Pathophysiological Disruption of the Endothelial Glycocalyx by Microplastic Contamination

Grant Proposal

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Author Note

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Abstract

Endothelial cells are a vital group of cells found in both blood and lymphatic vessels and are responsible for many physiological functions in the human body, the most significant being the production of the glycocalyx. The glycocalyx is a carbohydrate-rich fibrous layer that lines the surface of endothelial cells and plays several key roles in the human body. However, small plastic particles, known as microplastics, can be found in the blood vessels, potentially posing a threat to endothelial cells and the glycocalyx. These microplastics enter the body through various methods, including inhalation and ingestion. They are newly discovered and thus there is little research on them – but they are known to be very toxic.

The goal of this study is to investigate the effects these microplastics can have on endothelial cells, and consequently, the glycocalyx. This study utilizes two of the most common types of microplastics - polyethylene and polypropylene - to investigate the effects of microplastics on the viability and morphology of human lung endothelial cells. A trypan blue exclusion assay, ImageJ, and Cell Profiler were used to analyze cell viability and morphology. From this study, it was determined that microplastics do in fact affect both cell viability and cell morphology. This study aims to showcase the dangers of microplastics in hopes of conveying the true threat these small plastic particles pose. Through this, the study hopes to show the need for further research into microplastics and for an alternative form of plastic.

Keywords: microplastics, endothelial cells, glycocalyx, cell viability, morphology, triton X-100

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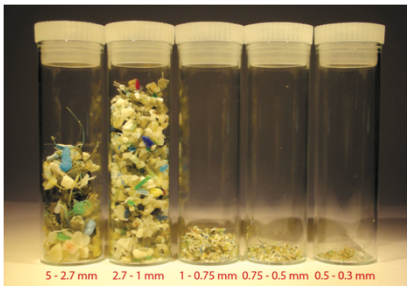

Figure 1. Microplastics collected from a Manta trawl of sizes ranging from 5 mm to 0.3 mm (Olabode & Fulmer, n.d.).

Microplastics are plastic particles that can range from 1 nanometer to 5 millimeters in size. These can be found everywhere: from the air we breathe to the water we drink. Even though they are so prevalent, microplastics were first discovered in 2004 by Marine Biologist Richard Thompson (Schmid et al., 2021). However, microplastics were not detected in the human body until 2022 when researchers in the Netherlands found microplastics in human blood samples (Roeloffs, 2024). Microplastics can be made of various materials and even though little is known about them, existing research about them shows their harmful effects.

Types of Microplastics

There are two main categories of microplastics: Primary and Secondary, which are differentiated by how they were created. Primary microplastics are produced by manufacturers to be small. They can come in the form of either pellets (called nurdles) or in the form of microbeads, which are often manufactured for the cosmetic and pharmaceutical industries (*Microplastics – Pollution Tracker*, 2016). Secondary microplastics, on the other hand, result from the breakdown of larger plastics – often beach litter or laundry (*Microplastics – Pollution Tracker*, 2016). Overall, there are 13 types of microplastics, differentiated by material. However, the most common types of microplastics are polyethylene, polypropylene, and polystyrene (Olabode & Fulmer, n.d.).

Effects of Microplastics

Microplastics are a new discovery, so little research has been done on them. Nevertheless, studies have shown that microplastics are extremely toxic, inducing oxidative damage, DNA damage, organ dysfunction, metabolic disorders, weakened immune responses, neurotoxicity, reproductive toxicity, and developmental toxicity (Li et al., 2023).

Endothelial Cells

Endothelial cells line all blood and lymphatic vessels found in the human body and thus play a key role in blood transport and human survival. In fact, these cells are often the first line of defense for any ingested toxins. Endothelial cells are most commonly found in arteries, veins, capillaries, and lymph capillaries. In lymph capillaries, endothelial cells function as a semi-permeable barrier, allowing for the transport of lymph fluid and facilitating the movement of immune cells to lymph nodes – which is necessary for the immune system to function (Cleveland Clinic, 2022). In arteries, veins, and capillaries, endothelial cells are responsible for producing glycocalyx, a vital substance for human survival (Villalba et al., 2021).

Vascular Endothelial Glycocalyx

The endothelial glycocalyx is a carbohydrate-rich, fibrous layer that lines the inner surface of endothelial cells and blood vessels, thus playing many vital roles in human survival (Reitsma et al., 2007). Functions of the glycocalyx include permeability and regulating the transport of water proteins and other essential molecules from the blood to the outside of blood vessels. Along with this, it can restrict certain molecules from passing through endothelial cells, thereby protecting blood vessels. This restriction can be attributed to the structure and negative charge of the glycocalyx (Jin et al., 2021).

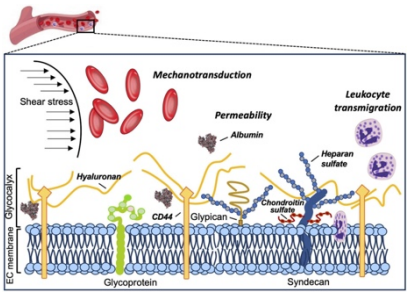

Figure 2. A diagram depicting the glycocalyx surrounding the endothelial cell membrane and performing its various functions (Villalba et al., 2021).

The glycocalyx is also necessary for inflammation. It plays an essential role in the occurrence and development of inflammations, a defense mechanism that allows the body to fight off infections and heal. When inflammation occurs, the glycocalyx is shed, making it easier for leukocytes to bind to endothelial cells. This binding is essential for immune response. The glycocalyx plays a key role in the anticoagulant process as well. Under certain conditions, the interaction between endothelial cells and blood cells can be altered, avoiding thrombosis. The glycocalyx can induce anticoagulant effects by interacting with antithrombin III, thrombomodulin, tissue factor pathway inhibitors, and other molecules. (Jin et al., 2021).

The glycocalyx is also necessary for sending signals. It detects changes in blood flow and then transmits that information to the endothelial cells, which enables the cells to respond with various morphological responses. The glycocalyx can also regulate apoptosis of the endothelial cells, allowing them to die whenever necessary and keeping them alive otherwise. A key function of the glycocalyx is cerebrovascular micro-homeostasis. The glycocalyx is responsible for maintaining the barrier function of the cerebral blood vessels. It regulates the permeability of the blood-brain barrier (BBB) and plays a key role in cerebrovascular coagulation and neuroinflammatory processes (Jin et al., 2021). This creates a need to study the effects of microplastics on these vital cells.

In this study, to determine the effects of microplastics on the glycocalyx, endothelial cells will be incubated with microplastics. Then, the survival and functioning of the cells will be measured before and after incubation using Trypan. By doing so, the dangers of microplastics for blood vessels will be determined, and information will be contributed to a field in which minimal research exists.

Section II: Specific Aims

This proposal's objective is to determine the effects that microplastics can have on endothelial cells and the glycocalyx. The central hypothesis of this proposal is that both polyethylene and polypropylene microplastics will have significant effects on cell viability and morphology. Specifically, the higher the concentrations of both types of microplastics, the less cell viability and the greater the morphological changes, but the results will be more significant for polyethylene microplastics in comparison to polypropylene as based on a study by Wang et al.

Specific Aim 1: Determine the appropriate concentration of Triton X-100 to be used as a positive control for endothelial cells, as different cells require different concentrations.

Specific Aim 2: Determine the differences in polyethylene and polypropylene microplastics' impacts on endothelial cell viability and morphology as different types of microplastics have different compositions.

Specific Aim 3: Determine if there is a level of microplastics that is safe for the endothelial cells and if various concentrations of microplastics have different effects on the cell viability and morphology of endothelial cells.

The expected outcome of this work is that the higher the concentration of microplastic, the less the cell viability and the greater the change to cell morphology. However, the effects will, overall, be greater for polyethylene than polypropylene microplastics.

Section III: Project Goals and Methodology

Relevance/Significance

Very little is known about microplastics and their effects overall. Endothelial cells play many key roles in the human body. By investigating the effects of microplastics on endothelial cells, more information will be contributed to a field in which there is minimal insight.

Innovation

Recent studies have shown that microplastics can have adverse effects on endothelial cells. For example, polystyrene microplastics can alter endothelial cell behavior inducing inflammation, which leads to cellular and bodily harm. (Vlacil et al., 2021). Other studies have shown that microplastics can reduce cell viability and increase oxidative stress responses in Caco-2 and HT-29 cells (Herrala et al., 2023). Thus, by determining the effects of various microplastics on endothelial cell viability and morphology, this study covers a broader area of concern in another group of cells that are key to the body.

Methodology

1) Create six experimental groups where various concentrations (10 µg/ml, 40 µg/ml, and 100 µg/ml) of both types of microplastics are created using dimethyl sulfoxide (DMSO) and integrated with media to be given to the cells.

2) Create one negative control group where the media given to the cells is not treated with anything and one positive control group where the media is mixed with a pre-determined concentration of Triton
3) Each group of cells will be incubated with the media for 24 hours and then analyzed using Trypan blue staining and a light microscope.
4) Images will be taken and analyzed using Cell Profiler

Specific Aim #1: Determine the appropriate concentration of Triton X-100 to be used as a positive control for endothelial cells as various cells require different concentrations.

The approach for this section will be to test various concentrations of Triton X-100. The concentrations being tested will be 0.001%, 0.01%, 0.1%, 1%, and 5%. These will be mixed with media and given to the cells. After being incubated for 24 hours, the cell viability and morphology will be tested.

Justification and Feasibility. Through testing various concentrations, the appropriate positive control can be determined. Triton X-100 is a widely used surfactant in scientific experiments to lyse cells. However, proper concentrations of Triton must be added to the cells, otherwise it can mess up the entire experiment setup and must be repeated. Cell viability and morphology are extremely sensitive and differ based on the type of cell. Slight variations can have significant impacts on the cells. As shown in Figure 3, even though the concentration was only varied by 0.02 mM, the results for 0.17 mM and 0.19 mM concentrations varied greatly (Koley & Bard, 2010). Thus, it is necessary to determine the appropriate level of Triton so as to not ruin the experiment.

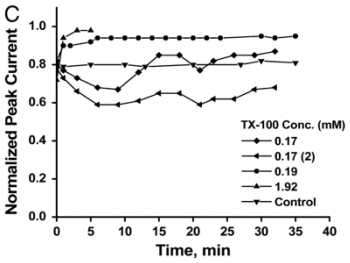

Figure 3. Normalized peak current plotted based on time for various concentrations of Triton X-100 and a control in HeLa cells (Koley & Bard, 2010).

Summary of Preliminary Data. From Figure 4, it can be seen that various Triton concentrations affect the cells differently. Concentrations such as 0.001% are too low and don't yield significant enough results. Concentrations such as 1% and 0.1% are too high and can affect the cells too much. However, Triton is necessary as it signifies complete cell death, as shown in Appendix 2: Preliminary Graph. Thus, this creates a necessity to determine the proper concentration to use for experimentation to have a proper control and be successful.

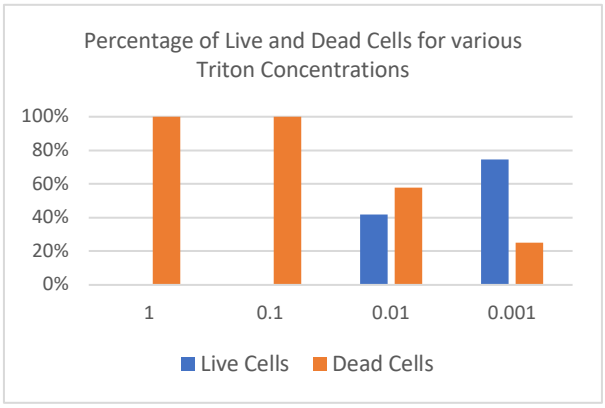

Figure 4. Percentage of Live and Dead Cells for four different Triton concentrations

Expected Outcomes. The goal of this aim is to determine the appropriate concentration of Triton X-100 in which the expected behavior is displayed but doesn't affect the entire experiment, thus leading to the expected outcome of one concentration leading to better results than the others. This knowledge will be used for the determination of the positive control when running actual experimentation.

Potential Pitfalls and Alternative Strategies. A potential pitfall with this strategy is that none of the chosen concentrations to test might actually work the best for endothelial cells. Thus, to fix this, more concentrations will need to be tested. An alternative strategy that could also be a supporting strategy would be to do research specifically on Triton X-100 use on endothelial cells and use the concentration used in those studies.

Specific Aim #2: Determine the differences in the effects of polyethylene and polypropylene microplastics on endothelial cell viability and morphology, as different types of microplastics have different compositions.

Justification and Feasibility: Different types of microplastics have different chemical compositions. Thus, these variations in chemical composition can lead to differences in the way they interact with the cell due to polarity. These differences can then lead to

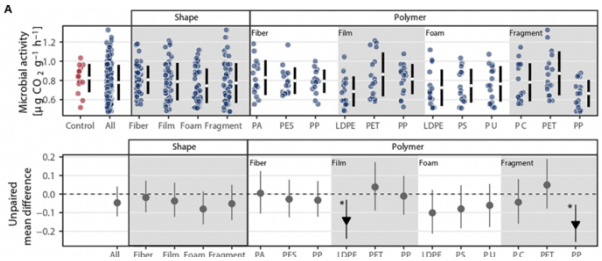

Figure 5. Microbial activity in *D.carota* in response to various microplastic shape and type (Lozano et al., 2021).

different results. As shown in Figure 5, the type of microplastic had a great impact on the microbial activity of the plant (Lozano et al., 2021). This variation brings up a need to determine the effects of multiple types of microplastics, as there is more than one kind in the air we breathe, food we eat, and water we drink. Thus, it's essential to consider multiple types as they have different effects.

Expected Outcomes: The goal of this aim is to determine how various microplastics can affect cell viability and morphology. Thus, the expected outcome is that one microplastic type (polyethylene) has a greater impact than another (polypropylene). This knowledge will be used to show that these results are widely applicable as it considers more than one type of microplastic.

Potential Pitfalls and Alternative Strategies: An alternative strategy is that instead of choosing two of the most common types of microplastics, research would be completed on which types are similar and different. By doing this, a wider variety of microplastics could be studied.

Specific Aim #3: Determine if there is a level of microplastics that is safe for the endothelial cells and if various concentrations of microplastics have different effects on the cell viability and morphology of endothelial cells.

Justification and Feasibility: Various concentrations of microplastics can affect the cells differently. In real life, not everyone's bodies will contain the same amount of microplastics, leading to variation. This leads to the potential that there are levels of microplastics that could be safe for the cells. As shown in Figure 6, a small difference in microplastic concentration can lead to a large difference in the function being measured (Wang et al., 2021). These differences bring up a necessity to investigate the effects of various concentrations of microplastics.

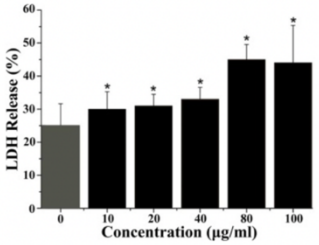

Figure 6. Percentage of LDH release at different concentrations of polyethylene (Wang et al., 2022)

Expected Outcomes: The overall goal of this aim is to determine how various concentrations of microplastics will affect the endothelial cells. Thus, the expected outcome is that the higher the concentration, the less the cell viability and the greater the morphological changes. This knowledge will be used to determine if

there is, in fact, a healthy level of microplastics or a level of microplastics that does not affect the cells negatively.

Potential Pitfalls and Alternative Strategies: A pitfall is that DMSO might cause an error in results. An alternative strategy is that instead of measuring various concentrations by dissolving them in DMSO, dissolving them in another substance. By using another liquid, such as water, the potential errors and chemical components associated with DMSO could be avoided.

Section III: Resources/Equipment

In order to do this experiment, an incubator will be used. This incubator will be kept at 37°C with 5% Carbon Dioxide - ideal conditions for cells (Wang et al., 2022). This equipment will be used to keep the cells alive once they have been thawed and will store the cells during the 24-hour incubation period. Additionally, the cells used will be sourced from Cell Applications Inc. cat# 540-05a and provided by the Dr. Mensah Lab.

Section IV: Ethical Considerations

All work will be performed under a laboratory hood to avoid inhalation of the microplastics. For similar reasons, proper PPE, including gloves, lab coats, and goggles, will be worn. Masks will be worn when dealing with microplastics. All biohazardous materials will be disposed of in the appropriate containers, including microplastics and human cells. The media used to dose the cells will be aspirated in 10% bleach and the human endothelial cells will be disposed of in 10% iodine. All experimentation will be performed in a BSL1 laboratory.

Section V: Timeline

The projected timeline will be as follows. The first three weeks of November will be spent finding a lab and finalizing the methodology. The last week of November and the first week of December will be spent collecting preliminary data which will aid in the actual data collection and analyzing it. The next three weeks of December and the first two weeks of January will be spent collecting actual data. The next week of January will be spent analyzing the data. The next two weeks will be spent finalizing all analyses and preparing for a presentation. This is a link to the outlined timeline: Link to Kan-Ban Chart.

Section VI: Appendix

Appendix 1: Grant Agency

This grant appeals to the National Institute of Environmental Health Sciences (NIEHS) agency. They issued a Notice of Special Interest for those projects regarding the effects of microplastics and nanoplastics on human health in 2022. The purpose of this Notice of Special Interest is to support research that aids in gaining a comprehensive understanding of the physiochemical characterization, exposure, and related human health effects of microplastics and nanoplastics (*NOT-ES-23-002*, n.d.).

Appendix 2: Preliminary Graph

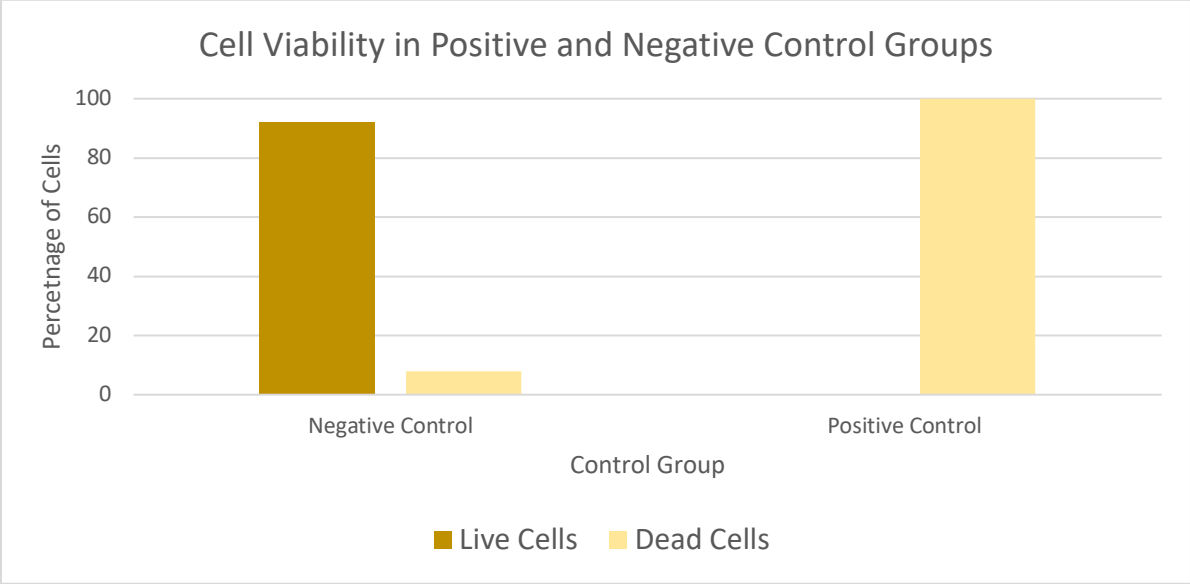

Figure 7. Cell Viability of Endothelial Cells after no treatment (negative control) and being treated with 1% Triton X-100 (positive control)

This graph shows the effects of Triton X-100 on the cells. From the graph, it can be clearly seen that when left normal, there is minimal cell death. However, when treated with Triton and everything else kept constant, there is complete cell death.

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