Research Plan/Project Summary:

- In this study, a Caco-2 cell model was used to replicate a baby's small intestines to study the effects of exosomes on the model. Necrotizing Enterocolitis is a severe disease that affects the intestines of preterm babies by attacking the baby's small intestines, resulting in increased inflammation of the small intestines wall. Exosomes are known to contain extracellular vesicles, which include anti-inflammatory factors and help protects the baby's immunity. Caco-2 cells are differentiative cells that transform into epithelial cells. The epithelial cells contain similar functions to exosomes by forming tight junctions between one another to access cellular communication and to prevent any seeping bacteria into the small intestines, this was measured by a Transepithelial Electrical Resistance device.
- For the experimentation, different exosomes have been collected from a variety of donor mothers, along with formula feeding milk. Using the Caco-2 cell model, the data compared was between the transwell inserts of these derived exosomes. The exosomes that were collected were preterm mothers breast milk, term mothers breast milk, and formula feeding milk. These different exosomes were being compared to one another to find statistical significance. Results showed that there was a statistical significance in TEER readings between when extracellular vesicles were present. However, there was very little statistical significance between the different extracellular vesicle groups: Preterm EVS, Term EVS, and Donor EVS. Thus, this information could be informative to mothers who have just delivered regarding benefits of extracellular vesicles and how they could potentially decrease their child's risk of NEC.

- A hypothesis was made that since Caco-2 cells are differentiative cells they transform into epithelial cells, very similar to enterocytes. These packed cells contain antitumoral and anti-inflammatory factors. Potentially, a baby dealing with NEC could use these anti-inflammatory exosomes to decrease the swelling of the baby's intestines, which later affects his/her health.
- The project was performed by several procedures.

Culturing and maintaining Caco-2 cells. Caco-2 cells were stored in a humidified incubator at 24 degrees Celsius. The cells were maintained in DMEM High Glucose with minus exosome fetal bovine serum (FBS) and DMEM High Glucose serum free media. Since the cells were placed in a transwell insert, the Caco-2 cells were exposed to the serum free media with DMEM High Glucose on the apical side. On the basal, or bottom of the well, the minus exosome with DMEM High serum was placed to prevent drying of the cells. The purpose of growing these Caco-2 cells were for the TEER readings later on in the experimentation.

Extraction of Exosomes. In my experimentation, the exosomes were being isolated by serial ultracentrifugation. After thawing donated colostrum samples from healthy mothers, they were subjected to 17,000 x g for 60 minutes at 4 degrees Celsius in Thermo Sci centrifuge (A27 -8x50 rotor). Next, the filtration process included filtering through 0.8 um, 0.45 um, and 0.22 um filters to remove any cell waste. The supernatants were centrifuged at 120.000 x g for 3 hours at 4 degrees Celsius in Beckman Coulter Optima XPN-90 Ultracentrifuge, with a SW50.2 rotor (6mm tube). Thus, ultracentrifugation forms a firm pellet of vesicles at the bottom of the tube. Next, I had to aspirate the supernatant with a pipette tip, avoiding contact with the pellet. Cold PBS was then added on the opposite side of the pellet. After, ultracentrifugation took place for 45 minutes at 120.000 x g at 4 degrees Celsius in Beckman Coulter Optima XPN-90

Ultracentrifuge. Lastly, the tubes were covered with parafilm and refrigerated overnight at 4 degrees Celsius.

Addition of Exosomes to Caco-2 Cell Model. After filtering thawed donated colostrum samples from healthy mothers, a BCA was performed. A BCA is a protein assay used for the quantification of the total amount of protein in a sample. Using the BCA Protein Assay Kit, I was testing to see the number of exosomes to obtain and use for the Caco-2 cell model. After establishing the quantity of exosomes to use in the cell model, the desired exosome amount was placed in different eppendorf tubes labeled K34W1 (control), K36W1 (term), K38W1(preterm), and TK13W1 (donor). Working under a fume hood, I pipetted 10 ul of the different eppendorf tube samples into the correct wells labeled on the Caco-2 cell model. I carefully placed the 10 ul of exosomes in the center of the transwell, where Caco-2 cells are present, along with the addition of new DMEM High Glucose serum free media. Next, the Caco-2 cell model was placed in a humidified incubator for 24 hrs.

- While performing this experimentation, some risk hazards were involved, such as handling the cancerous cells (BSL-1). Proper equipment was taken place by wearing a lab coat, gloves, working under a hood, and wearing closed shoes at all times.
- Data was measured by a transepithelial electrical resistant readings. The measurements were executed by very carefully placing the electrodes on both sides of the cell layer. This measures both the voltage and current across the cell monolayer without disrupting the cells. I recorded this measurement for each reading. Since a transwell insert contains three sections in one well, I calculated the TEER readings by measuring each of the three sections and compared those readings to other wells to see how compact the other cell junctions were.

- Bibliography:

Frost, B. L., Modi, B. P., Jaksic, T., & Caplan, M. S. (2017, January 1). New Medical and Surgical Insights Into Neonatal Necrotizing Enterocolitis: A Review. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27893069.

Lim, J. C., Golden, J. M., & Ford, H. R. (2015, June). Pathogenesis of neonatal necrotizing enterocolitis. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25854935.

Manca, S., Upadhyaya, B., Mutai, E., Desaulniers, A. T., Cederberg, R. A., White, B. R., & Zempleni, J. (2018, December 1). Milk exosomes are bioavailable and distinct microRNA cargos have unique tissue distribution patterns. Retrieved from https://nebraska.pure.elsevier.com/en/publications/milk-exosomes-are-bioavailable-and-distinct-microrna-cargos-have-.

de la Torre Gomez, C., Goreham, R. V., Bech Serra, J. J., Nann, T., & Kussmann, M. (2018, March 27). "Exosomics"-A Review of Biophysics, Biology and Biochemistry of Exosomes With a Focus on Human Breast Milk. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/29636770.

Pegtel1, D. M., & UMC, S. J. G. A. (n.d.). Exosomes. Retrieved from https://www.annualreviews.org/doi/abs/10.1146/annurev-biochem-013118-111902.

Admyre, C., Johansson, S. M., Qazi, K. R., Filén, J.-J., Lahesmaa, R., Norman, M., … Gabrielsson, S. (2007, August 1). Exosomes with immune modulatory features are present in human breast milk. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17641064.

Epithelial Cells in Urine: MedlinePlus Lab Test Information. (2019, September 30). Retrieved from https://medlineplus.gov/lab-tests/epithelial-cells-in-urine/.

No changes were made at the end of the research plan.