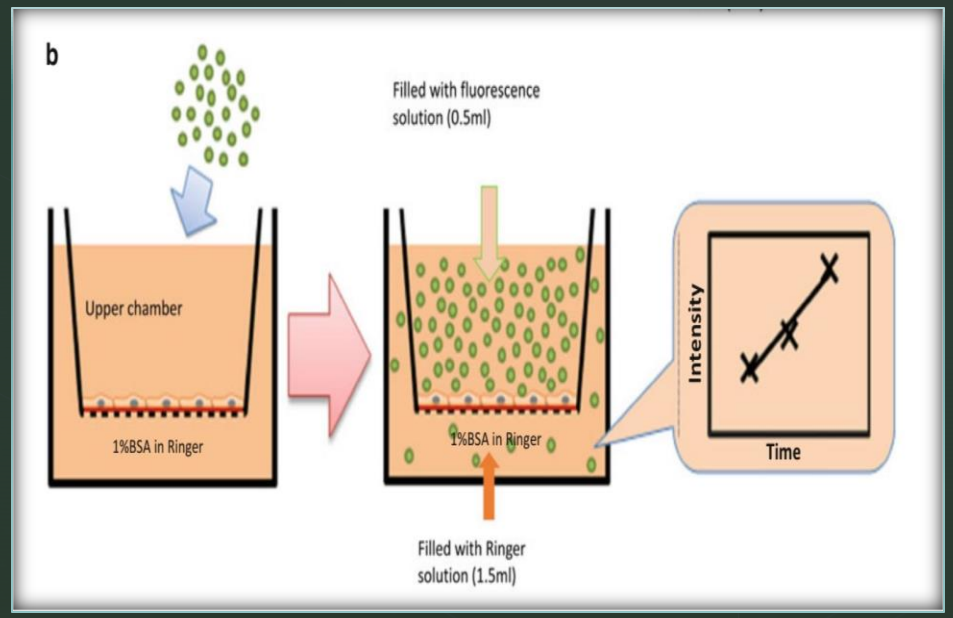



**Figure 3**: In vitro barrier models, "Transwell systems". The Transwell device features an upper donor side and a lower acceptor side, with the two compartments separated by a porous filter membrane of selected composition and pore size. When endothelial cells form a well-differentiated monolayer on these filters, added compounds (or nanoparticles in this case) in the upper chamber are internalized by the cells and if transcytosis occurs, they pass through the pores of the filters and reach the lower chamber.

(Feng et al., 2010)

# Results

**Figure 4**: The calculation of the relationship between the intensity of the Nanoparticles in the lower chamber in every 30 minute interval for Transwell B. The data doesn't represent a strong linear relationship in the increase of Nanoparticles in the lower chamber over time

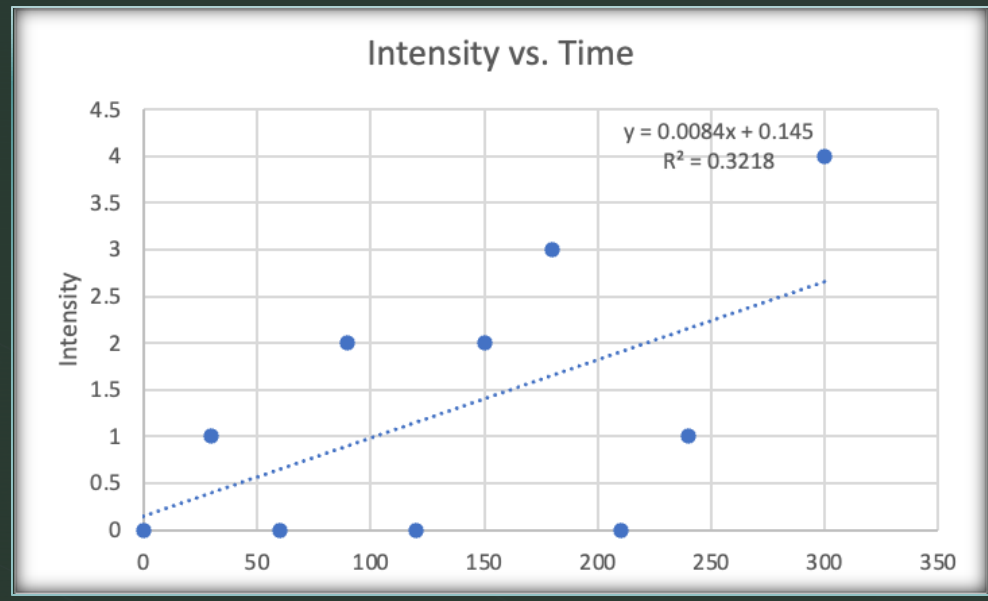


**Figure 5**: The calculation of the relationship between the intensity of the Nanoparticles in the lower chamber at every 30-minute interval for Transwell C. The data doesn't represent a strong linear relationship in the increase of Nanoparticles in the lower chamber over time

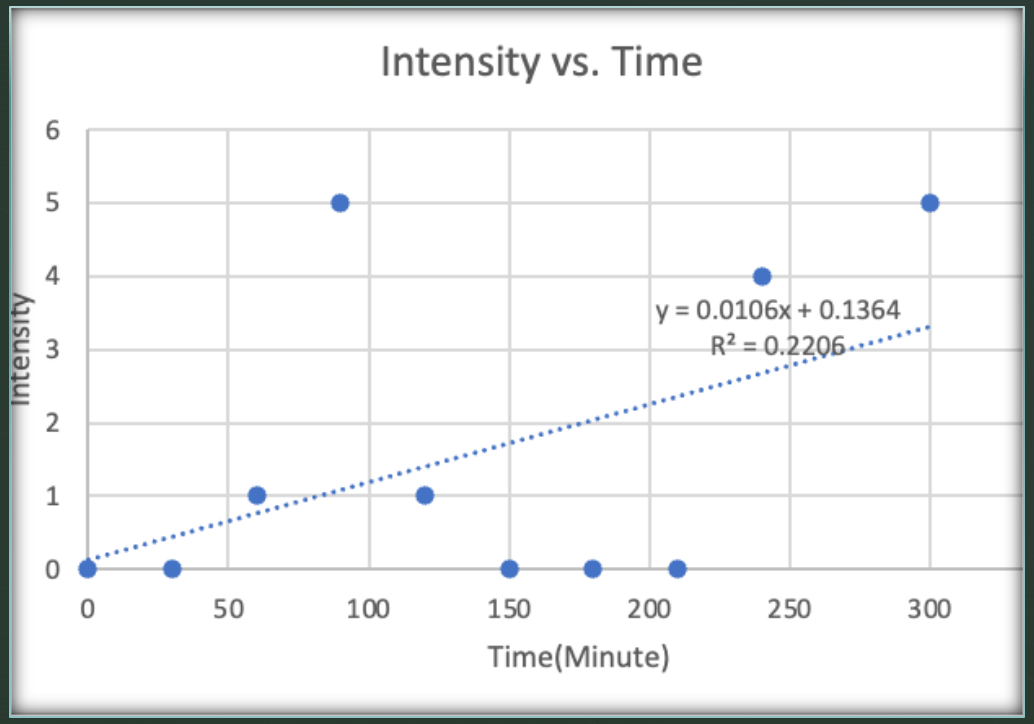


**Figure 6**: The calculation of the relationship between the intensity of the Nanoparticles in the lower chamber in every 30-minute interval for Transwell D. The data doesn't represent a strong linear relationship in the increase of Nanoparticles in the lower chamber over time

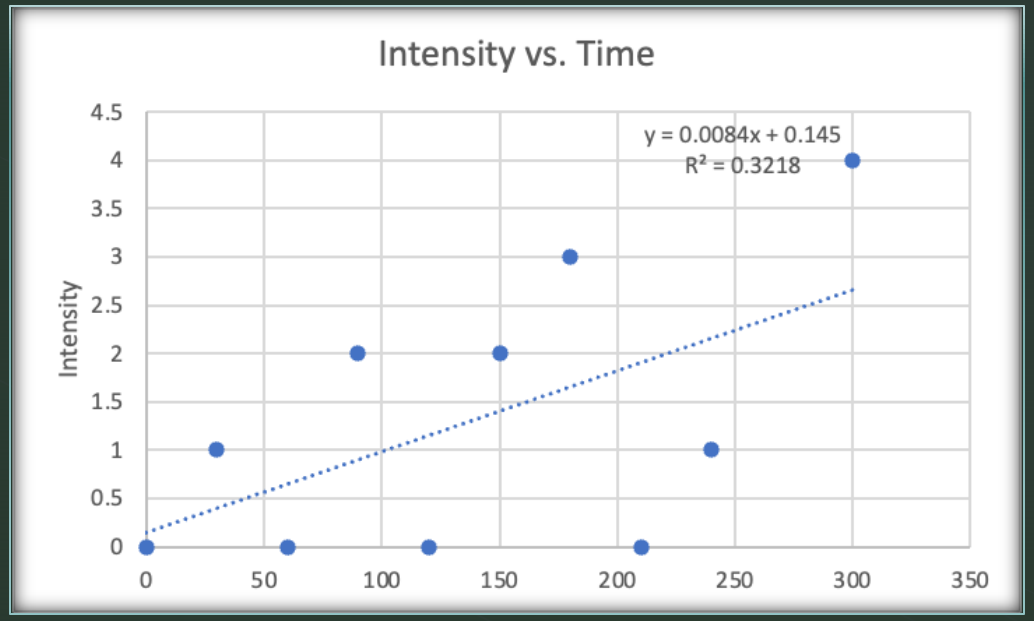
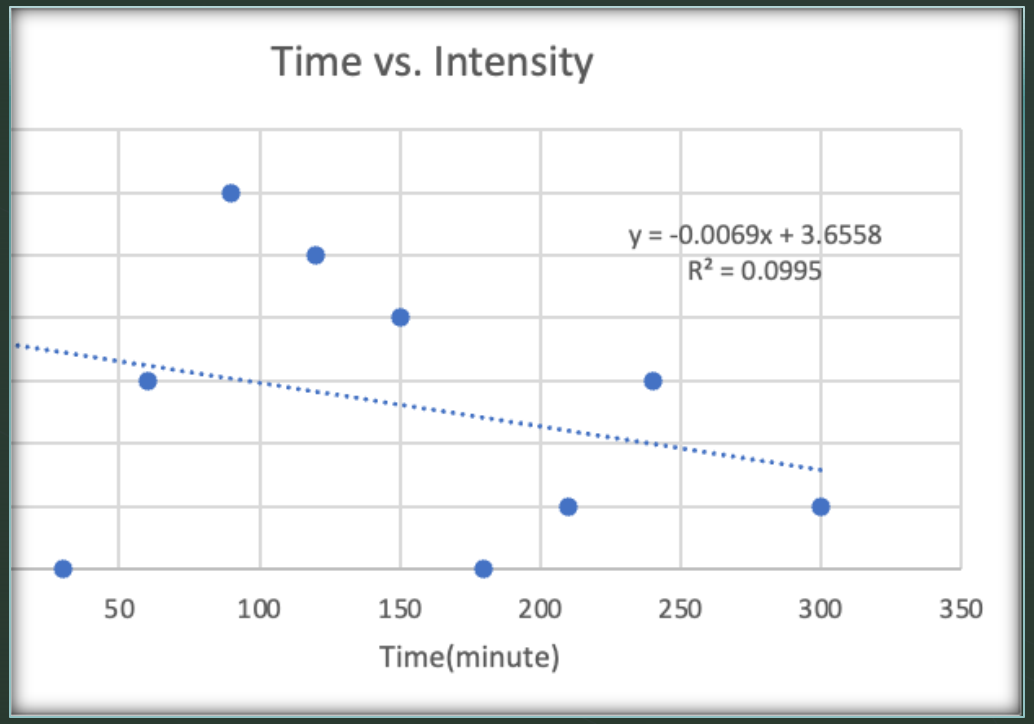


**Figure 7**: The calculation of the relationship between the intensity of the Nanoparticles in the lower chamber at every 30 minute interval for Transwell E. The data doesn't represent a consistent linear relationship in the increase of Nanoparticles in the lower chamber over time.



# The Concentration of Diluted Fluorescence

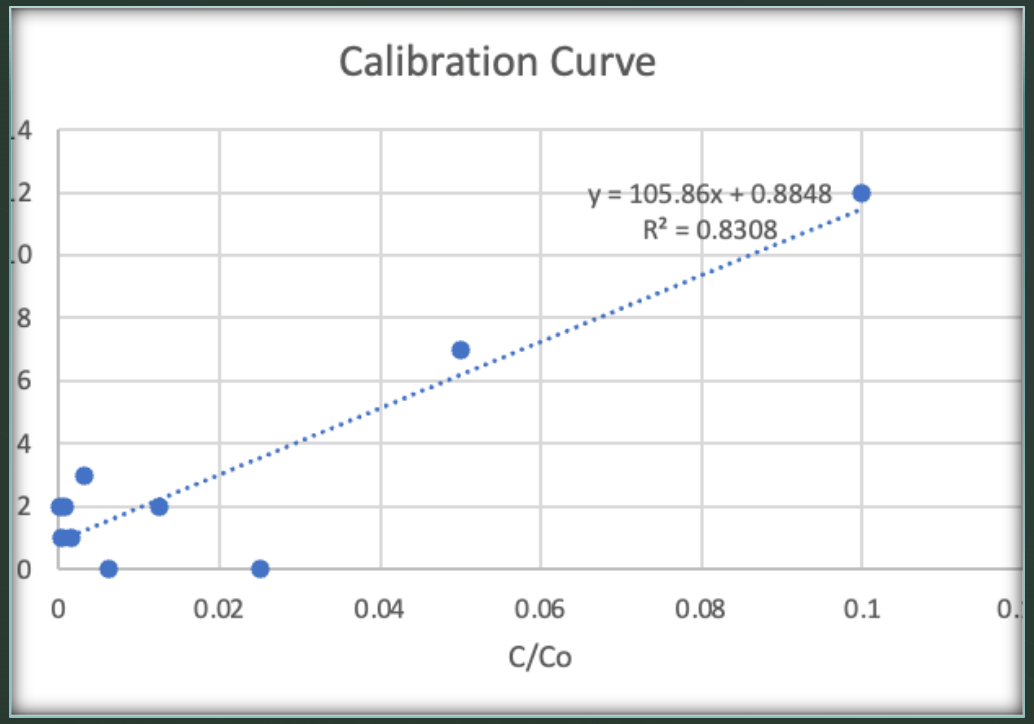
**Table 1**: List of concentrations for the diluted fluorescence solution used to calibrate the linear range of concentration vs. intensity curve for the fluorescence plate reader.

| $C_A$ | $C_B$ | 1% BSA | $C$ |
|---|---|---|---|
| $C_0$ | 0.1 mL* $C_A$ | 0.9 mL | 1 mL 0.1 $C_0$ |
| 0.1 $C_0$ | 0.5 mL* $C_A$ | 0.5 mL | 1 mL 0.05 $C_0$ |
| 0.05 $C_0$ | 0.5 mL* $C_A$ | 0.5 mL | 1 mL 0.025 $C_0$ |
| 0.025 $C_0$ | 0.5 mL* $C_A$ | 0.5 mL | 1 mL 0.0125 $C_0$ |
| 0.0125 $C_0$ | 0.8 mL* $C_A$ | 0.2 mL | 1 mL 0.01 $C_0$ |
| 0.01 $C_0$ | 0.75 mL* $C_A$ | 0.25 mL | 1 mL 0.0075 $C_0$ |
| 0.01 $C_0$ | 0.5 mL* $C_A$ | 0.5 mL | 1 mL 0.005 $C_0$ |
| 0.005 $C_0$ | 0.5 mL* $C_A$ | 0.5 mL | 1 mL 0.0025 $C_0$ |
| 0.0025 $C_0$ | 0.5 mL* $C_A$ | 0.5 mL | 1 mL 0.00125 $C_0$ |
| 0.00125 $C_0$ | 0.8 mL* $C_A$ | 0.2 mL | 1 mL 0.001 $C_0$ |

$C = C_B$ + 1% BSA

# The Calibration Curve

**Figure 8**: The diluted Cy5-PEG-PLGA solution used to calibrate the linear range of concentration vs. intensity curve for the Cy5-PEG-PLGA plate reader.



# Discussions

Our data doesn't support the hypothesis, which is "The intensity of the Nanoparticles in the lower chamber of the Transwell will increase over time which will indicate the permeability of Cy5-PEG-PLGA through the Blood-Brain-Barrier." Although the data shows that nanoparticles were passing through the in-vitro BBB over time, it's not a significant linear relationship.

One possible reason could be the adherence of nanoparticles to the Transwell filter due to its slightly negative charge. Secondly, nanoparticle agglomeration, which sometimes occurs in biological media as well as after nanoparticle internalization, could impair Transwell system transport studies since particle agglomerates may not be able to pass through the filter pores.

Another possible reason for the non-linear relationship could be after some time, the nanoparticles stop passing the BBB and when we collect the fluid from the lower chamber, we only get the particles that were already available from the first set of readings.

In previous studies, it was found that nanomaterials are capable to accumulate within the BBB(Weksler et al., 2013). Considering that export is generally absent for nanoparticles accumulating in the lysosomes and it will be important to consider in the future whether nanoparticle internalization and lysosomal accumulation could affect the BBB itself.

# Future Work

To correct this feature of our data we can attach Poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), and poloxamer (Pluronic) with the PEG-PLGA as excipients to further improve the stability and effectiveness of PLGA formulations(Chen et al., 2013).

We can also investigate if Peptides and other linkers can be attached on the surface of PLGA to provide targeting delivery which will increase the permeability of the PEG-PLGA nanoparticles across the BBB. In the future, we will also investigate the permeability in the 3D Blood-Brain-Barrier model.

# Conclusions

To confirm if the Blood-Brain-Model is efficient, we tested the permeability with Dextran-70K and Sodium Fluorescin for the first three weeks of the project and sophisticated result was found regarding the permeability of those molecules across the Blood-Brain-Barrier.

There should be progress in PLGA NPs as a vehicle to deliver drugs to the brain in a controllable and targeted manner. Unlike most NPs, PLGA NPs show a promising future to become a clinically and commercially feasible drug delivery system.

Clinical trials are needed to monitor the efficacy and toxicity of PLGA NPs. In order to figure out the efficient drug delivery system using PEG-PLGA NPs, we will continue to develop more in vitro BBB models and investigate the way we can better modify the PEG-PLGA NPs to enhance their ability to permeate through the BBB.

Uncertainties, such as drug encapsulation rate, assembly stability, particle size distribution stability, and in vivo pharmacokinetics, maybe the focus for our further future research and development.

# Acknowledgments

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