Summer 7-21-2022

The Effects of Polyethylene Terephthalate Nanoplastic Particles on the Metabolism and Expression of Cell Stress Markers in Human Caco-2 Cells

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Summer Undergraduate Research in Biology

Abstract

Humans interact with plastics daily due to their versatile use. Polyethylene Terephthalate (PET) is the most common type of plastic, and is used in the food and beverage packaging industries. As plastics degrade in micro- (<5 mm) and nano- (<1 µm) sized particles, these can enter the human body through ingestion, dermal contact and inhalation. Once micro- and nanoplastics are internalized, they can be absorbed through epithelial tissues in the lungs and intestines. The human epithelial adenocarcinoma Caco-2 cell line is an important model for intestinal cells. Although studies have shown that Caco-2 cells uptake PET nanoplastic particles, at low concentration there is no cytotoxic effects. Bioaccumulation is known to occur with nanoplastic uptake that may lead to higher intracellular levels of PET. Nothing is known about the effects of higher PET particle concentration in Caco-2 cells. This study examined the metabolism, and expression of the cell stress markers iNOS, KRAS, IL-1β, and COX-2 in human Caco-2 cells when exposed to 50, 100, and 200 µg/ml of PET nanoplastics for 72 hours. Although it was observed that KRAS, IL-1β, and COX-2 were upregulated in the PET treated samples relative to the control, there appeared to be no change in cell viability. These results suggest that PET nanoplastic particles do have an impact on the expression of cell stress markers in Caco-2 cells, but additional pathway mediators should be investigated to establish a clearer understanding of the effects of PET exposure on gut epithelial cells.

Introduction

Plastics consist primarily of polymers that can be found as primary plastics, which remain their manufactured size, or secondary plastics, which degrade from larger fragments. This degradation occurs through prolonged UV light exposure, microbial degradation, and physical abrasion.[1] When plastics are degraded to less than 5mm in diameter, they exist as microplastics, and further fragmentation results in nanoplastic particles which are less than 1 µm in diameter.[2] These small particle sizes raise a concern for human health as they are primarily taken up through ingestion.[2]

Polyethylene terephthalate (PET) is the most common type of plastic as it possesses versatile properties such as being lightweight, having gas barriers, and high-tensile strength.[3] These properties make PET useful and common in the food and beverage packaging industries.[3] Therefore, PET micro- and nanoplastics are prevalent in the environment and known to be ingested and inhaled. Once these small particles enter the body, they can be absorbed through epithelial tissues in the lungs and intestines. The human epithelial adenocarcinoma Caco-2 cell line is an important model of the intestinal barrier as the cells are heterogeneous and express functional and morphological characteristics of mature intestinal enterocytes.[4] Caco-2 cells are known to uptake PET nanoplastic particles.[5,6] PET particles at concentration up to 30 µg/mL do not affect cell viability, membrane integrity, or the production of pro-inflammatory cytokine proteins.[6] However, the effect on the expression of cellular stress markers or growth have not been investigated at higher concentrations that might be experienced with bioaccumulation. Research has shown that polystyrene nanoplastics can alter various proliferation and inflammation related genes in Caco-2 cells at higher concentrations,[7] but again these effects remain unknown when Caco-2 cells are exposed to higher concentrations of PET.

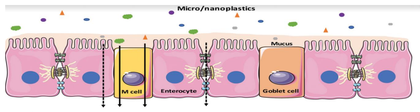


Figure 1. Diagram showing the endocytic uptake of micro- and nanoplastics by the intestinal epithelium.[1]

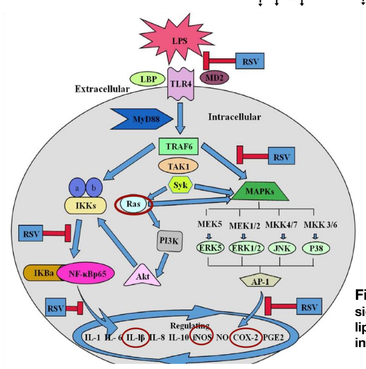


Figure 2. Model of Caco-2 stress signaling pathways induced by lipopolysaccharide. Targets of interest are circled in red.[8]

Results

Nile Red Stained PET Nanoplastic Uptake Assay

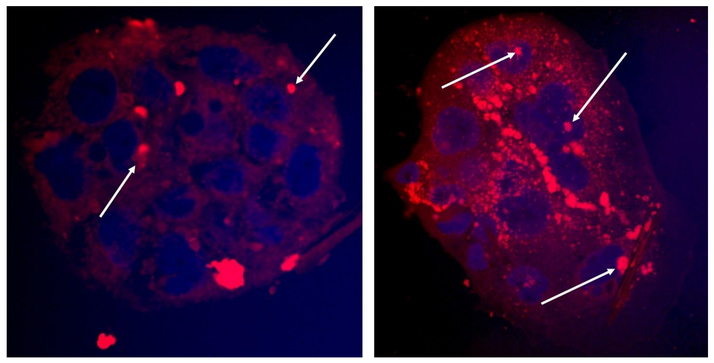


Figure 3. (Left) 60x images of Caco-2 cells treated for 72 hours with 200 µg/ml of Nile Red stained PET nanoplastic particles. Nuclei were stained with DAPI (blue). Images were captured using fluorescent microscopy. White arrows point to stained PET particles taken up by the Caco-2 cells.

Figure 5. (Below) qRT-PCR results for cell stress markers in Caco-2 cells exposed to PET treatments. Data for $\Delta\Delta C_T$ calculations are from the threshold (grey line) cycle numbers.

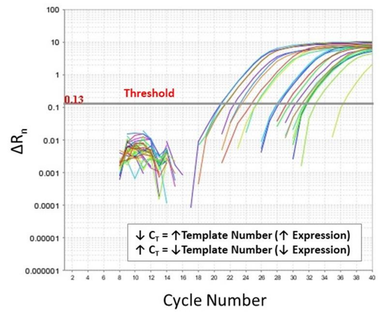
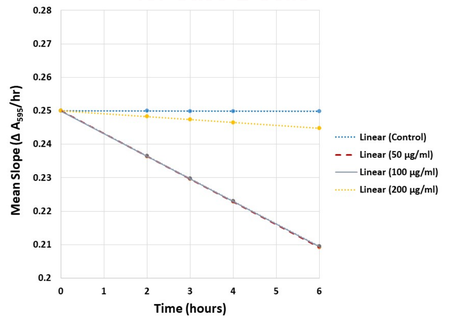


Figure 4. (Right) Graph depicting the normalized average rate of metabolic activity change (growth rate) measured by resazurin dye at A_{595} per hour in Caco-2 cells.



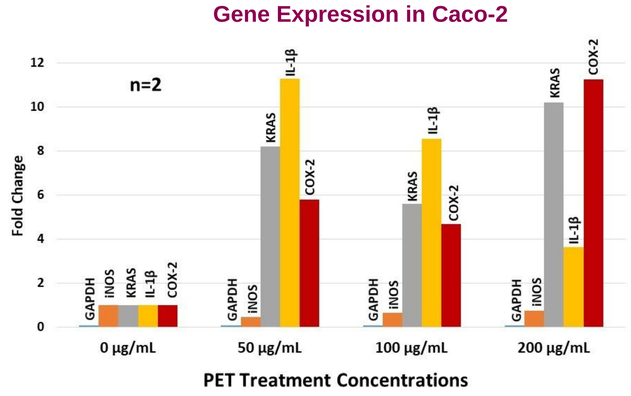


Figure 6. (Right) qRT-PCR analysis of the fold change in gene expression in Caco-2 cells after treatment with three different PET concentrations relative to the control. Fold change values are calculated as $2^{-\Delta\Delta CT}$. The fold change of KRAS, IL-1β, and COX-2 was greater than 2, and indicate probable increase in expression of these targets.

Question/Hypotheses

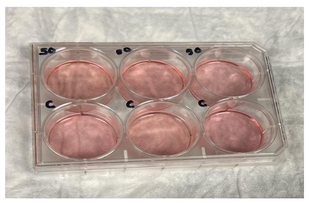
- **Do Caco-2 cells show significant changes in metabolic activity and the expression of specific stress markers when exposed to PET nanoplastics at greater than 30 µg/mL?**
- **There will be a decrease in cell viability of Caco-2 cells when exposed to PET nanoplastic particles.**
- **There will be a change in the expression of specific cell stress markers in Caco-2 cells when exposed to PET nanoplastic particles.**

Conclusions

- **Polyethylene terephthalate (PET) nanoplastic particles are taken up by Caco-2 cells.**
- **The fold change of KRAS, IL-1β, and COX-2 at 50, 100, and 200 µg/ml PET nanoplastic concentrations indicate probable increase in expression of these targets in Caco-2 cells.**
- **Cell viability of Caco-2 cells is relatively unchanged for any PET concentrations.**

Methods

Experimental Design

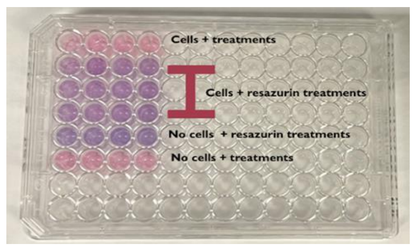


- 300,000 cells were seeded per well into two 6-well plates.
- Cells were treated with 0, 50, 100, and 200 µg/ml PET concentrations for 72 hours.

Total RNA Extraction and cDNA Synthesis

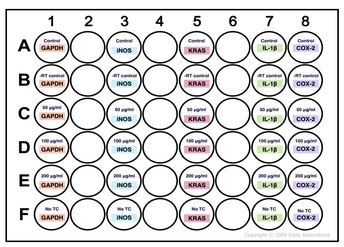
- Total RNA was isolated using the Qiagen RNeasy Plus Mini Kit and quantified on the NanoDrop.
- RNA was converted to cDNA using the High-Capacity RNA to cDNA kit.

Proliferation Assay



- Cell metabolic activity was quantified using the Resazurin Assay Kit (Abcam).
- Results were read by absorbance at 595 nm on a microplate reader to determine the cell metabolic activity change from 0 to 6 hours.

qRT-PCR Analysis



- Using the 48-well plate template depicted above, the control, no reverse transcriptase control (-RT), PET concentrations of 50, 100, 200 µg/ml, and the no template control (no TC) were added to their respective wells for qRT-PCR analysis.
- Primers for human iNOS, KRAS, IL-1β, and COX-2 were added to the treatments, and the amplifications of these targets were used to quantify the change in gene expression relative to the control.

Acknowledgements

This research was funded by the National Science Foundation REU-Site Grant DBI-1950350 and the Natural Science Division of Pepperdine University.

Special thanks to my incredible mentor Dr. Vandergon for all his guidance and support. I would also like to thank my lab partners David and Diana for their help along the way.

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