Proteomic analysis of Macrophage cells exposed to perfluorooctane sulfonic acid (PFOS)

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Abstract

Per- and polyfluoroalkyl substances (PFAS) persist in the environment and pose significant health risks. Previous studies show that exposure to Perfluorooctane sulfonate (PFOS), a prevalent type of PFAS, disrupts cell signaling pathways critical for immunity and cancer. Macrophages are innate immune cells that act as a major defense against foreign agents. In this study, RAW 264.7 Macrophage immune cells were cultured and incubated with various PFOS concentrations. Protein precipitation and trypsin digestion were performed on the collected cell lysates. Peptides were analyzed using Liquid Chromatography-Mass Spectrometry (LC-MS) techniques and the Proteome Discoverer software. Using the Panther Classification System, proteins were found to be important in several biological processes and pathways. Through protein identification and characterization, we hope to improve our understanding of the toxic effects related to PFOS exposure on immune cell function.

Introduction

Per- and polyfluroaklyl substances (PFAS) are widely distributed in consumer products that are used daily, including cookware, carpets, clothing, furniture, and fire-fighting foams (Dewapriya et al., 2023). It has been shown that these compounds pose significant health risks in humans, such as organ damage, weakened immune response, reproductive issues, and various types of cancers. (Perez et al., 2013). The USGS reported a study estimating that at least 45% of tap water in the United States has one or more of the 14,000+ types of PFAS (Smalling et al., 2023). Previous research has shown that PFAS can be bio-concentrated through food chains, with substantial contamination in fish (Sun et al., 2022).

Perfluoroctane sulfonic acid (PFOS), a type of PFAS, has the highest detection rate and concentration levels in both the environment and body tissues. The unique structure of PFOS is considered amphiphilic, containing a long hydrophobic fluorocarbon chain and a hydrophilic sulfonic acid functional group. The fluorocarbon chain is lipophilic, allowing for passive diffusion across cell membranes and accumulation in body tissues. The persistence of PFOS in the environment and body tissues can be explained by the strong carbon-fluorine bonds, which require significant amounts of energy to break.

Figure 1. Structure of perflurooctane sulfonic acid (PFOS)

Previous research has indicated a relationship between PFOS exposure and inflammatory markers in macrophages. More specifically, PFOS has been shown to stimulate the release of pro-inflammatory cytokines and inflammasome activation (Wang et al., 2021). However, the mechanisms by which PFOS affects immune system function are not fully understood. In this preliminary study, we designed an in vitro experiment that exposed RAW 264.7 macrophage cell cultures to variable PFOS concentrations for subsequent protein extraction and analysis using liquid chromatography mass spectrometry (LC-MS) techniques.

Methodology

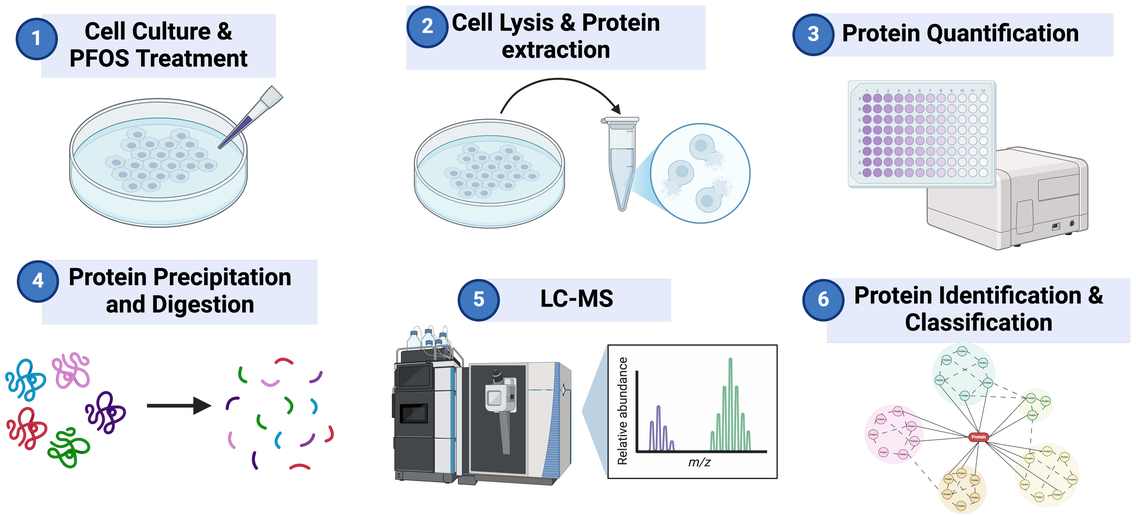

Figure 2. Overview of experimental and proteomic analysis workflow

Results

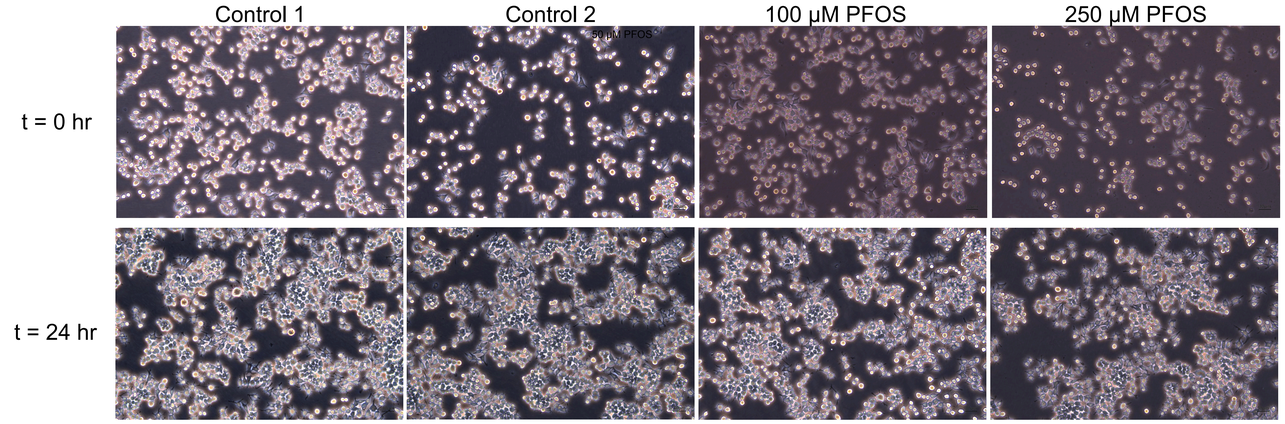

Figure 3. Microscopic images of raw 264.7 macrophage cell cultures with and without PFOS. The top row of images were taken immediately after cells were treated with or without PFOS (t = 0 hr). The bottom row of images were taken after 24 h of treatment and immediately before cell lysis.

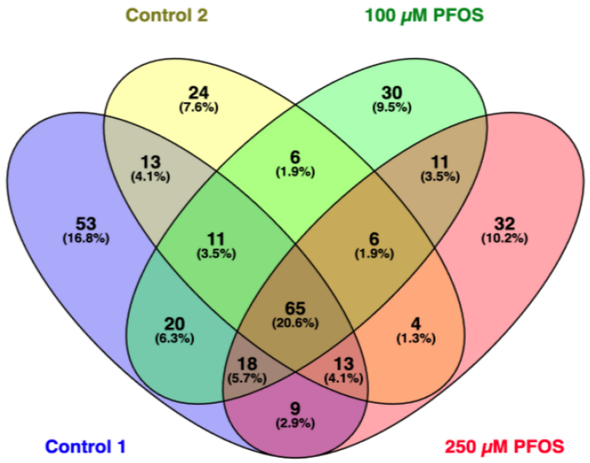

Figure 4. Number of peptides commonly and exclusively expressed in different treatments

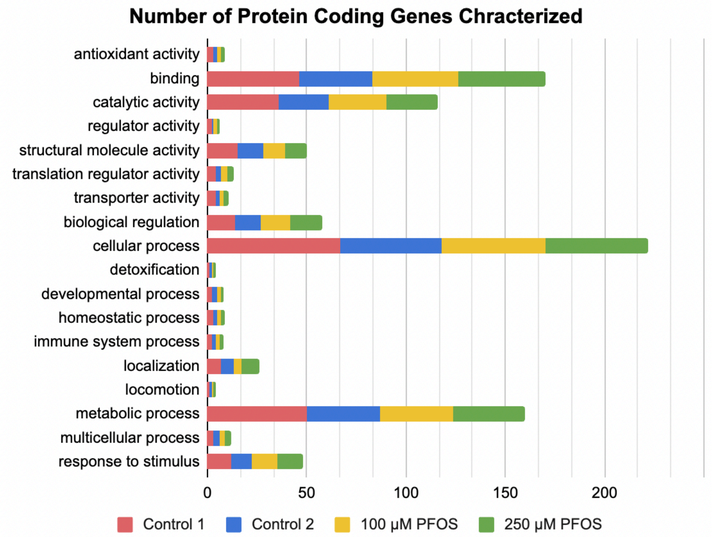

Figure 5. The number of protein coding genes identified and characterized in each treatment group using the PANTHER classification system.

Conclusion and Future Direction

- The identified proteins were successfully characterized using the Proteome Discoverer and PANTHER classification software
- Characterization showed protein coding genes primarily involved in binding activity, catalytic activity, cellular processes, and metabolic processes
- Analysis revealed proteins exclusive to PFOS treatment groups
- Small areas of localized cellular death were visualized immediately after PFOS treatment
- We hope to conduct future experiments using additional treatment groups to achieve a larger number of identified proteins for further characterization and expressional comparison

References

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