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# Studying the Stability of Collagen/Heparin Coatings to be Used in Cell Therapy Applications

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# Studying the Stability of Collagen/Heparin Coatings to be Used in Cell Therapy Applications

An Honors Thesis submitted in partial fulfillment of the

requirements of Honors Studies in Biology

By

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## ABSTRACT

This honors thesis aims to investigate the reusability and performance of cell coatings for cell therapy applications. Cell therapy, which involves the use of human cells to repair or replace damaged tissues, holds immense potential for medical advancements. However, ensuring the survival and functionality of transplanted cells remains a significant challenge. We focused on studying the effectiveness of coatings applied to cells for improved cell growth and viability. The research project involved the preparation of the coatings using a layer-by-layer method and the subsequent seeding of cells. The coated cells were then subjected to a series of experiments to assess their growth and survival rates. By trypsinizing and reseeding the cells, we aimed to explore the potential for reusing the coatings and their impact on cell performance. Our findings demonstrate that cell coatings offer a promising approach for enhancing cell therapy outcomes. The data revealed that the coated cells exhibited a higher number of cells than the control group, showing improved cell growth and survival. These findings have significant implications for the development of improved strategies for cell transplantation and tissue regeneration. This research contributes to the growing body of knowledge in the field of cell therapy and provides insights into the design and optimization of coatings for enhanced cell survival and function. The outcomes of this study have the potential to positively impact the field of regenerative medicine and pave the way for more effective cell-based therapies in the future

# INTRODUCTION

Cell therapy has emerged as a promising field in regenerative medicine, offering the potential to revolutionize medical treatments by utilizing human cells to repair or replace damaged tissues (Gage, 1998). Cell therapy harnesses the regenerative potential of the patient's cells. Doctors can harvest therapeutic cells from various sources including bone marrow, adipose tissue, and umbilical cord blood. The cells are then processed and injected back into your body at the site of injury. The goal of cell therapy is to restore or enhance tissue function by delivering viable and functional cells to the site of injury or disease (Bordignon, 1999). Successful cell therapy relies on the ability of transplanted cells to survive, integrate into the host tissue, and exert their intended therapeutic effects (Forbes, 2015). Cell therapy offers promising potential for treating a wide range of diseases and conditions, including cardiovascular disorders, neurodegenerative diseases, and autoimmune disorders (Regmi, 2019).

Several challenges hinder the effectiveness of cell therapy, including low cell viability, poor engraftment, and limited functional integration. Obtaining a reliable, abundant, and potent source of cells for therapy remains a challenge. Identifying suitable cell sources, developing standardized protocols for cell isolation, expansion, and characterization, and ensuring batch-to-batch consistency are critical factors in advancing cell therapy. The host immune system may recognize transplanted cells as foreign and mount an immune response, leading to rejection. Strategies such as immunomodulation, genetic engineering of cells to evade immune recognition or co-administration of immunosuppressive agents need to be explored (Regmi, 2019). These obstacles

necessitate the development of innovative strategies to improve the outcomes of cell therapy.

Human Mesenchymal Stem-Cells (HMSCs) are the cells used during this experiment. HMSCs hold solutions to some of the challenges in cell therapy. HMSCs can be derived from bone marrow, adipose tissue, umbilical cord tissue, and dental pulp. HMSCs help with autoimmune disorders and many other diseases related to bone/connective tissues. HMSCs are also used during transplants and can help prevent rejection. HMSCs have been shown effective in providing both anti-inflammatory and anti-microbial benefits for various diseases. The clinical potency of hMSCs is derived from their direct effects on pro-inflammatory and anti-microbial processes, as well as their ability to enhance host immunity to resolve infections and tissue damage (Bonfield, 2023).

One approach that has gained considerable attention is the use of cell coatings, which involve applying specific materials to the surface of cells to enhance their properties and interactions with the host environment (Wu, 2020). Cell coatings can modulate cell adhesion, protect against immune responses, provide a supportive microenvironment for cell growth and differentiation, and improve cell-matrix interactions (Green, 2012). The rationale behind cell coatings is to create a biomimetic environment that mimics the natural extracellular matrix (ECM) and promotes cell survival, proliferation, and functionality (Ren, 2019). By engineering the surface properties of cells, coatings aim to enhance their therapeutic potential and overcome the limitations associated with cell transplantation (Sacchetti, 2018).

Cell coatings can be applied using various techniques, including the layer-by-layer (LBL) method, which allows for precise control over coating thickness and composition (Sasaki, 2017). The LBL approach involves the alternate deposition of positively and negatively charged layers onto the cell surface, resulting in a multilayered coating (Zhang, 2007). The choice of coating materials is crucial and depends on the specific requirements of the cell therapy application (Gasperini, 2014). Common coating materials include polymers, proteins, nanoparticles, and biological molecules. Natural polymers include collagen, heparin, gelatin, and hyaluronic acid, as well as synthetic polymers like polyethylene glycol (PEG) and polycaprolactone (PCL) (Ullah, 2020). These materials can be functionalized with bioactive molecules, growth factors, or peptides to further enhance cell adhesion, survival, and differentiation (Pavlukhina, 2011). The coating materials in this experiment were the polymers heparin and collagen. Heparin and collagen are both components of the extracellular matrix. Heparin stores and releases growth factors. Collagen accelerates osteoblast differentiation and matrix mineralization.

Cell culturing is another important part of this research experiment. Cell culturing refers to growing and maintaining cells in a controlled laboratory environment. It involves providing cells with the necessary nutrients, growth factors, and conditions to support their growth and proliferation. The selection of appropriate cell types can lead to varying responses to coatings based on inherent characteristics and requirements. In monolayer culture, cells are grown as a single layer adhered to a flat surface, such as a petri dish or tissue culture flask. In suspension culture, cells are grown in a liquid medium without attachment to a substrate, typically using shaken flasks or bioreactors. In

a 3D culture, cells are grown in a three-dimensional matrix or scaffold to mimic the in vivo tissue environment more accurately. Cultured cells require a nutrient-rich media called growth media. This contains amino acids, vitamins, minerals, sugars, and growth factors. Fetal bovine serum (FBS) or other animal-derived sera are often added to the media to provide essential nutrients and promote cell growth. Antibiotics/Antimycotics are added to prevent bacterial, fungal, and mycoplasma contamination in the cell culture. Penicillin-streptomycin and L-Glutamine are the antibiotics in our fresh media (Alpha MEM). Cells are typically cultured in a controlled environment with a specific temperature and humidity to mimic physiological conditions. A sterile environment is maintained by working in a laminar flow hood to prevent contamination during cell handling. During subculturing, cells are transferred from one culture vessel to another to maintain their growth and prevent overcrowding. A cell passage refers to the number of times cells have been subcultured or passaged, which can affect their characteristics and behavior. Trypsin is a proteolytic enzyme that cleaves peptide bonds in proteins, and it is used to dissociate cells from the culture surface by breaking down cell adhesion proteins. Prolonged exposure to trypsin or using a concentration that is too high can result in cell death (Segeritz, 2017).

One key aspect to consider in cell coating research is the selection of appropriate cell types for investigation. Different cell types may exhibit varying responses to coatings, depending on their inherent characteristics and specific requirements (Verma, 2010). For instance, mesenchymal stem cells (MSCs), a commonly used cell type in regenerative medicine, can benefit from coatings that enhance their adhesion and differentiation capabilities (Liu, 2012). On the other hand, immune cells may require

coatings that modulate their interactions with the host immune system to prevent immune rejection (Boehler, 2011).

Previous studies have demonstrated the potential of cell coatings to improve cell survival, proliferation, and functionality. For example, Tan (2020) utilized a layer-by-layer coating approach to enhance the survival and proliferation of pancreatic islet cells for transplantation in type 1 diabetes. The coated islet cells exhibited improved viability and functionality. Similarly, Zhang (2020) investigated the application of hydrogel coatings to neural stem cells (NSCs) and observed enhanced cell survival, neuronal differentiation, and functional integration following transplantation into the brain.

## RESEARCH ORIENTATION AND HYPOTHESES

The objective of this study is to investigate the reusability and performance of cell coatings for cell therapy applications. Specifically, we aim to evaluate the impact of coatings on cell viability, proliferation, and functionality. By examining the stability and effectiveness of cell coatings, we seek to enhance our understanding of their role in improving cell therapy outcomes and pave the way for developing more effective and sustainable cell-based therapies. Previous studies have demonstrated improved cell survival, proliferation, and functionality with cell coatings.

To achieve this goal, we have formulated a specific research orientation/hypothesis to guide our study. We aim to evaluate the impact of cell coatings on cell proliferation and viability. We hypothesize that the presence of appropriate coatings will enhance cell viability by providing a supportive microenvironment that

mimics the natural ECM (Zhang, 2009). Moreover, we expect that coatings will facilitate cell proliferation, leading to increased cell numbers and improved therapeutic outcomes (Landry, 2018). We anticipate that cell coatings will create a favorable microenvironment that supports cell survival, mitigates cell death mechanisms, and enhances cell viability (Midgley, 2020). We expect to observe higher cell viability rates in coated cells than in uncoated cells, indicating coatings' protective and supportive effects. We predict that the coated cells will exhibit increased proliferation rates compared to uncoated cells, indicating the pro-proliferative effects of the coatings. Validate the hypotheses through rigorous experimentation and analysis

## MATERIALS AND METHODS

### Cell Culture

Human mesenchymal stem cells (HMSC) were obtained from a reliable cell repository. HMSC cells were selected for their potential therapeutic applications in cell therapy. HMSC cells were cultured in T25 flasks and T75 flasks containing a specific culture medium. The culture medium consisted of Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (FBS) and antibiotics (penicillin and streptomycin). The flasks were maintained in a humidified incubator at 37°C and 5% CO2.

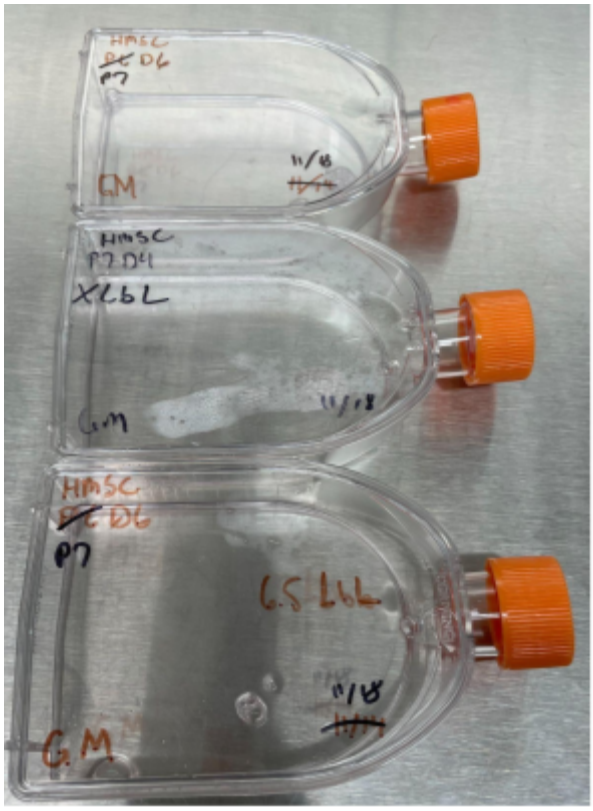



Figure 1. T75 Flasks in Experiment 1.

**Layer-by-Layer (LBL) Coating Preparation**

Buffers at pH 4 and 5 were used for optimal coating conditions. Acetate buffer (pH=4.0) is produced using 3.1 mL acetic acid (glacial) and 1.27 g sodium acetate. Acetate buffer (pH=5.0) is produced using 1.3 mL acetic acid and 5.29 g sodium acetate. LBL solutions were prepared using collagen (positive charge) and heparin (negative charge). Collagen solution was prepared by dissolving collagen in a 50 mL buffer (pH=4) at a concentration of 0.05 g. Heparin solution was prepared by dissolving heparin in a 50 mL buffer (pH=5) at a concentration of 0.05 g. Polyethyleneimine (PEI) is produced using 0.05 g PEI in a 50 mL buffer (pH=5.0). PEI provides a strong anchoring layer with a strong positive surface. The LBL method was employed to create coatings on

cell culture surfaces. T25 flasks were used as the culture vessels for this purpose. Initially, the flasks were filtered using a flask and vacuum. Then, the flasks were coated with a layer of PEI. Subsequently, alternating layers of collagen and heparin were applied using the layer-by-layer technique. Each layer was applied by adding a specific volume of the collagen solution or heparin solution and allowing sufficient time for adsorption before adding the next layer. Washes from the pH 5 buffer were performed between each layer. Washes are necessary because mixing solutions would create microparticles. The layering process was repeated until a 6.5 bilayer was achieved, meaning the last layer would be heparin. The 6.5 bilayer ensures the entire surface is covered. A 6.5 bilayer is also not too extreme in terms of cost and time. After the completion of the coating process, the coated flasks were placed under UV light and then washed with PBS to remove any unbound molecules.

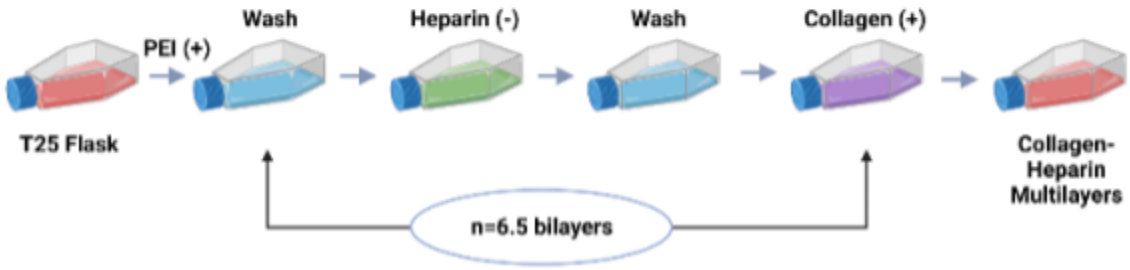



Figure 2. Depiction of layer-by-layer assembly using collagen and heparin onto T25 flask.

## Cell Media Preparation and Maintenance

Cell media were prepared by combining specific components, including DMEM, FBS, and antibiotics, in predetermined proportions. The media were formulated to

provide the necessary nutrients and growth factors required for cell culture. To maintain cell viability and provide fresh nutrients, cell media were changed periodically. The frequency of media change depended on the growth rate and specific requirements of the cells. Generally, every 2-3 days the cell media was changed. The old media were aspirated from the culture flasks, and fresh media were added to ensure a continuous supply of nutrients. New media was inserted based on working volume.

## Cell Passaging

Look under a microscope to ensure an 80% confluency and a maximum of 100% confluency for MSCs. The media is taken out and flasks are washed with PBS twice. To detach the cells from the culture surface for subculturing, trypsin was used. The culture medium was aspirated, and a sufficient amount of trypsin was added to cover the cells. The flasks were then incubated at 37°C for 10 minutes to allow the trypsin to act on the cells and detach them from the surface. After detachment, the trypsin activity was neutralized by adding 2 mL of fresh cell media. Centrigure is performed for 10 minutes at 200 G. The cells are aspirated to remove as much media from the pellet as possible. 2 more mL of fresh cell media are added to the pellet immediately. The cells were gently resuspended with the media by pipetting up and down to obtain a mixture. The cells are counted and then readded into new flasks based on seeding density, total live cells, and initial volume. The recommended seeding density of 5,000 cells/cm^2 was used to ensure optimal cell growth and proliferation.

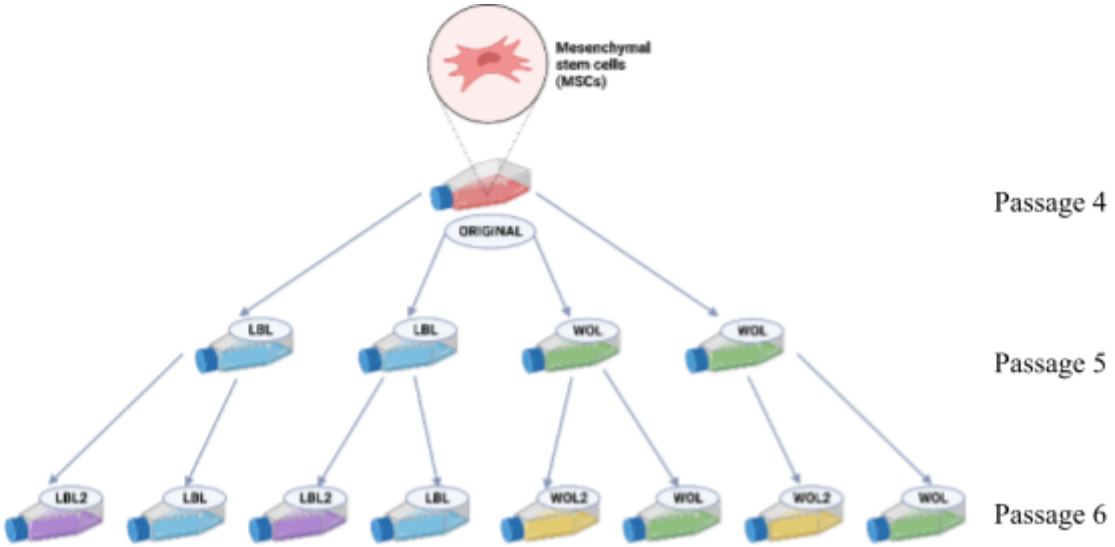



Figure 3. Flow Chart of Passages in Experiment 2. LBL = Layer-by-layer Samples, WOL = Without Layer-by-layer Samples, LBL2= Next Passage of Samples from the Layer-by-layer container, WOL2 = Next Passage of Samples from the without Layer-by-layer container. Same Color = Same Container

## Cell Counting

Trypan Blue dye was used to distinguish between live and dead cells. The dye was added to the cell suspension in a 1.5 mL tube, and the stained cells were observed under a microscope. The percentage of viable cells was determined by counting the unstained (live) cells and comparing them to the total cell count. Cell counting was performed using a hemocytometer, a specialized counting chamber. A small aliquot of the cell suspension was loaded onto the chamber, and cells within the defined counting area were manually counted under a microscope. The number of cells per unit volume was calculated based on the counting chamber's specifications. Photos were also taken at 10X magnification to observe the visual morphology.

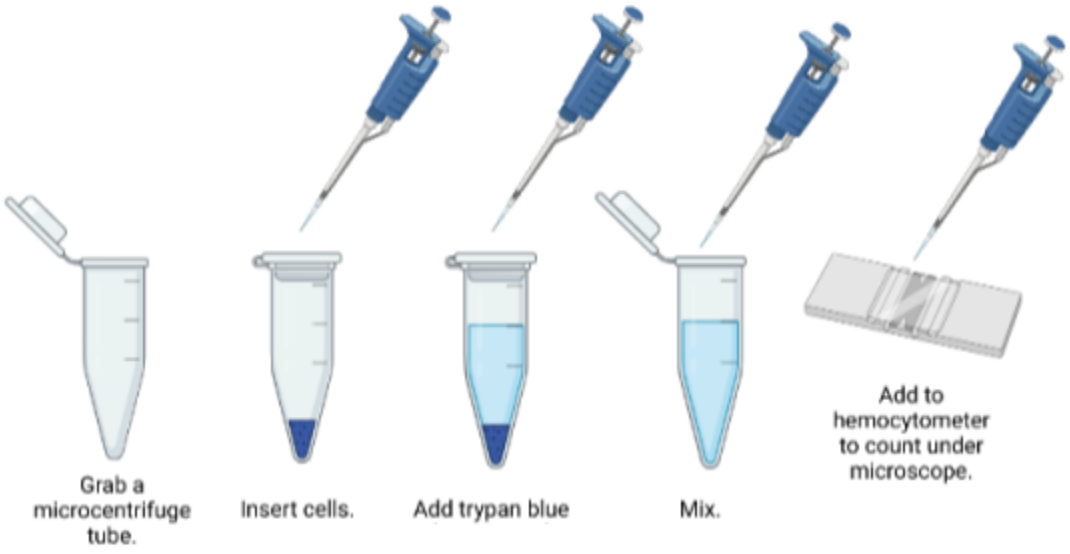



Figure 4. Depiction of Applying Trypan Blue.

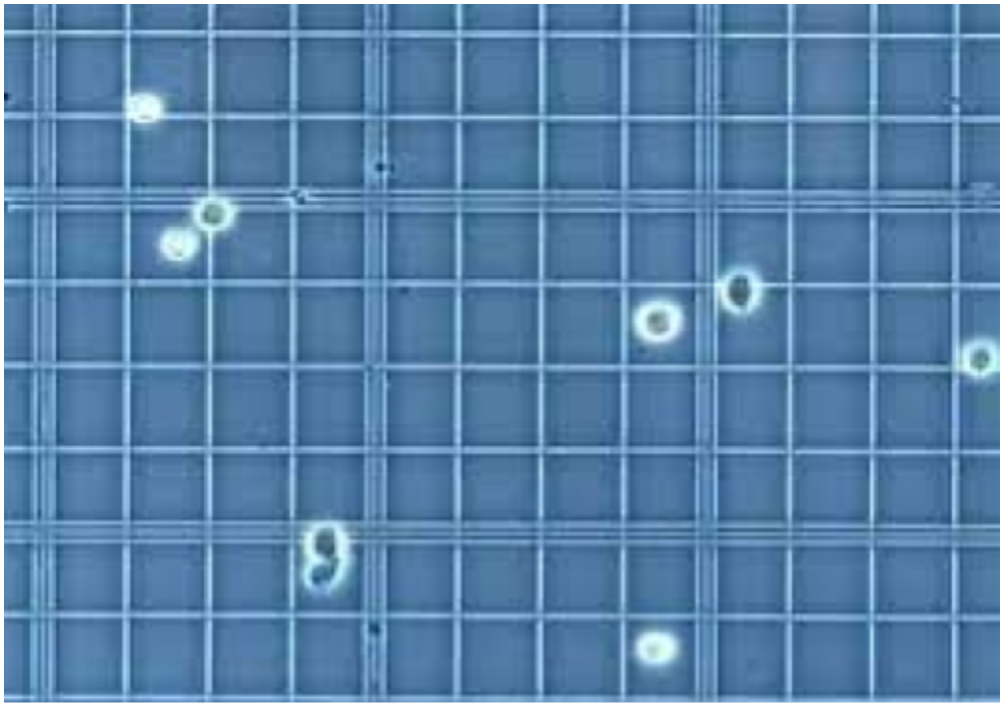


Figure 5. Trypan Blue Stain Showing Alive and Dead Cells (from Allevi, 2022)

## Statistical Analysis

All experimental data, including cell counts and viability, were noted. The data were collected from multiple replicates or independent experiments to ensure statistical robustness. The obtained results were interpreted and discussed in the context of the research objectives and hypotheses. Any trends, patterns, or significant findings were highlighted, and their implications were discussed.

Due to the nature of this study, conducting a comprehensive statistical analysis was not deemed appropriate due to the constraints of cost and time. This study involved extensive experimentation and data collection, requiring substantial resources and an extended period for completion. Given the limited budget and time frame available, allocating resources for complex statistical analyses would have been impractical. Instead, the focus of this research was primarily on qualitative observations and descriptive analysis to gain insights into the phenomenon under investigation. While statistical analyses could have provided additional insights and enhanced the rigor of the study, the feasibility of such analyses within the given limitations made them unfeasible. Thus, the findings of this study are primarily based on qualitative evidence, which still contributes valuable information to the field despite the absence of statistical analyses.

# RESULTS

## Experiment 1

The experiment began on November 14, 2022, with the layer-by-layer (LBL) method conducted over a 4-hour timespan. Heparin-terminated layers at a 6.5 bilayer were employed, involving successive increments of 7 mL of PEI, HEP (heparin), washes,

and COL (collagen) in a T75 flask. Following the layering process, the cells were exposed to UV light, treated with PBS, placed in an incubator, and subjected to trypsinization. Four days later, passage 6 was performed, accompanied by media preparation, image capture, and cell counting.

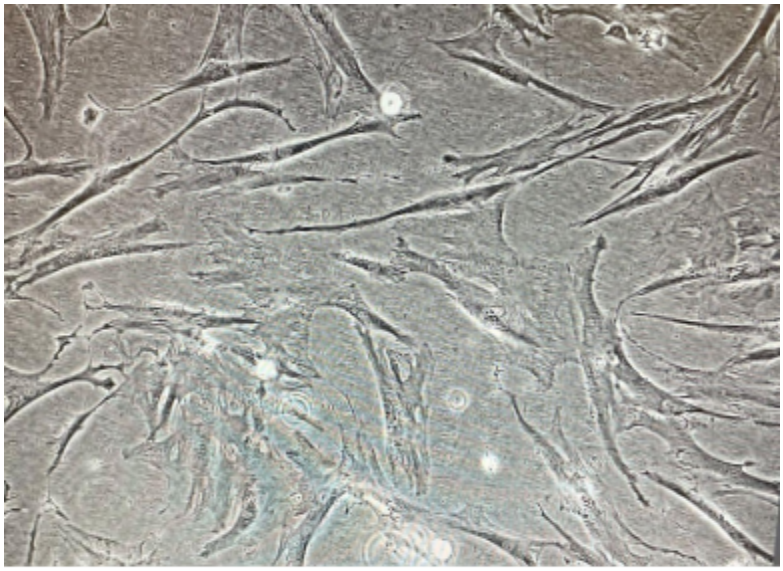
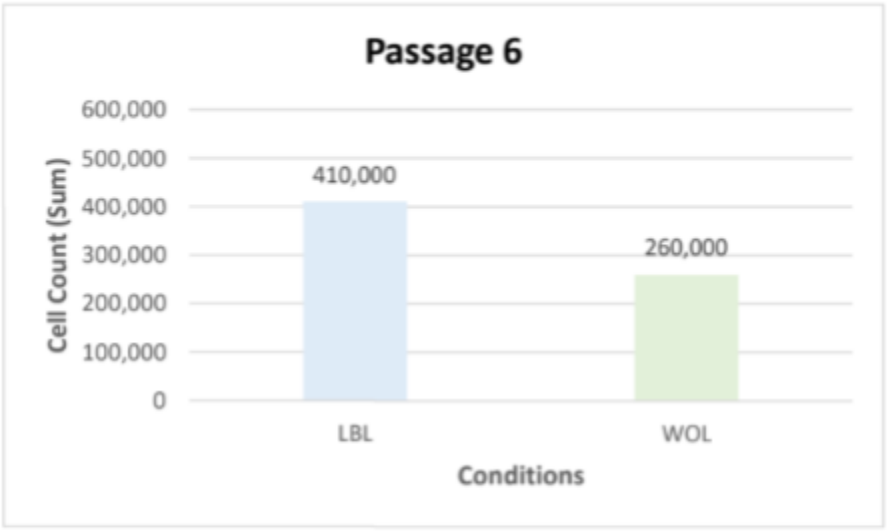


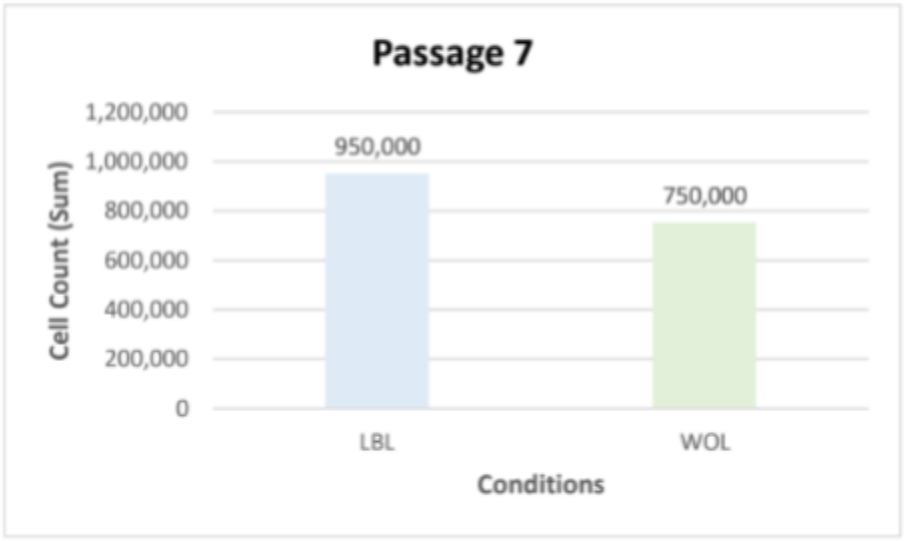
Figure 6. Final Image of LBL Cells in Experiment 1.

The initial cell count analysis revealed that the LBL group exhibited 410,000 cells, while the control group (WOL) showed 260,000 cells. The control group does not have the LBL coating. As the experiment progressed, the media was changed over the subsequent period. Twenty-five days later, the final cell count was performed for passage 7. The control group, which was reseeded with the same cells, displayed a cell count of 750,000. In comparison, the LBL group consisted of two flasks: one with reseeding, containing 500,000 cells, and another flask with 450,000 cells from the LBL group.

| Passage | Condition | Cell Count (Sum) |
|---|---|---|
| 6 | LBL | 410,000 |
| | WOL | 260,000 |
| 7 | LBL | 950,000 |
| | WOL | 750,000 |

Table 1. Experiment 1 Count Data.




Figures 7 and 8. Experiment 1 Layer-by-layer Coating vs. Without Layer-by-layer Coating

The data obtained from Experiment 1 demonstrates that the application of cell coatings, as observed in the LBL method, can significantly improve the chances of cell survival and reproduction. This can be observed through the higher combined cell count in the LBL containers when compared to the control group. It is noteworthy that reseeding on the LBL flask appears to be more effective in promoting cell growth than continued growth on the LBL surface.

## Experiments 2 and 3

Experiments 2 and 3 commenced with the layer-by-layer (LBL) method on February 21, 2023, starting with passage 4 cells. They were performed simultaneously. A seeding density of 5000 cells/cm^2 was employed, resulting in an initial seeding of 125,000 cells in a T25 flask. Cell media was prepared by combining 20% FBS and 80% cell media. A volume of 180 µL of the media was added to each flask, resulting in an initial volume of 4 mL.

The cell count analysis was performed by counting cells in four squares and employing a dilution factor of 2. The average cell count per square was 191 cells, resulting in a total cell count of 3.8 million cells. Subsequently, the cell media was changed three days later, and again three days after that. Two days later, passage 5 was conducted, with 750 µL of media added to each flask. For passage 5, the average cell counts were 700,000 for the LBL group and 680,000 for the WOL (without Layer-by-layer) group. Visually there was no significant difference but very slightly more confluent on LBL.

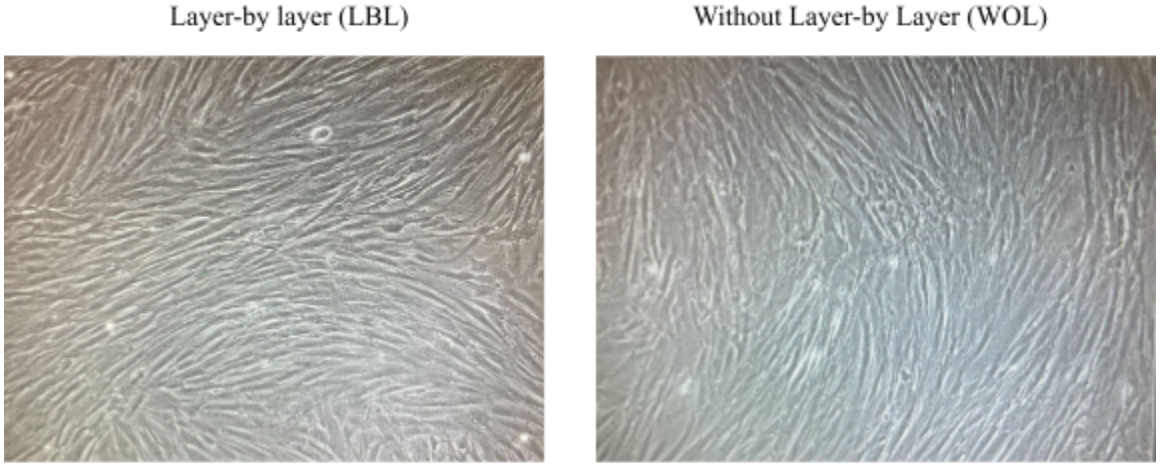



Figure 9. Coating Difference During Passage 5.

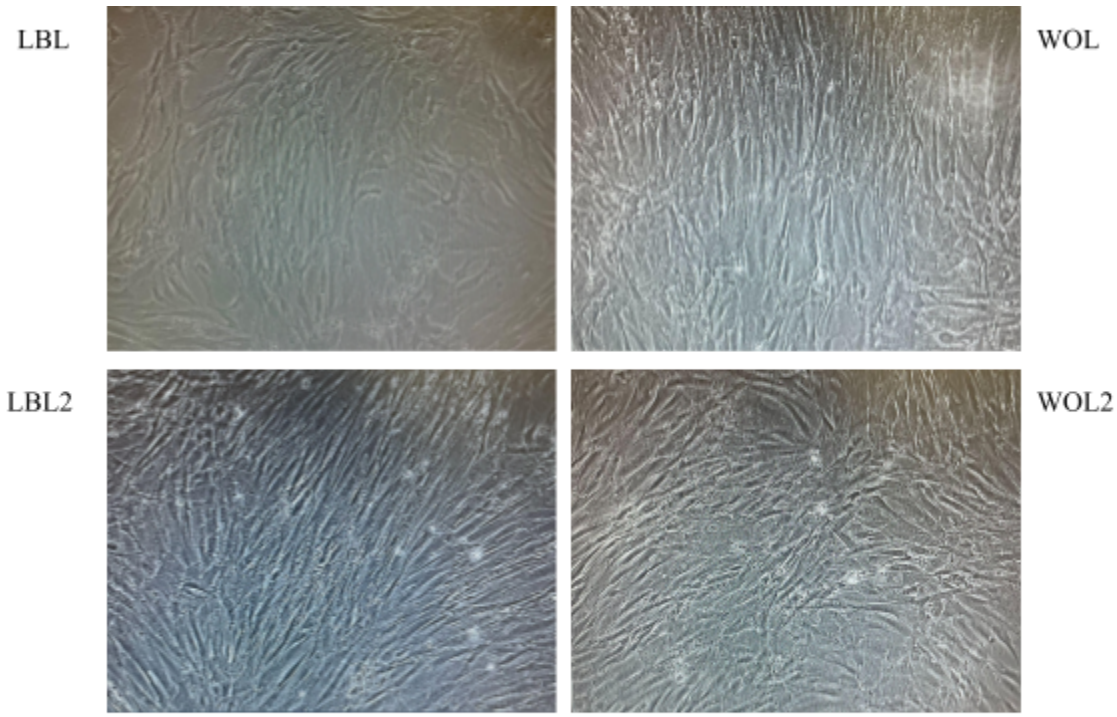


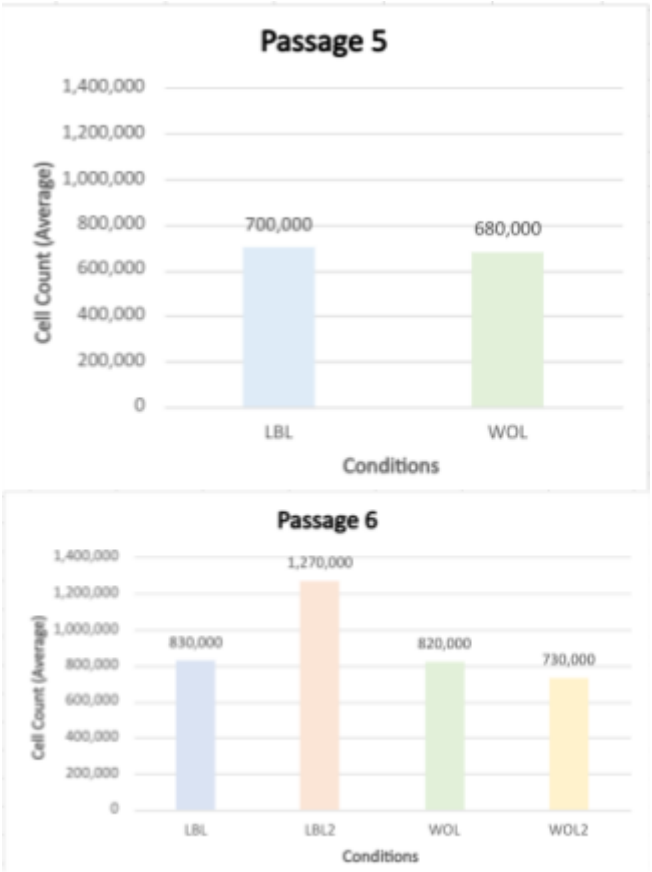

Figure 10. Cell Coating Comparison for Passage 6.

Continuing the experiment, the cell media was changed two days later, then three days later, and once again three days later. Four days later, passage 6 was carried out. The average cell counts at passage 6 were 830,000 for the LBL group, 820,000 for the WOLL

group, 1,270,000 for the LBL2 group, and 730,000 for the WOL2 group. Visually LBL2 was more confluent/compact.

| Passage | Condition | Average Cell Count |
|---|---|---|
| 5 | LBL | 700,000 |
| | WOL | 680,000 |
| 6 | LBL | 830,000 |
| | LBL2 | 1,270,000 |
| | WOL | 820,000 |
| | WOL2 | 730,000 |

Table 2. Experiment 2 & 3 Count Data.




Figures 11 and 12. Experiment 2 and 3 Averages.

Analyzing the cell count results from Experiment 2, it is evident that the LBL2 group demonstrated the most favorable outcomes in terms of cell growth. This indicates that passaging cells into a new flask with a layer-by-layer coating is beneficial for enhancing cell count. This is evident by the literal increases in the number for cell count. Although the average cell growth in the LBL and WOL groups was similar, the WOL group displayed higher variability in cell counts. This observation suggests that the layer-by-layer coating (LBL) may contribute to a higher probability of cell survival. This could be from cell proliferation or attachment. We did not test for that specifically so we cannot be certain. Further testing would need to be conducted using other lab kits. For example, an EdU Assay could be used to measure proliferation. For coated cells, we saw they did not increase as expected. This may be because the cells are eating the coating or they are mimicking the ECM. If they are mimicking the ECM, this means the coating taught the cells that they could mimic ECM even on an adherent surface. It doesn't seem to be negative to have the coatings considering it performed just as well. However, if you were wanting to save money and time, you'd perform sufficiently without the coating.

## DISCUSSION

The results of our study provide valuable insights into the durability and performance of cell coatings for cell therapy applications. The use of cell coatings has emerged as a promising approach to improve the survival and functionality of transplanted cells, addressing key challenges in cell therapy. In this research, we focused on evaluating the stability and effectiveness of coatings applied to cells using the layer-by-layer (LBL) method.

Our findings demonstrate that cell coatings offer a promising strategy for enhancing cell therapy outcomes. The data revealed that the coated cells exhibited a higher number of cells than the control group, showing improved cell growth and survival. This suggests that the coatings create a supportive microenvironment that enhances cell viability and proliferation. The coatings, composed of collagen and heparin layers, were designed to mimic the natural extracellular matrix (ECM) and provide a biomimetic environment for the cells. By modulating cell adhesion, protecting against immune responses, and improving cell-matrix interactions, the coatings promote favorable conditions for cell growth and function.

The choice of cell type is also crucial in cell coating research, as different cell types may respond differently to coatings. In our study, we utilized human mesenchymal stem cells (HMSCs) due to their potential therapeutic applications in regenerative medicine. The coatings applied to HMSCs resulted in enhanced cell viability and proliferation, supporting their potential for improving therapeutic outcomes. However, it is important to consider that the response to coatings may vary depending on the specific cell type and its inherent characteristics.

Comparing the results from Experiment 1 and Experiment 2, we observed variations in cell growth between the different experimental groups. In Experiment 1, the initial cell count analysis showed a higher cell count in the LBL group compared to the control group. This indicates the positive impact of the initial coating on cell growth and survival. In Experiment 2, the LBL2 group, where cells were passaged into a new container with the layer-by-layer coating, demonstrated the most favorable outcomes in terms of cell proliferation. This suggests that the reseeding process onto fresh coatings

further promotes cell growth and supports the notion of the coatings' durability and effectiveness.

The findings of this study contribute to the growing body of knowledge in the field of cell therapy and provide insights into the design and optimization of coatings for enhanced cell survival and function. The ability to improve cell viability, proliferation, and functionality through coatings holds great promise for advancing cell therapy approaches. By creating a biomimetic microenvironment, cell coatings can enhance the therapeutic potential of transplanted cells, ultimately improving tissue regeneration and patient outcomes.

## CONCLUSION

In conclusion, this honors thesis research focused on investigating the reusability and performance of cell coatings for cell therapy applications. The results of our study indicate that cell coatings, prepared using the layer-by-layer (LBL) method, offer a promising approach to enhancing cell growth, viability, and functionality. The coatings create a supportive microenvironment that mimics the natural extracellular matrix (ECM) and promotes cell survival and proliferation. The coatings provide long-lasting benefits such as cell growth and survival.

The data obtained from our experiments demonstrate that the coated cells exhibited a higher number of cells compared to the control group, indicating improved cell growth and survival. This has significant implications for the development of improved strategies for cell transplantation and tissue regeneration. This has significant implications for the development of improved strategies for cell therapy. Increasing the

number of cells can help ensure that an adequate dose of therapeutic cells reaches the affected area, increasing the likelihood of a positive outcome. By administering more cells, the chances are higher for cells to survive inside the body through immune responses. Cells, including hMSCs, often secrete various bioactive molecules and growth factors that can have beneficial effects on the surrounding tissue. With more cells, there is an increased secretion of these factors, enhancing the paracrine effects. This could promote tissue repair, modulate immune responses, reduce inflammation, and stimulate endogenous repair mechanisms.

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# APPENDIX

## Supplemental Figures

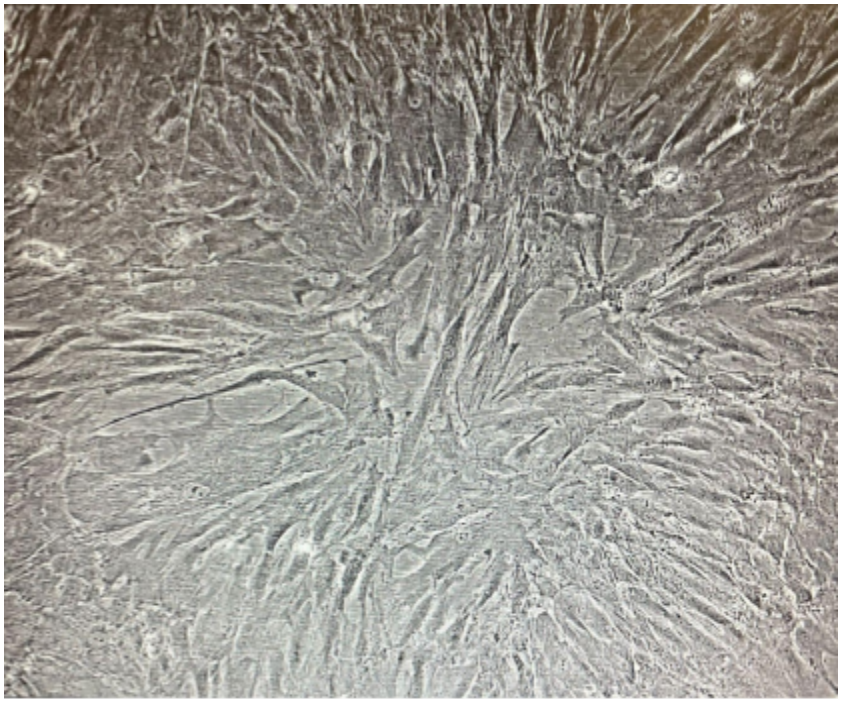


Figure 1. Initial Cells for Experiment 1.

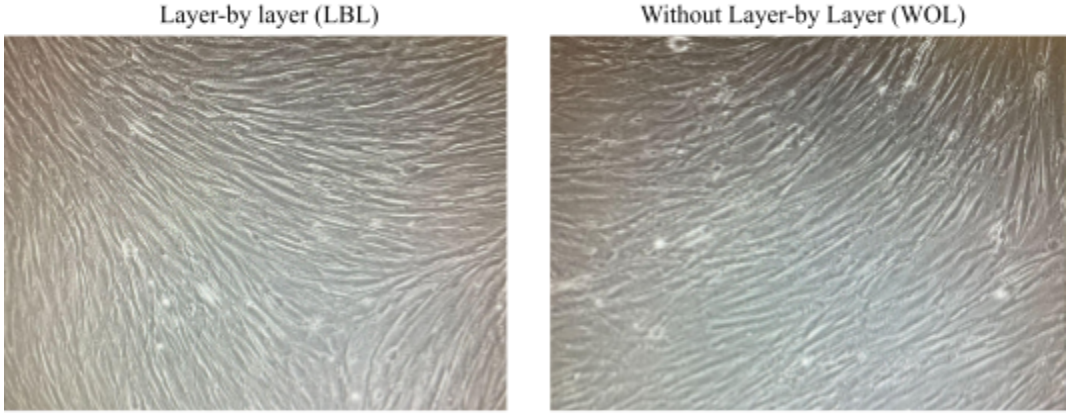



Figure 2. Coating Difference During Passage 5 (Experiment 2/3).

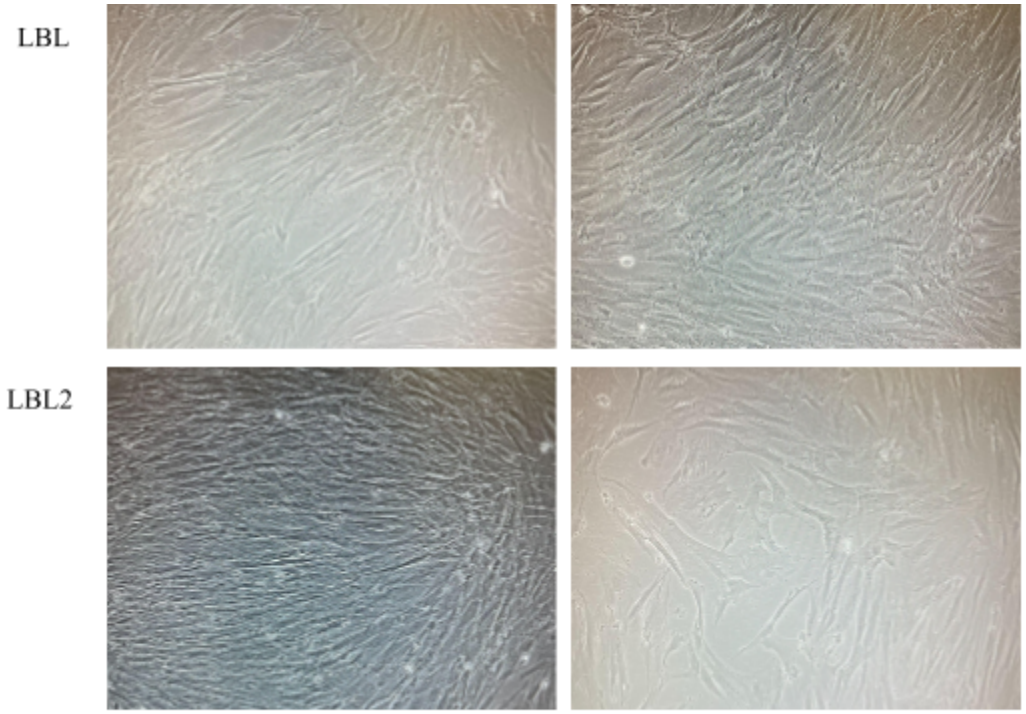
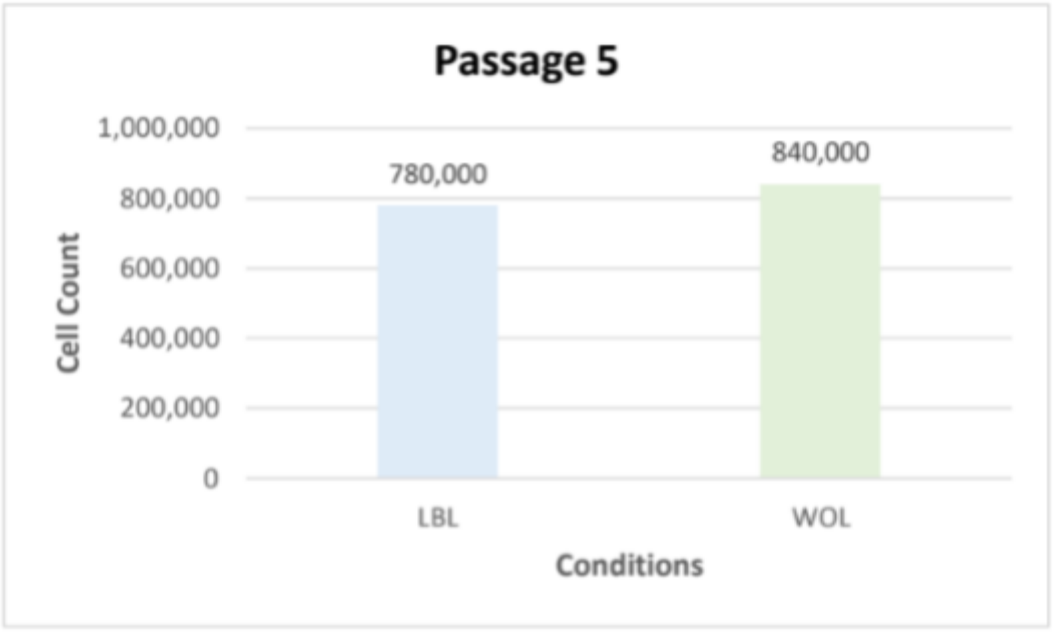
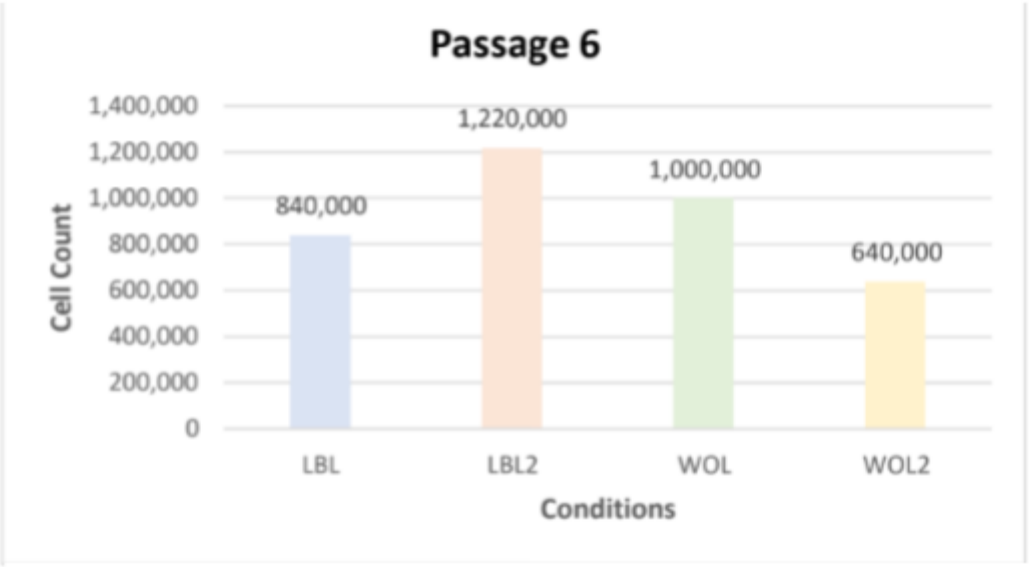
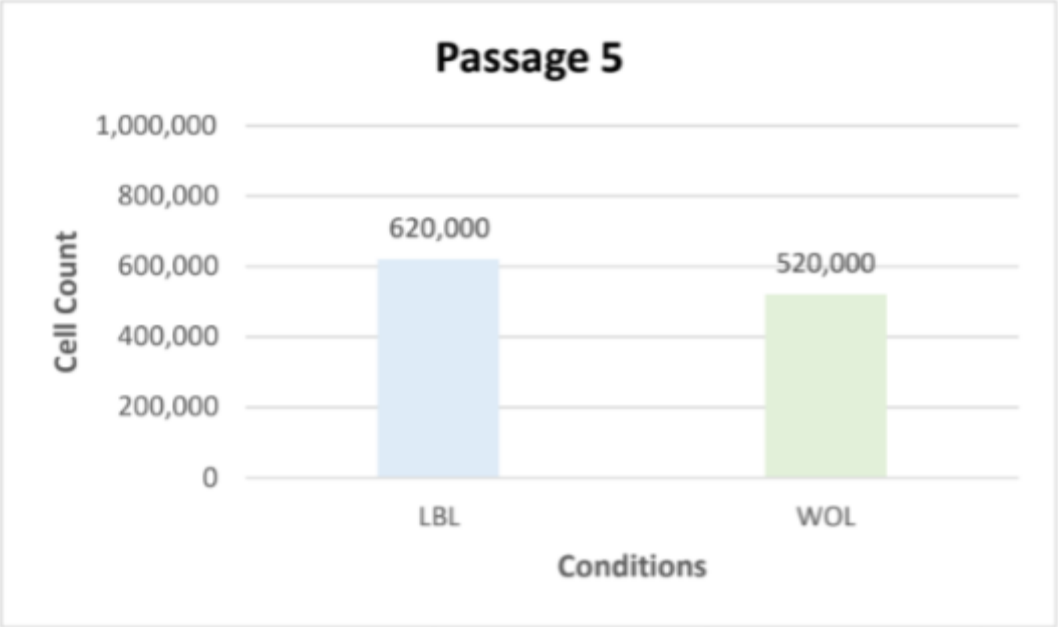


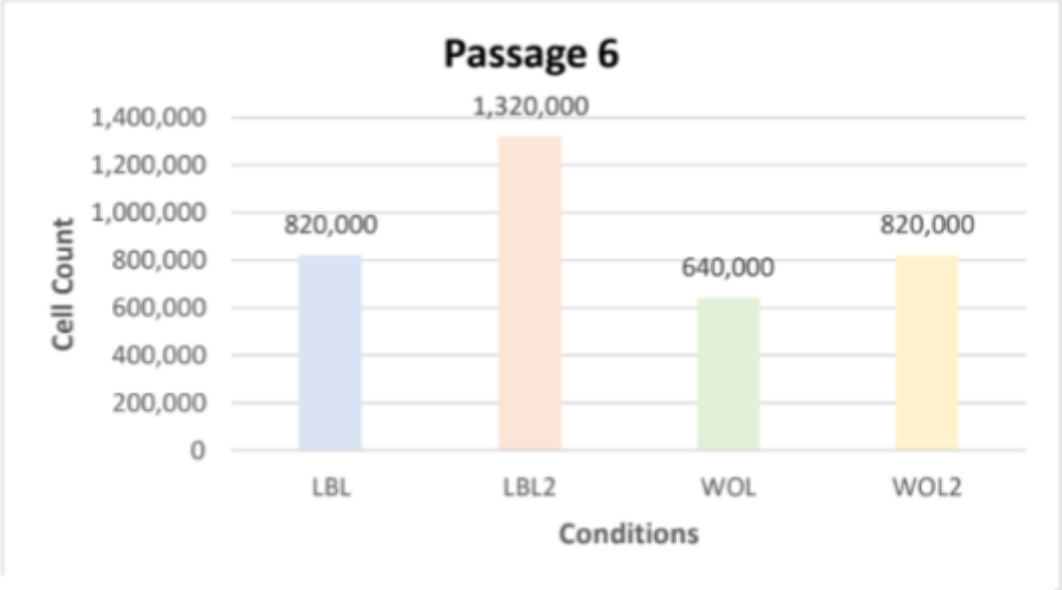

Figure 3. Cell Coating Comparison for Passage 6 (Experiment 2/3).




Figures 6 and 7. Experiment 3 Count.




Figures 4 and 5. Experiment 2 Count.

## Supplemental Tables

| Passage | Condition | Cell Count |
|---|---|---|
| 6 | LBL | 410,000 |
| | WOL | 260,000 |
| 7 | LBL | 500,000 |
| | LBL2 | 450,000 |
| | WOL | 750,000 |

Table 1. Experiment 1 Count Data.

| Passage | Condition | Cell Count |
|---|---|---|
| 5 | LBL | 780,000 |
| | WOL | 840,000 |
| 6 | LBL | 840,000 |
| | LBL2 | 1,220,000 |
| | WOL | 1,000,000 |
| | WOL2 | 640,000 |

Table 2. Experiment 2 Count Data.

| Passage | Condition | Cell Count |
|---|---|---|
| 5 | LBL | 620,000 |
| | WOL | 520,000 |
| 6 | LBL | 820,000 |
| | LBL2 | 1,320,000 |
| | WOL | 640,000 |
| | WOL2 | 820,000 |

Table 3. Experiment 3 Count Data.