Investigating the Inflammatory Effects of Microplastics on Human Health Using 3D Liver Models

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Introduction

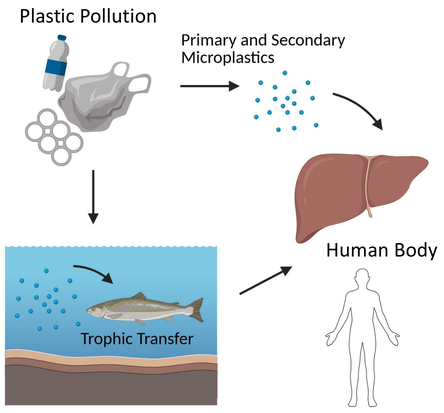
The Ocean Cleanup/EPA, via Shutterstock

Plastics in the Ocean

The Great Pacific Garbage Patch has an area of **1.6 million square kilometers**, approximately three times the size of France.[2]

Microplastics account for **94%** of the estimated **1.8 trillion pieces** floating in the area[2]

Microplastics in the Environment



Routes of Entry into the Body

Ingestion

- Microplastics have been detected in **more than 100** brands of table salt.[3]

Inhalation

- Humans inhale a **credit card's amount** of microplastics **every week**.[1]

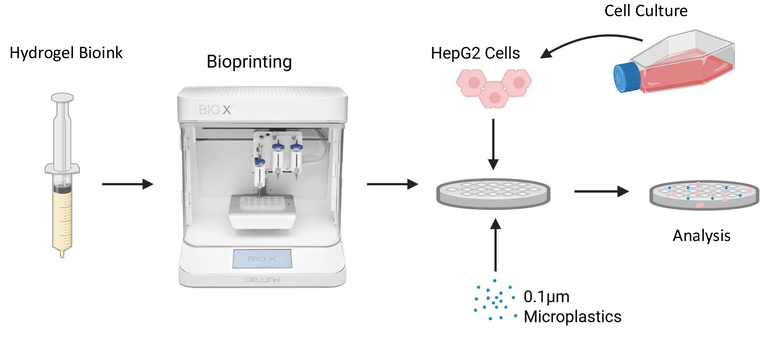
Dermal Contact

- Toxic plastic additives, such as flame retardants can enter the skin through contact.

Research Objectives

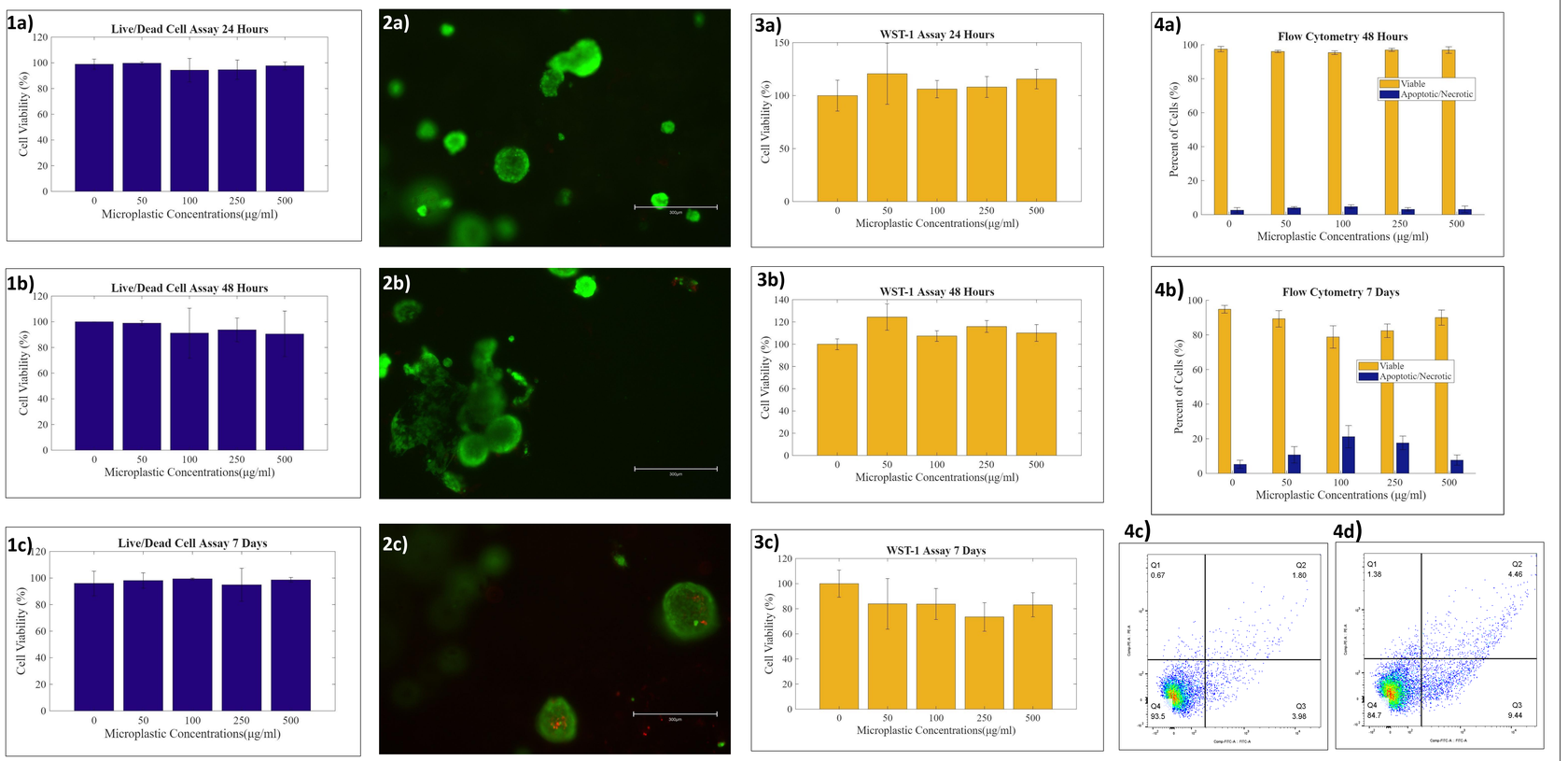
Investigate the inflammatory effects of microplastics on the liver using a novel 3D model approach that better simulates cell to cell interactions in the liver.

Methods



3D liver models were developed and treated with various concentrations of microplastics, and multiple assays (live/dead, WST-1 and flow cytometry for apoptosis) were performed to analyze cell viability and inflammatory responses after incubation for different time points.

Results



1) Cell viability calculated from Live/Dead Cell Assay at various time points a) 24 Hours after microplastic exposure, b) 48 Hours after microplastic exposure, and c) 7 days after microplastic exposure.
2) Images taken using an EVOS Fluorescent Microscope of cells after 7-day exposure to a) 0 µg/ml microplastics, b) 100 µg/ml microplastics, and c) 500 µg/ml microplastics. Green fluorescence indicates live cells, and red fluorescence indicates dead cells. Scale = 300 µm.
3) Cell Viability calculate from a WST-1 assay at various time points a) 24 Hours after microplastic exposure, b) 48 Hours after microplastic exposure, and c) 7 days after microplastic exposure.
4) Results of apoptosis analysis using an Annexin V/FITC assay for flow cytometry at various time points a) 48 Hours after microplastic exposure and b) 7 Days after microplastic exposure. The gold bar indicates the percentage of cells that are viable, and the navy bar indicates the percentage of cells undergoing early apoptosis, late apoptosis and necrosis. Quadrants 1-3 of c) and d) indicate cells that are apoptotic and necrotic, whereas quadrant 4 indicates viable cells. Both graphs were collected from samples treated with 500 µg/ml microplastics at time points c) 48 hours after microplastic exposure and d) 7 days after microplastic exposure.

Conclusions

- Scaffolds for the 3D liver models were 3D printed using a combination of Gelatin Methacrylate (GelMA) and Poly(ethylene glycol) Dimethacrylate (PEGDMA).
- HepG2 cells were successfully cultured on the 3D printed scaffolds.
- Upon treatment with 0.1 µm polystyrene microbeads, dose-dependent decrease in cell viability was observed on day 7 using WST-1 assay.
- Flow cytometry studies confirmed that a greater number of cells entered the apoptosis/necrosis stage at higher microplastics concentrations on day 7, when compared to the 48h timepoint.

Future Studies

- Higher Concentrations (1000 and 2000 µg/ml)
- Longer Time Points (14 days and 21 days)
- Different cell types (ex: Kupffer Cells)
- Different types of microplastics (ex: environmental samples, microfibers)
- Investigate indicators of cell stress (ex: cytokine release, reactive oxygen species (ROS))

References

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Acknowledgements

This work is supported by the U.S. National Science Foundation under Award #2348968, REU Site: URI Plastic Initiative at the University of Rhode Island, and the Donald R. Wilson Jr. Family Foundation. Partial support for professional development activities was provided by EPSCoR Cooperative Agreement #OIA-2433276 and the RI Commerce Corporation through the Science and Technology Advisory Committee. Any opinions, findings, conclusions, or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the funding partners.