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Analytical techniques for assessing microplastic-induced physiological damage in tissues

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Abstract

Microplastic contamination is a growing environmental concern with potential health risks. This study aims to investigate the physiological effects of microplastics and develop strategies to mitigate their impact. The research utilizes advanced analytical techniques to identify, visualize, and characterize microplastics in tissues. Methods such as FTIR and Raman spectroscopy, X-ray Micro-CT, CLSM, LM, TEM, SEM, Fluorescence Microscopy, IHC, and biochemical assays are employed. The findings reveal that microplastics can induce oxidative stress, inflammation, and cellular damage, which may lead to various pathologies. The implications of this research lie in understanding the complex mechanisms underlying microplastic toxicity and developing effective environmental and public health policies. Standardizing protocols and establishing dose-response relationships are suggested for future research. Overall, this study contributes to the comprehension of analytical techniques to analyse microplastic-induced physiological damage and its implications for human health.

Keywords: Microplastics, microplastic toxicity FTIR, raman spectroscopy, X-ray Micro-CT, CLSM, LM, TEM, SEM, fluorescence microscopy, IHC

1. Introduction

Microplastic contamination, as represented by fragments of plastic of less than 5 mm in size, has been an emergent environmental issue. These particles are of diverse origin such as the breakdown of larger plastic material, personal cosmetics, and chemical fibers, and have become ubiquitous in marine as well as terrestrial environments (Ahmed, M. B., *et al.*, 2021; Andrady, A. L. 2017) [2, 6]. Microplastics have been reported to be present in an extremely diverse array of organisms ranging from plankton to the human being and present serious issues regarding their capacity to interfere with biological processes and induce physiologic damage. The persistent presence of such particles in the environment, coupled with their tendency to adsorb toxic chemicals, further contributes to their potential toxicities. (Al-Thawadi, S. 2020; Anbumani, S., *et al.*, 2018) [3, 5].

The widespread occurrence of microplastics in biological systems emphasizes the necessity of thoroughly investigating their physiological effects. Microplastics can interact with tissues and organs, causing oxidative stress, inflammation, and cell damage. These effects can interfere with normal cellular processes and are associated with different pathologies, such as cancer, neurodegenerative diseases, and cardiovascular diseases. Moreover, the small size of microplastics facilitates their passage through biological barriers, thereby posing the risk of accumulation in vital organs and impairing their function. Thus, the investigation of physiological effects of microplastics is essential for the development of effective strategies to counteract their adverse effects on health. (Geyer, R., *et al.*, 2017; Wright, S. L., *et al.*, 2017; Prata, J. C., *et al.*, 2020, Avio, C. G., *et al.*, 2017) [15, 47, 36, 7].

Analytical techniques are required for the assessment of the nature and degree of tissue injury caused by microplastics. These methods enable investigators to visualize and measure structural changes, identify markers of inflammation and oxidative stress, and characterize the localization and composition of microplastics in tissues. By providing critical information regarding cellular and molecular damage mechanisms of microplastic toxicity, these methodologies are crucial for further progress in comprehending health hazards of microplastic exposure. The following sections outline various analysis techniques that make

up an integrative toolkit to investigate microplastic-induced physiological damage.

2. Microplastic Localization, Visualization, Identification, and Characterization in Tissues

Advanced analytical methods enable Identification of microplastics within biological samples and precise characterization of microplastics in tissues, providing information about their chemical composition, size, shape, and distribution. Fourier Transform Infrared Spectroscopy (FTIR) and Raman Spectroscopy are analytical techniques used to identify and characterize the chemical composition of microplastics within tissues. These methods rely on the interaction of infrared or Raman light with the material to produce characteristic spectra, which can be used to identify specific polymers (Xu, J. L., *et al.*, 2019) ^[48].

FTIR Spectroscopy is technique to measures the absorption of infrared radiation by the sample, producing a spectrum that serves as a molecular fingerprint. FTIR can identify different types of plastics (e.g., polyethylene, polystyrene, polyvinyl chloride) based on their characteristic absorption bands (Rathore, C., *et al.*, 2023) ^[38]. Micro-FTIR and FTIR imaging enable the analysis of microplastics as small as 10-20 μm within tissue sections (Mikulec, V., *et al.*, 2023) ^[33].

Complementary to FTIR, Raman spectroscopy measures the inelastic scattering of monochromatic light by the sample. It provides information about molecular vibrations and can identify plastic polymers with high specificity. Raman has the advantage of minimal sample preparation and can analyze particles as small as 1 μm , making it suitable for detecting smaller microplastics (Käppler, A., *et al.*, 2016) ^[23].

X-ray Microtomography (Micro-CT) is an imaging technique that provides 3D visualization of microplastics within tissues and allows researchers to examine the spatial distribution and morphology of microplastics without damaging the sample. Micro-CT generates high-resolution 3D images by taking multiple X-ray projections of the sample from different angles and reconstructing them computationally. This technique can reveal the three-dimensional distribution of microplastics within organs and tissues, providing insights into their accumulation patterns and potential migration pathways. The non-destructive nature of Micro-CT allows for subsequent analyses of the same sample using other techniques, enabling multi-modal characterization of microplastic-tissue interactions (Parobková, V., *et al.*, 2025; de Paiva Teles, Á., *et al.*, 2024) ^[34, 11].

Confocal Laser Scanning Microscopy (CLSM) is a powerful imaging technique that provides high-resolution, 3D images of microplastics within tissues (Li, L., Luo, Y., *et al.*, 2020) ^[26]. CLSM is particularly useful for studying the interactions between microplastics and cellular structures, as well as for tracking the movement of microplastics within living organisms allowing for the real-time observation of microplastic uptake, intracellular trafficking, and potential exocytosis. By using optical sectioning, CLSM eliminates out-of-focus light, resulting in sharper images with improved contrast compared to conventional fluorescence microscopy. This technique can be combined with fluorescent labelling of microplastics and cellular structures to visualize their spatial relationships with subcellular resolution (Zhu, Y., *et al.*, 2024) ^[49].

3. Examining tissue morphology and assessing microplastic-induced damage

Light Microscopy (LM) and Haematoxylin & Eosin (H&E) staining are fundamental techniques for examining tissue morphology and serve as the first step in assessing microplastic-induced damage. LM provides an overall view of tissue architecture, while H&E staining differentiates between cellular components, highlighting structural changes, inflammation, and necrosis. Haematoxylin stains nucleus blue, while eosin stains cytoplasm pink, allowing for clear visualization of tissue organization and cellular abnormalities (Abdel-Zaher, S., *et al.*, 2023) ^[1]. In tissues exposed to microplastics, H&E staining can reveal inflammatory cell infiltration, tissue fibrosis, necrotic areas, and alterations in cell morphology. These histopathological changes provide valuable insights into the severity and nature of tissue damage, establishing a foundation for more specialized analyses (Hamed, M., *et al.*, 2021) ^[17].

Building upon the observations from light microscopy, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) provide high-resolution images of tissue ultrastructure. TEM is particularly useful for detecting intracellular localization of microplastics and examining subcellular damage, including alterations in organelles, membrane integrity, and cytoskeletal organization (Trianni, A., *et al.*, 2024) ^[42]. SEM offers detailed surface morphology of tissues, revealing physical interactions between microplastics and cellular surfaces (Wang, Z. M., *et al.*, 2017) ^[46].

These advanced imaging techniques can identify subtle changes that are not visible with conventional light microscopy, such as disruption of cell junctions, alterations in mitochondrial structure, and changes in endoplasmic reticulum morphology. By providing nanometer-scale resolution, TEM and SEM are invaluable for elucidating the mechanisms of microplastic-induced cellular damage (Jeong, C. B., *et al.*, 2016) ^[22].

Fluorescence Microscopy complements electron microscopy by enabling the visualization of microplastics within tissues through fluorescent labelling. This technique is particularly valuable for tracking the distribution and movement of microplastics in samples. Many plastic polymers exhibit autofluorescence, but specific fluorescent dyes like Nile Red can enhance detection sensitivity and specificity (Maes, T., *et al.*, 2017; Karakolis, E. G., *et al.*, 2019) ^[31, 24].

By using different fluorescent markers, researchers can simultaneously visualize microplastics and cellular structures, allowing for the assessment of spatial relationships and potential interactions. Time-lapse fluorescence microscopy can also be employed to study the dynamic processes involved in microplastic uptake, translocation, and elimination in living tissues (Karakolis, E. G., *et al.*, 2019) ^[24].

Moving from structural to functional analyses, Immunohistochemistry (IHC) and Immunofluorescence (IF) are techniques used to identify specific protein markers in tissues. These methods involve the use of antibodies to detect proteins associated with inflammation, oxidative stress, and apoptosis. By visualizing these markers, researchers can assess the biological responses to microplastic exposure and understand the underlying mechanisms of tissue damage (Gaspar, L., *et al.*, 2023) ^[14].

In microplastic toxicology studies, Inflammatory markers

(CD68 for macrophages, myeloperoxidase for neutrophils) (Pechiappan, H. 2021; Shao, W., *et al.*, 2024)^[35, 39], and apoptotic markers (cleaved caspase-3, TUNEL staining) (Wan, S., *et al.*, 2024; Lu, T., *et al.*, 2024)^[44, 30] can also be used.

4. Measuring Oxidative Stress in Microplastic-Exposed Tissues

Once cellular and tissue damage is identified through microscopic techniques, biochemical assays provide quantitative measurements of the molecular changes induced by microplastics. Oxidative stress is a primary mechanism through which microplastics exert their toxic effects, making its assessment crucial for understanding microplastic-induced damage. Oxidative stress is a key indicator of cellular damage caused by microplastics. Several assays are used to evaluate oxidative stress, including:

MDA Assay and TBARS Test measure the levels of malondialdehyde, a byproduct of lipid peroxidation. Elevated MDA levels indicate membrane damage due to oxidative stress, a common consequence of microplastic exposure. The colorimetric TBARS assay measures MDA-TBA adduct formation at 532 nm, providing a quantitative assessment of lipid peroxidation (Jeong, C. B., *et al.*, 2016; Hsieh, S. L., *et al.*, 2021; Hamed, M., *et al.*, 2020; Bristow, P. T. 2024)^[22, 19, 18, 9].

Oxidative stress can cause protein carbonylation, which is measured using 2,4-dinitrophenylhydrazine (DNPH) derivatization followed by spectrophotometric analysis. Increased protein carbonyl content indicates protein oxidation and potential functional impairment (Dalle-Donne, I., *et al.*, 2003; Michailidou, K., *et al.*, 2024)^[10, 32].

Comet Test (Single Cell Gel Electrophoresis) is assay to visualize DNA damage in individual cells. Cells are embedded in agarose, lysed, and subjected to electrophoresis. Damaged DNA migrates further, creating a "comet tail" whose length and intensity correlate with the degree of DNA damage. The Comet Test can detect single and double-strand breaks, providing a sensitive measure of genotoxicity induced by microplastics (Tang, K. H. D. 2025; Berber, A. A. 2019; Farag, A. A., *et al.*, 2023)^[41, 8, 13].

TUNEL Assay is a technique that identifies apoptotic cells by labeling the terminal end of nucleic acids. The enzyme terminal deoxynucleotidyl transferase (TdT) adds dUTPs to the 3'-OH ends of fragmented DNA, which can then be visualized using various detection methods. The TUNEL Assay detects apoptotic cell death by labeling fragmented DNA allowing for the assessment of tissue-specific vulnerability to microplastic-induced damage (Lu, C., *et al.*, 2023; Li, T., *et al.*, 2024)^[29, 27].

8-OHdG Assay is a technique in which 8-OHdG is a product of DNA oxidation and a biomarker of oxidative DNA damage. It can be measured using ELISA or HPLC techniques. Elevated 8-OHdG levels suggest potential mutagenic and carcinogenic effects of microplastic exposure (Sincihu, Y., *et al.*, 2023; Ding, R., *et al.*, 2024)^[40, 12].

Antioxidant Enzymes: superoxide dismutase, catalase, glutathione s-transferase, and glutathione peroxidase (GPx) and glutathione (GSH) assays assess the activity of enzymes involved in the antioxidant defense mechanisms. SOD catalyzes the dismutation of superoxide radicals, CAT decomposes hydrogen peroxide, and glutathione (GSH) acts

as a cellular redox buffer. Changes in the activity of these enzymes reflect the cellular response to oxidative stress induced by microplastics. For instance, initial increases in SOD and CAT activities may indicate an adaptive response, while decreased activities after prolonged exposure suggest antioxidant system depletion (Jaikumar, I.M., *et al.*, 2021; Köktürk, M., *et al.*, 2024)^[21, 25].

5. Assessment of Inflammatory response due to microplastic exposure

The Enzyme-Linked Immunosorbent Assay (ELISA) is used to measure the levels of inflammatory cytokines such as IL-6, TNF- α , and IL-1 β . These cytokines are key mediators of inflammation and their elevated levels indicate an inflammatory response to microplastic exposure. ELISA provides quantitative data on cytokine concentrations, allowing for the assessment of inflammation severity and progression (Grellner, W., *et al.*, 2000; Liu, C., *et al.*, 2021)^[16, 28].

In microplastic toxicology, specific cytokine profiles can indicate different pathological processes:

- IL-1 β and TNF- α elevations suggest acute inflammatory responses (Pechiappan, H. 2021)^[35].
- IL-6 increases may indicate chronic inflammation (Pulvirenti, E., *et al.*, 2022)^[37]
- TGF- β upregulation can signal fibrotic changes (Wang, S., *et al.*, 2023)
- IL-10 alterations reflect attempts to regulate inflammatory processes (Pechiappan, H. 2021)^[35].

The temporal patterns of cytokine expression can provide insights into the evolution of the inflammatory response to microplastic exposure (Hwang, J., *et al.*, 2020)^[20].

6. Conclusion

The study of microplastic-induced physiological damage in tissues and organs is a critical area of research that requires the use of advanced analytical techniques. Methods such as FTIR Spectroscopy, Raman Spectroscopy, X-ray Microtomography (Micro-CT), Confocal Laser Scanning Microscopy (CLSM), Light Microscopy, Transmission Electron Microscopy, Fluorescence Microscopy, Immunohistochemistry (IHC) & Immunofluorescence (IF), Lipid Peroxidation, Protein Carbonyl Content, Comet Test, TUNEL Assay, 8-OHdG Assay, Assessment of Antioxidant Enzymes, and Inflammatory Cytokines provides valuable insights into the structural, functional, Physiological and Metabolic changes caused by microplastics. The integration of these techniques allows assessment of microplastic-tissue interactions, from the identification, visualization, localization and characterization of microplastics to the evaluation of cellular and molecular responses. This multi-faceted approach is essential for understanding the complex mechanisms underlying microplastic toxicity.

Future research directions should focus on standardizing analytical protocols, developing more sensitive detection methods, and establishing dose-response relationships between microplastic exposure and physiological effects. Additionally, studies examining the long-term consequences of chronic microplastic exposure are needed to fully understand the health implications of this pervasive environmental pollutant.

By employing these analytical techniques, researchers can better understand the health risks associated with

microplastic pollution and develop strategies to mitigate their impact on biological systems, ultimately contributing to more effective environmental and public health policies.

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