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Analysis of bacterial biofilm formed on intraradicular posts attached to an intraoral device

Análise do biofilme bacteriano formado em pinos intrarradiculares fixados a um dispositivo intraoral

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ABSTRACT

Objetive: This study aimed to evaluate, in situ, the microbiological composition of the biofilm formed on intraradicular posts attached to removable intraoral devices. **Methods**: For this, ten participants wore an upper intraoral device containing three types of intraradicular posts nickel-chromium metal, fiberglass, and acrylic resin-over twenty-eight days. The posts were fixed to the device palatally and with the long axis parallel to the longitudinal axis of the acrylic. Throughout the experimental period, all participants were instructed to follow the same oral hygiene and device care pattern. After this period of use, a portion of the post-samples was evaluated to quantify the

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number of microorganisms using the culture method. The other portion of the posts was analyzed through scanning electron microscopy to assess microbial colonization. The data obtained were recorded in an Excel spreadsheet and subjected to Kruskal-Wallis and Dunn statistical analyses. **Results**: No statistically significant difference was observed between the posts and the different media. All posts showed contamination in all media. Microbial colonies were identified through Scanning Electron Microscopy on all three intraradicular posts analyzed. **Conclusion**: Posts exposed in the oral cavity for twenty-eight days demonstrate a high microbial load regardless of their composition.

Indexing terms: Endodontics. Microbiology. Microscopy, electron, scanning. Post and core technique.

RESUMO

Objetivo: Este estudo teve como objetivo avaliar, in situ, a composição microbiológica do biofilme formado em pinos intrarradiculares fixados a dispositivos intraorais removíveis. **Métodos**: Para isso, dez participantes usaram um dispositivo intraoral superior contendo três tipos de pinos intrarradiculares – metal de níquel-cromo, fibra de vidro e resina acrílica – por um período de vinte e oito dias. Os pinos foram fixados palatalmente ao dispositivo, com o eixo longo paralelo ao eixo longitudinal do acrílico. Durante o período experimental, todos os participantes foram instruídos a seguir o mesmo padrão de higiene bucal e cuidados com o dispositivo. Após esse período de uso, uma parte das amostras dos pinos foi avaliada para quantificar a quantidade de microrganismos por meio do método de cultura. A outra parte dos pinos foi analisada por meio de microscopia eletrônica de varredura para avaliar a colonização microbiana. Os dados obtidos foram registrados em uma planilha do Excel e submetidos às análises estatísticas de Kruskal-Wallis e Dunn. **Resultados**: Nenhuma diferença estatisticamente significativa foi observada entre os pinos e os diferentes meios. Todos os pinos apresentaram contaminação em todos os meios. Colônias microbianas foram identificadas por microscopia eletrônica de varredura em todos os três tipos de pinos intrarradiculares analisados. **Conclusão**: Pinos expostos na cavidade oral por vinte e oito dias demonstram uma alta carga microbiana, independentemente de sua composição.

Termos de indexação: Endodontia. Microbiologia. Microscopia eletrônica de varredura. Técnica para retentor intrarradicular.

INTRODUCTION

Oral bacteria can form biofilms on solid surfaces and remain suspended in nutrient-containing fluids [1]. Biofilms represent microbial lifestyles that manifest in various configurations on biotic and abiotic surfaces [2]. Eighty percent of human infections are estimated to originate from biofilms [3]. One of the most adopted definitions for biofilms is that they consist of sessile microbial communities adhered to a surface and composed of cells entangled in a self-produced extracellular polymeric matrix substance [4]. The matrix plays a crucial role in the microbial biofilm community's morphology, ecology, survival, and resistance [5]. Biofilms tend to protect bacterial cells from host defense mechanisms and the effects of antibiotics. Moreover, they release planktonic bacterial cells, which can cause acute diseases [6].

There are various ways in which microorganisms can approach the surface of dental materials, including Brownian motion, fluid movement, deposition, and interaction with other cells [7]. Given the complexity of microbial interaction, no precise model can describe the process of bacterial adhesion to different dental materials.

In situations where the tooth exhibits fragility due to previous endodontic treatments, fractures, or extensive restorations, the remaining coronal portion may be compromised. In this context, it becomes necessary to create restorations to restore the shape, function, and aesthetics of the tooth and protect the remaining tooth structure. Thus, using intraradicular retainers is justified as a means to provide retention and support for future restoration, especially when there are significant losses of dental structure and weakening of the root canal [8].

The risk of contamination of exposed intraradicular posts in the oral cavity is a concern as it breaks the aseptic chain in endodontic treatment, a series of procedures and practices aimed at maintaining a sterile environment during root canal treatment, and should be avoided. To date, no study has evaluated how the process of biofilm formation occurs on the surface of intraradicular posts exposed to the oral environment. The exposure of posts in the oral cavity creates a favorable environment for the adherence of bacteria and fungi, forming biofilms that can compromise the structural integrity of the retainer and trigger infectious processes.

Therefore, it becomes relevant to investigate in situ the microbial composition and structure of biofilms that form on the surfaces of intraradicular posts exposed to saliva in the oral cavity through an in situ assay, considering different materials.

METHODS

This study received approval from the Research Ethics Committee (Protocol CAAE 65690522.3.0000.5347).

Participant selection

Ten participants were selected for this study based on a sample size calculation aimed at detecting a difference of at least 1.5 standard deviation units between the means of the measurements observed in the groups. The objective was to achieve a statistical power of 90%, with a significance level of 5%. This calculation was based on a previous study by Aires et al. [9].

The participants were of both sexes, aged between 18 and 25 years, and had good oral health, including adequate oral hygiene, low caries index, and normal salivary flow.

Exclusion criteria included individuals who were pregnant, had active caries, gingivitis, or periodontal disease, were using orthodontic appliances or undergoing dental whitening, had local or systemic pathologies, were taking any medication, were allergic to the material used, or disagreed with the terms of the research. Exclusion criteria were related to the development of allergic reactions or others during the use of oral devices, leading to the suspension of the individual's participation in the study.

All participants expressed their consent to participate in the research through a Consent Form. The consent form included detailed information about the study methodology, its objectives, participant selection and exclusion criteria, risk analysis, and compensation procedures, ensuring participants' freedom to withdraw their consent at any time.

Intraradicular posts tested

Thirty intraradicular posts were analyzed, divided equally into three groups, each containing ten posts of a specific material type: fiberglass post (Exacto – Angelus Indústria de Produtos Odontológicos S/A, Londrina, Paraná, Brazil), cast nickel-chromium metal post, and temporary acrylic resin post.

Each manufactured intraoral device was equipped with three types of intraradicular posts. Before insertion into the devices, the posts underwent an additional sterilization process in an autoclave, using a 40-minute cycle at 240°F (126°C) and a pressure of 20 psi.

Fabrication of the intraoral palatal device

White type III gypsum models (Asfer Indústria Química Ltda., São Caetano do Sul, São Paulo, Brazil) obtained from the upper arches of the participants through anatomical molding with Jeltrate® Plus alginate (Dentsply, Petrópolis, Rio de Janeiro, Brazil) were used.

The intraoral devices were fabricated from these models using chemically activated pink acrylic resin (Jet, Artigos Odontológicos Clássico, São Paulo, SP). These devices underwent finishing and polishing to prevent irritation and plaque buildup, thereby preventing changes in gingival tissues.

The samples of intraradicular posts were inserted into the acrylic resin with wax, ensuring the material did not cover them. The posts were positioned so that their long axis was parallel to the long axis of the acrylic resin, aiming to minimize the volume of the intraoral device.

Participant guidance

Participants used the intraoral device for twenty-eight days, with post-collection on the twenty-ninth day. Throughout the experiment, participants were instructed not to use mouthwashes. Instead, they were instructed to brush their teeth with a standardized Colgate-Palmolive Company toothpaste and to use soft Reach toothbrushes from Johnson & Johnson. The intraoral devices were not brushed with toothpaste on the surface containing the intraradicular posts, but only in the region in direct contact with soft tissues. Participants did not use systemic antimicrobial agents.

Each participant received instructions, a bottle of saline solution, and a plastic case from Dental Morelli Ltda. (Sorocaba, SP) to store the intraoral device and packages of sterile cotton from Cremer S.A. (Blumenau, Santa Catarina, Brazil).

Participants were instructed to maintain regular oral hygiene and continuously use the device, except during food or beverage intake. During these periods, the device was kept in a plastic case, wrapped in cotton, and moistened with saline solution.

Any discomfort reported by participants during the installation of the intraoral devices or the experiment was promptly diagnosed and treated to eliminate the cause of irritation.

Microbiological collection and analysis

The intraradicular posts were carefully removed from the intraoral devices using previously sterilized dental forceps in a laminar flow hood. Seven posts of each type were allocated for microbiological analysis, while the remaining three were examined by Scanning Electron Microscopy (SEM).

The biofilm present on each post was collected and deposited in Eppendorf tubes to initiate microbiological processing. These samples were transferred to test tubes containing 1 mL of Brain Heart Infusion (BHI) broth.

Within a laminar flow hood, the tubes were agitated using a vortex mixer (model MA 162 – Marconi, São Paulo, SP) for 60 seconds to ensure proper dispersion of microorganisms. Subsequently, serial dilutions (1/10, 1/100, 1/1,000, and 1/10,000) were performed using saline solution. The seeding and counting method was employed by Naghili et al. [10]. Twenty-five microliters of each dilution were seeded, in duplicate, on the following culture media:

• Brain Heart Infusion Agar (Himedia Labs, Curitiba, Paraná, Brazil) was incubated in a microbiological incubator at 37°C for two days to grow facultative anaerobic microorganisms.

• Mitis Salivarius Agar (Himedia Labs, Curitiba, Paraná, Brazil) containing 1% potassium tellurite solution, incubated at 35-37°C for 18-48 hours under microaerophilic conditions, for the presumptive identification of *Streptococcus sp*. (blue colonies). Black-blue colonies were not considered, as they suggest the presence of *Enterococcus faecalis*.

• M-Enterococcus Agar (Himedia Labs, Curitiba, Paraná, Brazil) supplemented with 1g/l esculin was incubated at 37°C for two days to grow *Enterococcus spp*.

• Sabouraud Dextrose Agar (Himedia Labs, Curitiba, Paraná, Brazil) with 0.5g/l chloramphenicol was aerobically incubated at room temperature (approximately 26°C) for 48 hours and at 37°C for an additional three days for the growth of fungi.

After the incubation period, the number of Colony Forming Units (CFUs) was determined in each drop, from each dilution. The total number of CFUs/ml of broth was then calculated using the standard formula:

$CFU_{total} = (CFU_{per\ drop} \times dilution \times 40)$ CFUs/ml.

Given:

▪ CFU_{total} = total number of CFUs per ml.

▪ $CFU_{per\ drop}$ = number of CFUs counted in the drop plated on the specific medium. As there are two drops for each dilution, the one showing a countable number of CFUs between 10 and 50 was considered.

▪ Dilution = dilution factor applied (if 10^{-2}, it was converted to 100).

▪ 40 = value corresponding to the aliquot withdrawn for plating from a dilution and its proportionality with the volume of the tube it originated from.

Identification at the species level for the CFUs was not conducted. Thus, the total microbial load corresponding to each non-selective (BHI) or selective (Mitis Salivarius agar, M-Enterococcus agar, Sabouraud agar) culture medium was described.

Scanning electron microscopy

Three posts of each type of retainer analyzed were subjected to the fixation, mounting, and metallization process according to the Center for Microscopy and Microanalysis protocol.

The scanning electron microscope used was the Cam Scan MV2300 (Electron Optic Services Inc., Ottawa, Ontario, Canada), operated by the responsible technician under the supervision of a research team member. Microbial colonization was visualized at magnifications of 10,000 and 20,000 times.

Statistical analysis

The data obtained in CFU/ml were transformed logarithmically, and the Shapiro-Wilk normality test was used to check the data distribution.

The Kruskal-Wallis and Dunn tests were employed to assess the microbial load. The significance level was set at 5% ($p \leq 0.05$). All statistical analyses were conducted using GraphPad Prism version 10.1.2 for Windows, GraphPad Software, Boston, Massachusetts USA (www.graphpad.com).

RESULTS

Analysis of CFU presence in biofilms

The data do not follow a normal distribution, according to the normality analysis using the Shapiro-Wilk test.

Regarding the cultivated microbial groups, different types of posts exhibit similar CFU counts for the same culture medium (Kruskall-Wallis and Dunn test, $p > 0.05$) (Figure 1). These results indicate that, despite variations in the types of intraradicular posts, the quantity of adhered microorganisms of the cultivated species to the post surfaces did not vary statistically significantly among the different materials tested.

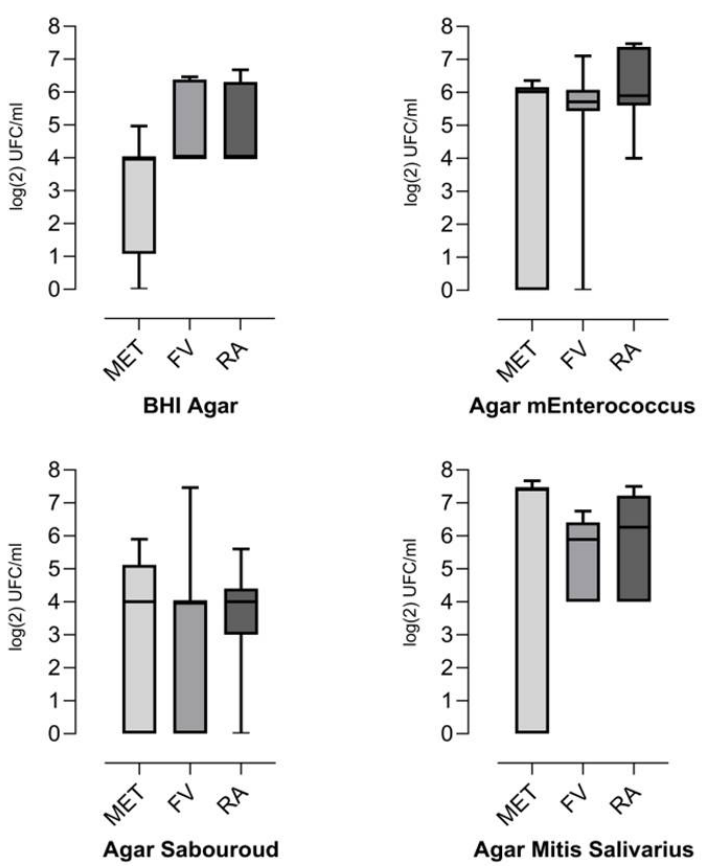

Figure 1. Boxplot comparing the analysis of posts and media.

The CFU counts of the studied species in different media were similar for the same type of post (Kruskall-Wallis and Dunn test, $p>0.05$). This indicates no more significant contamination in one culture media for the same type of material. These results suggest that, regardless of the culture medium used, the microbial load on the intraradicular posts was similar, which may indicate that the microorganisms had a similar capacity for growth and colonization under the different conditions provided by the culture media.

Analysis in SEM

In the SEM analysis, it was possible to identify the presence of microbial colonies on the three types of intraradicular posts analyzed (Figure 2). This visually confirms the adhesion and growth of microorganisms on the surface of the posts, regardless of their composition.

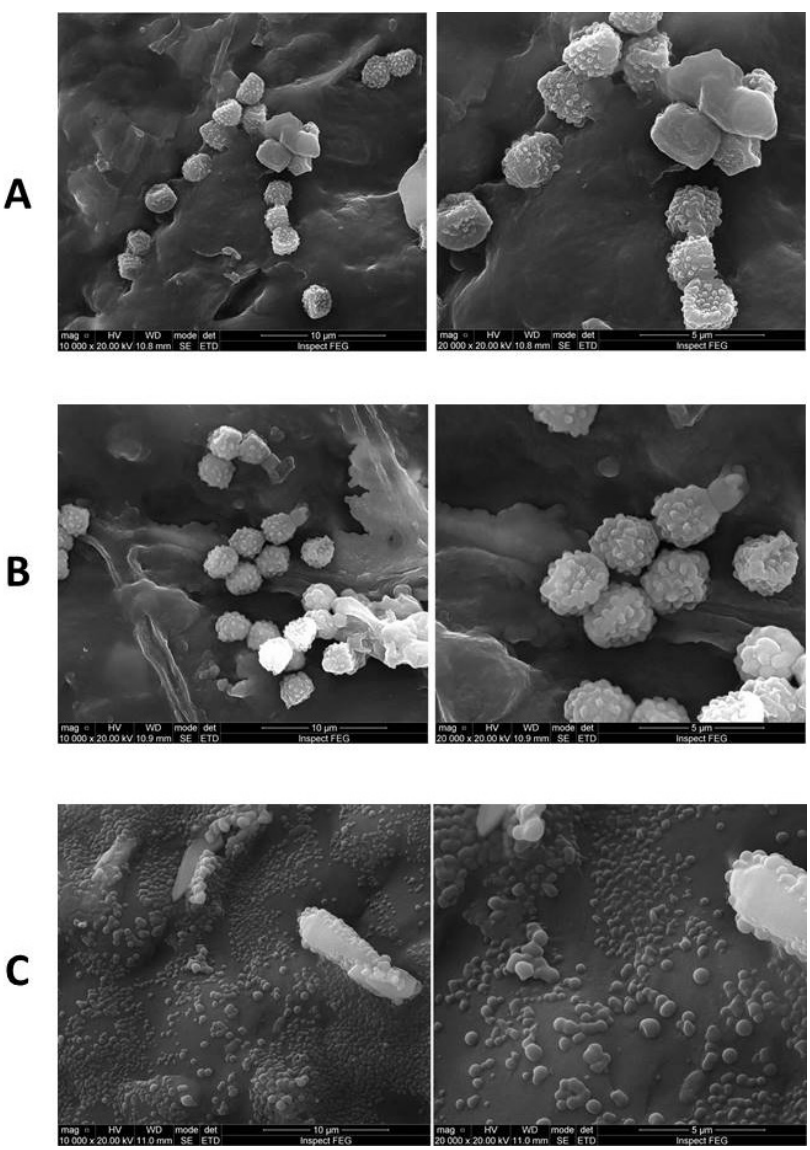

Figure 2. SEM image of the microbial colony formed on fiberglass posts (A), nickel-chromium cast metal (B), and temporary acrylic resin (C).

This observation reinforces the results obtained in the microbiological analyses. It suggests that all types of intraradicular posts studied have a propensity for microbial colonization when exposed to the oral environment for a prolonged period. This visual evidence complements the quantitative analyses and provides a more comprehensive understanding of the observed phenomenon.

DISCUSSION

The use of intraradicular posts in dentistry is a common practice in endodontic procedures, playing a crucial role in the structural restoration of teeth with significant tissue loss. The exposure of these posts to the oral cavity can introduce a new challenge to subsequent restorative procedures and raise concerns about microbial contamination. However, no exact parameters define how microbial establishment occurs on intraradicular posts when exposed to the oral cavity. Therefore, the present study contributes to understanding this dynamic process of biofilm establishment on dental materials exposed to the oral cavity.

The selection of the three types of intraradicular posts (fiberglass, nickel-chromium cast metal, and temporary acrylic resin) was due to their widespread use in the clinical routine of dentists. These materials represent standard options for the structural restoration of teeth with significant tissue loss in endodontic procedures. Fiberglass is often chosen due to its ability to mimic the elasticity of natural dental tissue, offering an aesthetic and functional option. On the other hand, nickel-chromium cast metal posts are traditionally used for their strength and durability. Temporary acrylic resin posts are employed temporarily during the restoration process until a definitive solution is applied. By addressing these three types of intraradicular posts, the study aims to provide relevant insights into the interaction between the most common materials in clinical practice and the oral microbiota, thus contributing to a more comprehensive understanding of the challenges associated with microbial contamination in this context.

The choice of culture media to identify facultative anaerobes, *Enterococcus spp., Streptococcus sp.*, and fungi is directly related to the microorganisms commonly found in endodontic infections. Facultative anaerobes are often associated with these infections due to the anaerobic conditions in root canal systems. *Enterococcus spp*. and *Streptococcus sp.* stand out as widely recognized etiological agents in endodontic infections. According to Lima et al. [11], streptococcal species are frequently found in endodontic infections. *Streptococcus mutans*, usually associated with dental caries, was identified in 70% of patients with primary and secondary endodontic infections. Regarding *Enterococcus spp.*, Pinheiro et al. [12] emphasized that *Enterococcus faecalis* is a microorganism in endodontic infections, often isolated from previously treated root canal systems (45.8%). Furthermore, the presence of fungi, such as *Candida albicans*, can also contribute to the complexity of these infections [13]. Therefore, the careful selection of culture media aims to provide a conducive environment for the growth and identification of these microorganisms, allowing for an accurate assessment of microbial load on intraradicular posts and their relationship with endodontic infections.

By adopting a 28-day exposure period of the posts in the oral cavity for microbial analysis, the study aimed to provide a representative assessment of the microbial load on intraradicular posts after prolonged exposure to the oral cavity, thus contributing to a more comprehensive understanding of the factors influencing microbial contamination in this clinical context. According to a study by Matoso et al. [14], after 2-3 weeks, it is already possible to observe the formation of multispecies biofilms with a higher degree of maturation.

The study results' analysis revealed a high microbial load adhered to the surface of the retainers, regardless of their composition. This finding highlights the possibility of microorganisms' colonization

of retainers, indicating that microbial contamination is a common challenge faced in clinical practice. Microorganisms with variable morphological characteristics were observed in the analysis of the posts, similar to those presented in studies [15,16] that evaluated extracted teeth with root canal infections.

Several studies have utilized SEM to observe the topography and structure of the formed biofilm [17,18]. According to Baldasso et al. [16], SEM provides a detailed analysis of the characteristics and location of the biofilm and bacterial morphology, including cocci, rods, filaments, and spirochetes, along with the visualization of human cells. It was observed that cocci and rods are the most common bacterial forms in SEM images. This finding is consistent with previous studies demonstrating a high prevalence of Gram-negative rods and Gram-positive cocci in primary endodontic infections [19,20].

One limitation of this study was the need for follow-up for periods shorter than 28 days, which would have allowed for the investigation of biofilm formation and colonization by each analyzed microbial species. Future research aims to develop a composite or substance capable of being applied to intraradicular posts to hinder the adherence of microorganisms to their surface without compromising the properties of the materials and the subsequent rehabilitative procedures following their application. This could significantly contribute to the maintenance of periapical health and increase the durability of restorative procedures.

CONCLUSION

Based on the study results, it can be concluded that intraradicular posts exposed in the oral cavity for twenty-eight days, regardless of their composition, demonstrated a high microbial load organized into biofilm on their surface.

Conflict of interest: The authors declare that there are no conflicts of interest.

Collaborators

FL Rodrigues and NP Plein, methodology, resources, writing – original draft. LH Burnett Júnior, investigation, methodology, supervision, writing – review & editing. F Montagner and SB Luisi, conceptualization, data curation, projetct administration, supervision, writing – review & editing. TAF Melo, conceptualization, investigation, methodology, resouces, data curation, projetct administration, supervision, writing – review & editing.

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