engineering proceedings

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Detection of Microparticles in Tissue and Saliva Samples from the Oral Cavity of Cancer Patients: Identification Methodology Using Selected Analytical Methods †

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† Presented at the 5th International Conference on Advances in Environmental Engineering, Ostrava, Czech Republic, 26–28 November 2025.

Abstract

The paper focuses on describing a methodology for detecting microparticles in saliva and oral tissues using FTIR microscopy and scanning electron microscopy. The aim is to compare samples from patients with oral cavity carcinoma and healthy individuals, identify the presence of microparticles, and assess their potential association with carcinogenesis.

Keywords: microparticles; FTIR microscopy; oral squamous cell carcinoma

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Academic Editor: Adriana Estokova, Natalia Junakova, Tomas Dvorsky, Vojtech Vaclavik and Magdalena Balintova

Published: 22 December 2025

Citation: Valigůrová, A.; Čabanová, K.; Matějková, P.; Belobradová, J.; Vaculová, J.; Hurník, P.; Štembírek, J.; Buchtová, M.; Halfar, J.; Brožová, K.; et al. Detection of Microparticles in Tissue and Saliva Samples from the Oral Cavity of Cancer Patients: Identification Methodology Using Selected Analytical Methods. *Eng. Proc.* **2025**, *116*, 40. https://doi.org/10.3390/engproc2025116040

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1. Introduction

Squamous cell carcinoma (SCC) is the most common malignancy affecting the oral cavity worldwide, representing a significant health burden [1]. Despite advances in understanding its multifactorial etiology, including tobacco and alcohol use, the role of emerging environmental pollutants, such as microplastics, remains largely unexplored [2]. Microplastics, defined as plastic particles smaller than 5 mm, have been detected in various environmental matrices and biological samples, raising concerns about their potential impacts on human health [3,4].

Studies have reported the presence of microplastics in human tissues and fluids, suggesting possible bioaccumulation and interactions with cellular structures that may influence disease processes, including carcinogenesis [5,6]. However, methodologies for the reliable detection and characterization of microplastics in biological matrices such as oral tissues and saliva are still under development.

Fourier-transform infrared (FTIR) microscopy is a powerful technique widely used for microplastic identification due to its ability to provide detailed chemical composition and morphological information at the microscale. This study aims to present a robust methodology for detecting and characterizing microplastics in tissue and saliva samples

from patients diagnosed with oral SCC, utilizing FTIR microscopy and complementary analytical methods. The results are expected to contribute to a better understanding of the potential role of microplastics in oral cancer pathogenesis.

2. Methods

Main text paragraph (M_Text). To determine the presence, quantity, and chemical composition of microplastics in oral squamous cell carcinoma (SCC) tissues and saliva, an analytical approach was designed combining standardized sample collection, chemical pretreatment, filtration, FTIR microspectroscopy, and statistical analysis. All measurements were performed under standardized conditions to ensure reproducibility.

2.1. Sample Collection and Preparation

Biopsy samples of tissue and saliva were collected from patients with oral SCC prior to the initiation of any oncological treatment. Control samples of saliva and healthy oral mucosa were obtained from patients without a cancer diagnosis. All samples were processed under clean laboratory conditions to minimize contamination.

2.2. Chemical Pretreatment

To remove organic material and facilitate microplastic isolation, tissue and saliva samples were chemically digested in a 30% potassium hydroxide (KOH) solution. Tissue samples were digested for one week, while saliva samples were digested for one month, both in a thermostat maintained at approximately 30 ± 2 °C.

2.3. Filtration and Microscopic Inspection

The digested samples were first filtered through cellulose acetate membrane filters (Thermo Fisher Scientific, Waltham, MA, USA, pore size 0.45 μm, diameter 47 mm). Subsequently, the samples were subjected to an ultrasonic bath and then filtered again through aluminum oxide membrane filters (Whatman, Phoenix, AZ, USA, pore size 0.2 μm, diameter 25 mm) to capture microparticles for subsequent analysis. The filters were then placed in a desiccator to remove moisture.

2.4. Infrared Spectroscopy

To determine the material composition of the particles captured on the filters, a key analysis was carried out using FTIR microspectroscopy. Dry filters were analyzed using a fully automated FTIR microscope for chemical imaging Bruker LUMOS II (Billerica, MA USA), which combines Fourier-transform infrared spectroscopy with microscopy and allows measurement of particles down to approximately 10 μm, with the lower limit depending on the morphology of the particles. Given the particle size and the type of filter, transmittance mode was chosen, using a FPA detector.

3. Expectations and Hypotheses

The main aim of this study is the preparation of biological samples and the optimization of a combination of analytical methods that enable reliable and accurate identification of microplastics in complex biological materials, such as tissues and saliva. A properly established sample preparation procedure ensures effective removal of biological material while preserving the integrity of microplastics for subsequent analysis.

4. Preliminary Results

Fourier-transform infrared (FTIR) spectroscopy methods represent an effective and reliable tool for the detection and characterization of microplastics in complex biological

matrices, such as tissue and saliva samples. The developed methodology offers high sensitivity and specificity, enabling accurate and reproducible assessment of microplastic presence [7].

Preliminary data obtained using this methodology suggest that microplastics can indeed be detected in the oral tissues and saliva of patients with squamous cell carcinoma. Microplastic particles larger than 0.45 μm were detected in the analyzed samples.

We further hypothesize that the identified microplastics will predominantly include widely used polymers such as polyethylene (PE), polypropylene (PP), and polystyrene (PS), reflecting their ubiquity in the environment and known prevalence in biological samples. These findings may suggest either a potential contribution of microplastics to carcinogenic processes, for example, through induction of chronic inflammation, oxidative stress, or genotoxic effects, or alternatively that their accumulation is a consequence of altered tissue permeability, vascularization, or phagocytic activity associated with the cancerous state.

5. Conclusions

We successfully developed and optimized a combination of procedures and methods suitable for detecting microplastics in biological samples, such as oral tissues and saliva. The developed methodology effectively removes biological material from the samples without damaging the filters or the microplastic particles themselves, resulting in the analysis of only the present microplastics.

The methodology is ready for application in larger-scale studies that may provide new insights into the potential role of microplastics in oral carcinogenesis—either as potential contributors to tumor processes through inflammation, oxidative stress, and genotoxic mechanisms, or as secondary accumulations caused by changes in tumor tissue. Further and more detailed analyses are needed to clarify these relationships.

Author Contributions: Conceptualization, A.V.; methodology, A.V., K.Č., P.M., J.V. and J.B.; formal analysis, K.B. and J.H.; investigation, A.V., K.Č., J.B., J.Š., M.B. and P.H.; writing—original draft preparation, A.V.; writing—review and editing, A.V., K.B., J.H., K.Č. and S.H.; visualization, A.V.; supervision, K.Č., J.Š. and S.H.; funding acquisition, A.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Student Grant Competition financed by VSB—Technical University of Ostrava within the project "Research on detection, identification and elimination of micropollutants in the environment." (no. SP2025/007).

Institutional Review Board Statement: The study was approved by the Ethics Committee of the Faculty Hospital of Ostrava (document number 1/2025), and the research was conducted in accordance with the Ethical Principles for Medical Research Involving Human Subjects (Declaration of Helsinki).

Informed Consent Statement: All patients involved in the research signed an informed consent for their participation in the study.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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