Evaluation of Microplastics in saliva of orthodontic patients with clear aligners with poor and good oral health

Gokul G L[1], Sivakamavalli J [2], Shantha S[*1]

[1] Department of Orthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 77, Tamil Nadu, India

[2] Lab in Biotechnology and Biosignal Transduction, Department of Orthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 77, Tamil Nadu, India

***Corresponding Author:** sivakamavalli.sdc@saveetha.com

ABSTRACT:

BACKGROUND:

AIM: Isolation, characterization and identification of microplastics from clear aligners of patients with poor and good oral health.

MATERIALS AND METHODS: Sample collection: plaque and saliva from patient with aligners (Poor and good oral hygiene). Microplastics isolation; functional characterization via FTIR and microplastics morphology (SEM).

RESULTS:

The organic matter in the orthodontic samples is digested by hydrogen peroxide method, where 30% of H_2O_2 is used for digestion process, following the digested samples were subjected to filtration process with 5mm pore size and 47 diameter filter membrane. The filtrate was then subjected to light microscopy to confirm the presence of microplastics in the given orthodontic samples.

The FTIR spectra of isolated microplastics showed higher prevalence of polyamide (PA) and less cellophane (CP) with 70% of PA. The SEM reveals the surface morphology of isolated microplastics from orthodontic samples as fibers, filaments, pellets, spheres and other irregular structures. The EDX analysis reveals the presence of carbon predominantly with other components (Si, Cr, Fe and Ni).

CONCLUSION: The study confirms the presence of microplastics harboured in saliva and plaques samples from orthodontic patients with clear aligners.

KEYWORDS: Microplastics, Orthodontics, Saliva, Plaque, Debonded components of orthodontic appliance, SEM, FTIR

INTRODUCTION:

Environmental contamination poses a significant threat to public health, compromising general well-being through various avenues such as diseases, microbial infections, and mutations. Recently, microplastics have emerged as a notable public health concern, predominantly found in man-made items, contributing to genetic and health-related issues in eukaryotic systems.

These microplastics can infiltrate the human body through various means, prompting an exploration into their presence in dental appliances used by orthodontic patients. Orthodontic treatment involves the physical realignment of teeth using mechanical appliances typically made of metallic, ceramic, or non-degradable thermoplastic polymeric materials, thereby making the existence of microplastics in saliva apparent. The primary sources of microplastic contamination in the oral environment are toothpaste and composite restorative materials. Toothpaste containing plastic particles of 5 mm in size contributes to primary microplastics, while secondary microplastics originate from resin-based composite restorative materials degrading within the oral cavity or being released during finishing and polishing procedures. The widespread use of microplastics in personal care products often goes unnoticed, despite the potential hazards they pose. Prolonged exposure to toothpaste containing microbeads can lead to tooth enamel and dentine erosion, while gingivitis and periodontitis can arise from microbeads becoming trapped in the gingival sulcus.

Moreover, the use of resin-based composites in dental procedures can result in the leaching of harmful monomers, such as Bisphenol A-glycidyl methacrylate (Bis-GMA), which poses environmental risks upon disposal. Studies indicate that microplastics not only harm aquatic life but also permeate the food chain through bioaccumulation, potentially leading to the extinction of aquatic species. Although some nations have enacted legislation to restrict the use of microplastics in healthcare items, many regions lack stringent regulations. To mitigate the growing threat of microplastics to the environment, an educational and regulatory approach to their usage is imperative. Furthermore, the surfaces of orthodontic appliances, comprising various materials, can serve as breeding grounds for microbial biofilms, exacerbating oral health issues if hygiene is neglected. This study aims to identify microplastics possibly trapped on thermoplastic aligners, shedding light on their presence in orthodontic appliances and their potential health implications.

MATERIALS AND METHODS:

Dental sample Collection

The samples were collected from aligner patients with poor oral hygiene (POH) with Debris score 3 and good oral hygiene (GOH) with debris score 0. The sample included saliva with dental plaque, and the sample size is 2 POH and 2 GOH. Care was taken to collect the appliance without disturbing the plaque content on the aligner. The collected sample was contained in a sterile container with saline buffer **(Greene, 1967)**. The patient was also made to spit a minimum of 5 ml saliva to collect additional washing of remnant plaque debris following hand scaling in another sterile glass container to avoid plastic contamination. Swab from each container was placed in a 5 mL sterile container containing 2 mL normal saline (NS) for further study.

Microplastics Isolation

To digest organic matter, approximately 15 mL of 30% hydrogen peroxide was introduced into the sample. These containers were subsequently positioned inside a shaking incubator set at 45°C, with 75 rpm, for a 24-hour incubation duration. Following the incubation period, the mixture was allowed to naturally reach room temperature and was left undisturbed for a span of 48 hours. To ensure complete dissolution of any clumped salt & food debris, 850 mL of

distilled water, previously filtered, was added. The resulting liquid was then subjected to vacuum filtration using cellulose nitrate filter paper with 5-millimetre pores and a 47mm diameter. At designated time intervals, the filter papers were changed and left to air dry at room temperature to facilitate the examination and characterization of microplastics (MPs).

Light Microscopy

Whether quantity or quality varied observation, the physical characteristics of the MPs were also noted. On such occasions, selected fragments of MPs were chosen specifically for further characterization.

Fourier Transform-Infrared Spectroscopy:

Based on microscopic observation, selected fragments of MPs were subjected to FT-IR analysis (Thermo Nicolet iS5) in transmittance mode. The spectrum range was set in between 4000-400 cm1 and the output data were then compared with standard library (Hummel Polymer and Additives) to identify the polymer type. Further, the spectrum with a match quality index > ¼ 0.7 were taken into consideration and matches below <0.6 were considerably rejected.

Scanning Electron Microscopy:

The analysis was carried out by using a Scanning Electron Microscope instrument (JEOL). The selected fragments from light microscopy were subjected to SEM analysis for understanding the surface morphology (i.e. sphere, filament, fiber, microbeads, clusters).

RESULTS:

The isolation of microplastics from orthodontic samples was done by hydrogen peroxide method, where 15 ml of 30 % H_2O_2 is added to the mixture of saliva and plaque sample suspended in saline until effervescence appeared, after 24 hrs of incubation in room temperature the suspension was clear and several micro particles floated on the surface of the suspension. The floated microplastic particles are filtered using Whatman filter paper and the paper is changed periodically. Then the light microscopy images of filtered microplastics from the suspension provided basic insights to the presence of microplastics in the orthodontic samples (POH and GOH). Through the light microscopy images it is evident that microplastics are present and are in different shapes such as fibres, filaments, pellets, microbeads, spheres and irregular shapes.

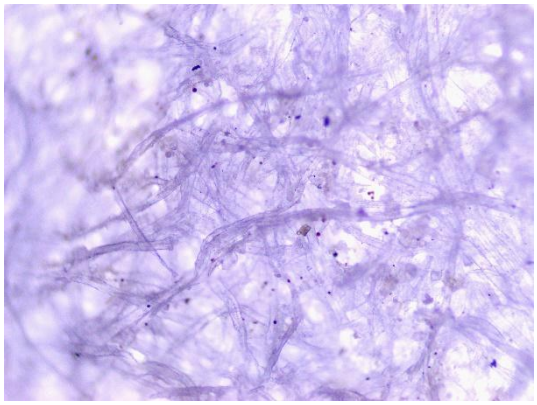
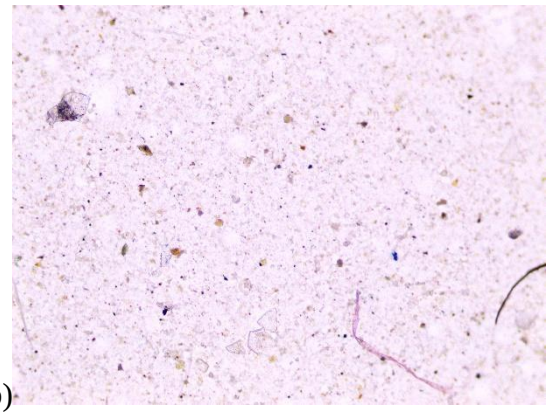
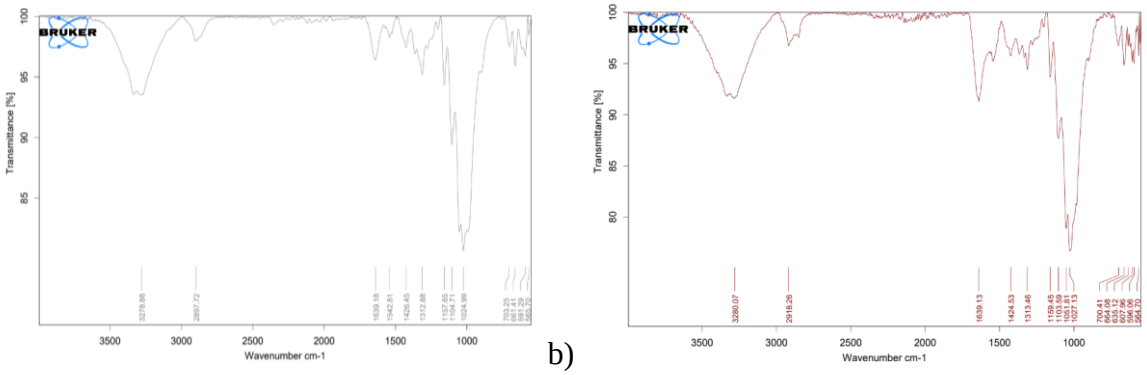
a)  b) 

Figure 1: Light microscopy images of isolated microplastics, In the above figure, a) Patients with aligners (poor oral health), b) Patients with aligners (good oral health)

FTIR evaluation of the microplastics from the orthodontic patient with clear aligners having poor and good oral health showed the existence of many functional groups in it. The bands in

poor oral health noted at 1639.18, 1542.81, 1024.99, 703.25, 661.41 and 565.70, likewise, the bands of good oral health noted at 1639.13, 1424.53, 1159.45, 1027.13, 700.41, 607.96 and 564.70 showed the presence of N–H and functional groups of proteins. Other peaks in the spectra showed the existence of various functional groups like, a high peak at 3278.88 (POH) and 3280.07 (GOH) indicated O–H functional group and numerous smaller peaks in between 3000 and 2000 cm-1 indicated minimal C-H functional group. The FT-IR histogram presented in Figure 3 reveals the detection of four primary polymer types—polyamide (PA), polypropylene (PP), polystyrene (PS), and cellophane (CP)—showing an impressive 91% matching identity. This comprehensive polymer analysis definitively confirms that the identified microplastics have a human-made origin. Among the dental samples, the prevalence of microplastics followed this sequence: polyamide > polystyrene > polypropylene > cellophane. Moreover, our study detected a minimal presence of other microplastics, such as Polyethylene terephthalate (PET) and polyethylene.



a) b)

Figure 2: FTIR spectra of the oral samples of orthodontic patients (Saliva + plaque) with aligners a) POH b) GOH

Our analysis revealed a diverse composition of MPs, encompassing fibres, fragments, pellets, and dusty materials of unidentified origin. Concurrently, the most prevalent types of microplastics identified were fibres, fragments, and pellets (as depicted in Figure 3). In our research, we documented a wide range of microplastic (MP) sizes, with the smallest particles measuring as small as 2.6 millimetres and the largest ones reaching up to 3.3 millimetres in size. This variation in microplastic sizes underscores the diversity and complexity of these particles within the samples we examined. Moreover, we utilised scanning electron microscopy (SEM) to visually validate the existence of microplastics (MPs), as illustrated in Figure 4.

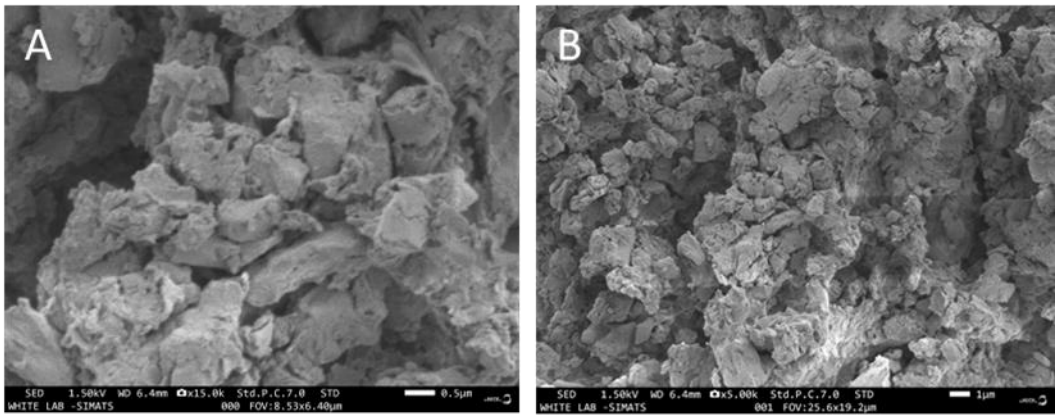


Figure 3. SEM images of microplastics isolated from aligners of orthodontic patients (Saliva + plaque) under different magnification a) POH b) GOH

The SEM/EDX analyses delivered detailed images depicting the surface structure of fibers and fragments examined, along with their unique elemental composition patterns. This data served the purpose of sifting through potential microplastics while eliminating non-plastic elements.

Discussion:

This study attempts to provide evidence of the presence of microplastics in oral samples of orthodontic patients on Aligner therapy. on debonding, which provides further evidence that microplastics are harboured on the complex assembly of orthodontic appliances. Although no studies have been conducted on the harm of microplastics directly exposed to humans through orthodontic appliances, studies have shown that people are more susceptible to oral diseases as it may act as a substratum to host pathogenic bacterial biofilms. The presence of microplastics in saliva and plaque supports the finding that compared to low-concentration exposures, workers in some positions exposed to high concentrations of PS and PA are more prone to present similar symptoms, including oral biofilm formation, dental caries etc. Previous studies have demonstrated the prevalence of microplastics in air environments and found that polyester and man-made fiber accounted for more than 90% (17–19). A study conducted by Zhang et al. 2018, the result described the levels of PET and PC in orthodontic appliances in different dental resins and adhesives of different origin, which showed that the PA- and CP-based microplastics are ubiquitous in orthodontic appliances from different countries, and the concentrations of PA-based microplastics were significantly higher whereas the CP are lowest(20). Our results showed that polyester (73% was PA) had the second highest exposure level of microplastics in the dental samples, while the level of CP was the lowest (Figure 3). The SEM micrographs shows different morphological structures of microplastics such as pellets, fibers, filaments and other irregular shapes. Likewise, in a study conducted by (Huang et al. 2022) the presence of microplastics in human sputum is reported.

Conclusion: This study is the first report to confirm the presence of microplastics in saliva and plaque of orthodontic patients with aligners (POH & GOH). Whereas there is not much of a considerable difference in the content of MPs in POH and GOH.

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