Section IV: Discussion

In experimentation, the negative control is just plain media and serves as a control that shows no changes to cell viability and morphology. Through this, majority of human error can be accounted for. The 0.1% Triton and 0.01% Triton both served as positive controls for significant changes to cell viability and morphology. Using preliminary experimentation, it was determined that the ideal positive control would be 0.1% Triton or higher for complete cell disruption and 0.01% Triton for measurable cell disruption.

When microplastics are introduced to the human cells, the cells display initial resistance to intaking the microplastics. However, they are unable to keep up this resistance and soon, the cells start to intake microplastics rapidly. These microplastics can then cause the cells to denature and undergo apoptosis after a certain level of denaturing. However, higher levels of microplastic lead to increased denaturing, which causes the cells to die instead.

This can be seen in Figures 3, 4, 5, and 6. Figure 3 depicts the changes in morphology through cell area. Cell area can be used to show changes to the glycocalyx surrounding the cell along with the endothelial cell membrane. A higher value than the negative control indicates that the cell expanded, and a value lower indicates the cell shrunk. The negative control has a fold change result of 1, as is normal since everything is being compared to that. However, from the graph, it can be seen that the results are more significant for the 10µg/mL concentrations, having p-values less than 0.001 and 0.01, than the 40µg/mL and 100 µg/mL concentrations, where only one was statistically significant with a p-value less than 0.05. This implies that microplastics of lower concentrations cause the cells to expand, however, once the cells start to be exposed to even greater concentrations, they start to shrink again. This can severely affect the cells and denature them quickly.

Figure 4 depicts the changes in morphology through cell perimeter. Similar to cell area, cell perimeter shows changes to the glycocalyx but focuses mainly on the cell membrane. A higher value than the negative control indicates the cell membrane was forced to expand and a lower value indicates the cell membrane was forced to shrink. From the graph, it can be seen that the results were much greater than the negative control for the 10 μg/mL and closer to the negative control for the 100 μg/mL. This implies that the cells were forced to expand and then shrink when exposed to higher concentrations of microplastics, which can severely alter the cell and leave behind irreversible damage.

Figure 5 depicts the changes in morphology through cell maximum radius. This metric is similar to cell perimeter and shows changed to the cell membrane and shape. A higher value than the negative control indicates the cell shape was forced to elongate while a lower value than the negative control indicates the cell was forced to shrink. From the graph, it can be seen that the results for 10 μg/mL and 40 μg/mL were more significant than the results for 100 μg/mL. It can also be seen that the results drop, increase, and then drop again. This implies that the cells were elongated and then forced to change shape again by shrinking. Experiencing such great changes can lead to permanent damage to the cells.

Figure 6 depicts the changes in cell morphology through eccentricity. This metric is similar to cell maximum radius and shows changes to cell shape. A higher value that the negative control indicates the cells were less circular and a lower value indicates the cells were more circular. From the graph, the results for 40 μg/mL were lower than the negative control but the results for 10 μg/mL and 100 μg/mL were greater than the negative control. This indicates that the cell was forced to change shape multiple times. Such rapid changes to shape can harm the cell greatly and lead to malfunctioning of the cell.

Due to increased denaturing, the cells eventually undergo apoptosis. This is as a result of natural body mechanisms in which cells are programmed to commit apoptosis when they are damaged beyond repair.

Figure 2 depicts the cell viability of endothelial cells after being subjected to microplastics, which highlights this effect. The negative control has minimal cell death, indicating that there was minimal human error and thus the results are dependent on the factor that is changing, which is the treatment of the endothelial cells. However, as the concentrations increase for both microplastics from 10 µg/mL to 40 µg/mL to 100µg/mL, the cell viability decreases. This is seen through the increase in percentage of dead cells.

In this experiment, two types of microplastics were used: polyethylene and polypropylene. Microplastics are classified by the types of materials and as a result, different microplastics have different chemical compositions. This means that they interact with cells differently and as such, have different effects on the cells.

Figure 2 highlights these results. While the results for both polypropylene and polyethylene is greater than that of the negative control and 0.01% Triton, the overall cell death is all concentrations of polypropylene was greater than polyethylene. Figure 3 also shows this as the results for the polypropylene microplastics had greater significance from the negative control than the results for the polyethylene microplastics. Additionally, the results for the various concentrations of polypropylene microplastics had higher degrees of variation than that of the polyethylene microplastics. This was consistent across Figures 4, 5, and 6. This highlights that there is variation in the effects of different microplastics and in the case of endothelial cells, polypropylene microplastics seem to have a more significant impact.

Based on the analysis, it can be determined that microplastics have a significant impact on both cell morphology and cell viability, which supports hypothesis 1a. However, the results vary significant across various concentrations where cell viability is decreased as concentrations increases and cell morphology experiences great changes as concentrations differ which disproves part of hypothesis 2a. The results also vary across types of microplastics and in the case of endothelial cells, polypropylene microplastics have a more significant impact, which disproves hypothesis 3a.

A limitation is that this experiment was only conducted once and so the results came from the same sample. This restricts the results of this study to only this cell group and potentially implies that the results cannot be applied to the whole population. However, many cells were taken from the sample to prevent as much of the limitation from restricting the study

Past work has shown that polyethylene microplastics are more toxic to tomato plant cells than polypropylene microplastics (Shi et al., 2022). However, the results from this study indicate that polypropylene microplastics are more toxic to endothelial cells. This highlights the fact that each organism is different and thus microplastics affect each organism differently.

Implications and Applications

The results for this study are critical in showcasing the dangers of microplastics and the need to further investigate them. Highlighting the differences both concentrations and types of microplastics can cause emphasizes the need for detailed research into the field and the need for precision when investigating. The results in this study are consistent with current research in microplastics in which it has been found that microplastics are toxic to plant cells and other types of human cells.

Future Research

One of the main functions of the glycocalyx is to detect signals from the blood flow and send it to the endothelial cells so they can react accordingly. It does this through a set of proteins, of the primary ones being Heparan sulfate proteoglycans. Therefore, further research can be done by subjecting the endothelial cells to shear stress to simulate blood flow before and after microplastic incubation and analyzing the expression of heparan sulfate. This would allow for a deeper understanding into the extent to which microplastics affect endothelial cells and thus the body's response to changes in blood flow.

Additionally, it is critical to determine ways to eliminate these microplastics from the human body. The harmful effects of microplastics in the human body can only be combatted through the removal of microplastics. As such, it is necessary to conduct further research into ways to remove microplastics of various sizes and types from the human body.

Section V: Conclusion

This study aimed to discover how various concentrations and types of microplastics affected the cell viability and morphology of the endothelial cells. To do so, a trypan blue exclusion assay and CellProfiler was used. From this study, it was found that both cell viability and morphology are affected by the presence of microplastics. As concentrations increase, cell viability decreases. However, the greatest change in morphology can be seen for lower concentrations. This implies that the change is so great for higher concentrations of microplastics that it denatures the cells so much, they undergo apoptosis. It was also discovered that polypropylene microplastics are more toxic for endothelial cells than polyethylene microplastics, prompting the research into why it is so. Microplastics are something that are truly dangerous and further research needs to be conducted to determine the extent to which they are harmful.

Microplastics are man-made and must be removed by man before the health effects start to truly affect everyone.