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Research Article

A Comparative Evaluation of Microbial Adhesion of Oral Pathogens to Three Different Types of Post system: *An Invitro* Study

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Abstract

Objective: This study evaluated the adhesion probability of three common microflora present in the oral cavity as *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans* to the three frequently used post systems: Stainless Steel metallic post, non-metallic Composite Fiber post and Quartz Fiber post.

Materials and Methods: To compare the adhesion rate, fifteen posts; five of each material type were tested after impregnating them in salivary substitute for 1 hour, and each post type was cultured with the three different pathogens separately using BHI and sucrose broth respectively at 37°C incubation for 4 weeks. Miles and Mishra standard methodology were followed to form microbial biofilms and results were analyzed by counting of colony-forming units (CFUs/mL).

Results: The highest probability of bacterial adhesion for *Staphylococcus aureus* followed by *Candida albicans* was seen with quartz fiber post. However, the adherence ratio when evaluated statistically was not significant with P-value > 0.05. The evaluation of mean values of each indicates a slightly increased adhesion of *Staphylococcus aureus* (1.94×10^4) and *Candida albicans* (12.10×10^6) to quartz post. In case of Stainless steel, the mean values of *S. aureus* (1.05×10^4) showed a slight decrease in adhesion when compared to composite fiber post (1.21×10^4). *Candida albicans* population adhered more to stainless steel (6.63×10^6) when compared to composite fiber post.

Conclusion: In this pilot study, maximum growth of microorganisms to quartz fiber post material was observed, although a further large sample size is required to corroborate the findings. If confirmed, with larger data sample, the quartz fiber post can be avoided in cases of periapical inflammation and retreatment cases requiring posts.

Keywords: *Post and Core Failure; Microbial Adhesion; Post Materials; Stainless Steel Post; Composite Fiber Post; Quartz Fiber Post*

Abbreviations

ATCC: American Type Collection Center; BHI: Brain Heart Infusion Agar; CFU: Colony Forming Units

Introduction

A dental post is recommended in cases with extensive loss of coronal structure and the post is used to provide retention and resistance for an artificial crown. It is also used as a platform for retentive attachment systems and non-retentive overdenture abutments [1]. The ideal properties of a good dental post material include fatigue resistance, possess elastic modulus similar to dentin, and protect crown margin seal from coronal leakage. The post should be preferably radiopaque with good esthetics and biocompatibility. The type of post materials varies from a metallic post which is prefabricated or custom made. The prefabricated post is available like stainless steel, titanium and Brass post. The custom made post can be made from gold alloys, chrome-cobalt alloys, or nickel-chromium alloys [2].

The nonmetallic most are fiber posts which are composite fibers embedded in an epoxy resin matrix. Different fibers can be embedded in a resin matrix. The commonest fibers are reinforced composite, glass, or quartz, or carbon fiber. Among the types of fibers, Carbon Fiber is not esthetic although they have good mechanical properties. Hence the commonest used posts are fiber-reinforced glass or quartz posts. These posts have excellent esthetic properties, flexural and fatigue strength, modulus of elasticity similar to that of dentin, and hence are easy to handle allowing single-visit therapy. The main advantage of fiber post is that they are biocompatible, relatively cheap, and can be easily removed if necessary. The other group of the post is Zirconia composed of partially stabilized zirconium dioxide ceramic material that shows excellent chemical stability, good esthetic, radio-opacity, and superior light transmission properties. The drawback is that they are brittle, cannot be etched, thus bonding to resins is less predictable. Because of their susceptibility to fracture, and the increased risk of root fracture, along with the difficulties associated with bonding, they are not routinely used in the clinical situation [3]. Failures of fiber posts were mainly due to post-loss of retention, while metal post failures were mostly related to root fracture, post-fracture, and crown and/or post-loss of retention. In conclusion, metal posts and fiber posts present similar clinical behavior at short to medium-term follow-up [4]. Remaining dental structure and ferrule increase the survival of restored root-canaled teeth, some studies showed that post type did not significantly influence the survival of restorations. These results can help dentists answer the important question of how best to rehabilitate endodontically treated teeth with no remaining coronal wall [5]. Amongst all post types, Studies have shown that the most widely accepted post material is composite fiber posts [6]. The combination of aesthetic and mechanical benefits of fiber post has made its use more frequent. As for the study conducted by these authors, fiber posts showed favorable marginal seal properties and hence can substantially reduce microbial leakage and contamination in endodontically treated teeth [6].

Microflora in the oral cavity comprises a diverse group of organisms that are mostly harmless however, bacteria are the most predominant group among all. Some bacteria have been implicated in oral diseases such as caries and periodontitis, Anaerobic *Streptococcus mutans* for example can cause periodontal inflammation and dental caries [7,8]. Staphylococci were isolated from plaque samples of individuals with dentures and can be seen in immunocompromised patients and patients suffering from oral infections. Moreover, *Actinobacillus* present in periodontal pockets can result in localized and generalized aggressive periodontitis, while *Candida albicans* fungal pathogens can spread directly from the oral cavity to the throat and stomach. In our study, we have selected three different types of post materials and three commonest microorganisms present in oral flora to analyze the adhesion properties of post materials to these selected organisms [9].

Aim of the Study

To identify the dental post material that has the least adhesion of microorganisms on its surface after 4 weeks of impregnation in artificial salivary substitute, simulating the human oral cavity at body temperature. This study also attempts to find the safest post system to be used in retreatment cases due to infected canals.

Materials and Methods

Materials used

Post materials (Figure 1):

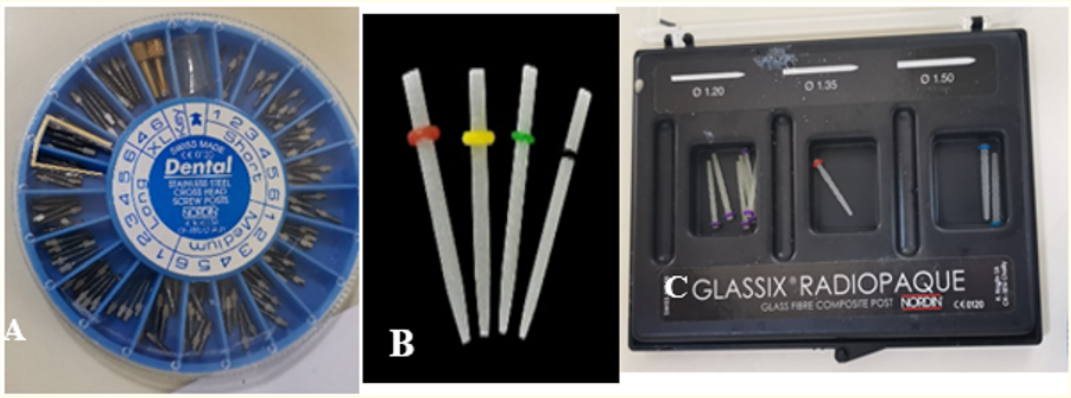

Figure 1: *(A) Stainless steel post. (B) Quartz fiber post. (C) Glass composite post.*

- Group A - Stainless Steel post (15 posts)
- Group B - Composite Fiber post (15 posts)
- Group C - Quartz Fiber post (15 posts).

Salivary substitute

A standard brand of salivary substitute (MOI-STIR) was used for experimentation to simulate conditions of the oral cavity with the following composition: water, Glycerin, Sodium CMC, Methylparaben, Potassium chloride, Dibasic sodium phosphate, Propylparaben, Calcium Chloride, Magnesium chloride, and Sodium chloride.

Organisms and media

- BHI and sucrose - growth medium - 20 ML of BHI and 3% sucrose.
- Broth - culturing medium - 20 ML of broth.
- *Staphylococcus aureus* ATCC-29213, (2). *Streptococcus mutans* ATCC-25175 and (3). *Candida albicans*. ATCC- 24433.

Methods

All experiments were carried out in a Microbiology laboratory inside a biosafety hood, as shown in the methodology chart of figure 2. Five posts from three different post materials (Stainless Steel, Composite Fiber, and Quarts Fiber) were placed into three containers

with the culture of oral pathogens: *Staphylococcus aureus*, *Streptococcus mutans*, and *Candida albicans* respectively. In the first step, the posts were soaked in a salivary substitute at room temperature for 1 hour (Figure 5B). Then each oral pathogen was cultured in BHI Broth medium (Figure 3), containing 20 ml of the liquid culture medium and 20 ml of the growth medium (BHI and 3% sucrose). Subsequently, three posts of different materials were incorporated into distinct containers containing 3 different microorganisms. The containers were briefly mixed and kept at (37°C) for 4 weeks.

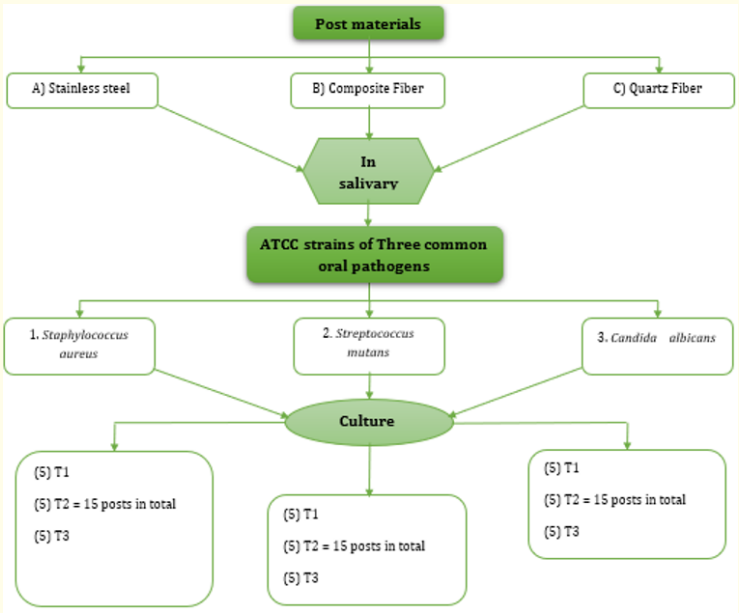

Figure 2: *Methodology chart.*

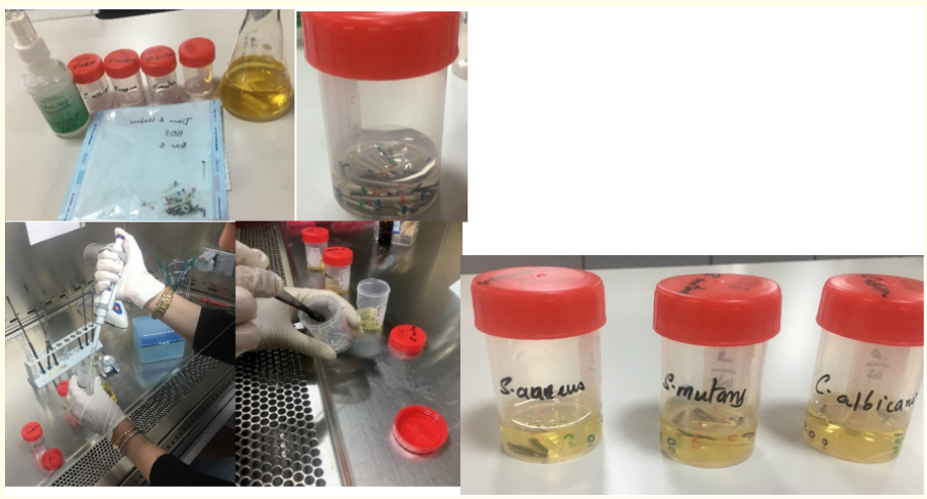

Figure 3: *(A) Materials used, (B) Post materials kept in salivary substitute for 1 hour, (C) ATCC strains of oral pathogens cultured separately with the broth medium, (D) Post materials containing Staphylococcus aureus, Streptococcus mutants and Candida albicans culture.*

Experiment

A standard methodology was followed for microbial biofilm formation using standardized strains of *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans*. The *Candida albicans* strains were subcultured onto Sabouraud dextrose agar, while the strains of *Staphylococcus aureus* and *Streptococcus mutans* were picked in brain heart infusion agar (BHI). The microorganisms were incubated in a bacteriological incubator at 37°C for 24 hours. Then all tests with *Streptococcus mutans* strains were incubated in a bacteriological oven under microaerophilic conditions (5% CO_2). After the incubation period, microorganism colonies were suspended in sterile saline solution (0.9% NaCl) and set in a spectrophotometer for obtaining a standardized suspension containing 10^6 cells/mL. The parameters of optical density and wavelength were, respectively, 0.284 and 530 nm for *Candida albicans*, 0.374 and 490 nm for *Staphylococcus aureus* and 0.620 and 398 nm for *Streptococcus mutans*. For biofilm formation, the broth was used, which is composed of 20g trypticase, 2g NaCl, 3g K_2HPO_4, 2g KH_2PO_4, 1g K_2CO_3, 120 mg $MgSO_4$, 15 mg $MnSO_4$, and 50 g $C_6H_8O_7$, dissolved in 1000 mL of distilled water. The broth was sterilized by autoclaving at 121°C for 15 minutes. The sterilized specimens were placed in the first row of 24-well plates with the aid of sterile tweezers, and 0.1 mL of each microbial suspension was inoculated in each well of the plate for the formation of the multispecies (heterotypic) biofilm. The plates were incubated in a bacteriological incubator maintained at 37°C for 48 hours.

CFU Counting (CFU/mL) was used to remove the non-adherent microbial cells, 2 mL of broth were substituted by 2 mL of sterile saline solution in each well, and the plate was stirred for five minutes with an orbital shaker. The CFU count was performed on the whole sample size of each group that contains 15 posts. For this, specimens were individually placed in falcon tubes containing 10 mL of sterile saline solution and then homogenized for 30 seconds. After that each microorganism was diluted with sterile saline solution and placed in selective media as follows: *Candida albicans* in Sabouraud dextrose agar with 50 mg/L of chloramphenicol, *Staphylococcus aureus* in NaCl BHI agar, and *Streptococcus mutans* in Mitis Salivarius agar plus 0.2 IU/mL of bacitracin and 15% sucrose. The plates were incubated at 37°C for 48 hours, and the plates containing from 30 to 300 colonies were counted for the CFU number. Since samples were contaminated by a biofilm composed of three microorganisms (*S. mutans, C. albicans, and S. aureus)*, for each sample, the definitive CFU/mL value was obtained by summing each CFU/mL value. Biofilm analysis was done by counting microorganisms according to Miles and Mishra method (Figure 4) for viable cell counting, to determine the number of colonies forming units in a bacterial suspension or homogenate.

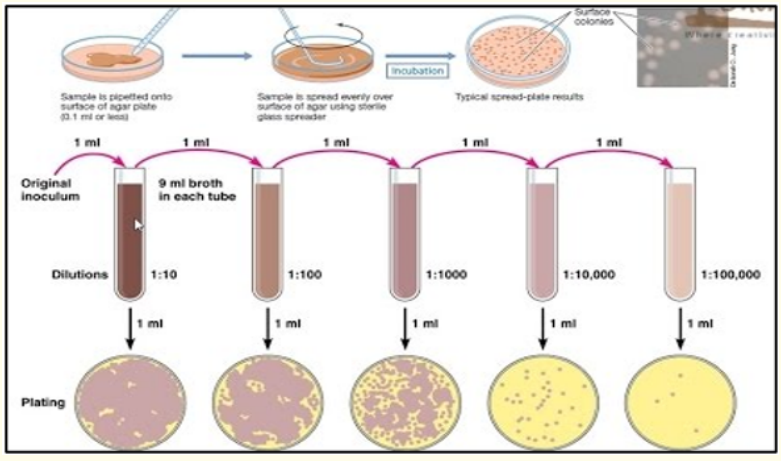

Figure 3: *Bacterial counting using Miles and Mishra dilutions to agar plates.*

Results

Results listed in table 1 and 2 have shown that the highest probability of bacterial adherence is to quartz fiber post, which was proved by the maximum growth of *staphylococcus aureus* and other mentioned microflora on the surfaces of this material (Figure 5). Lesser probability of *Candida albicans* adhesion (Figure 6) to quartz/glass fiber post but still *Candida* has the highest rate of growth to it. However, *Streptococcus mutans* is the only microflora that did not show any adherence reaction to any post system (Figure 7).

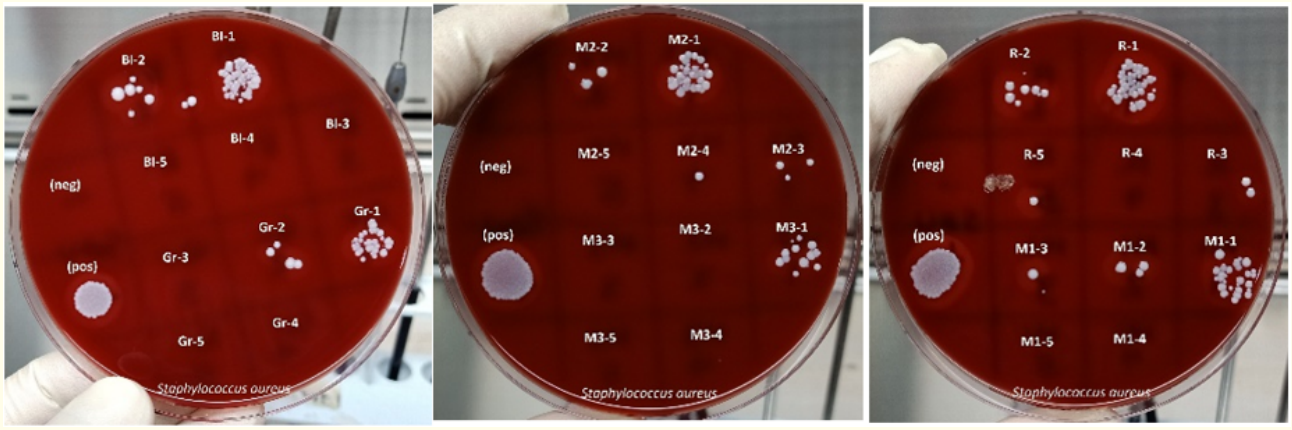

Figure 5: *Miles and Mishra counting of blood agar plates with Staphylococcus aureus.*

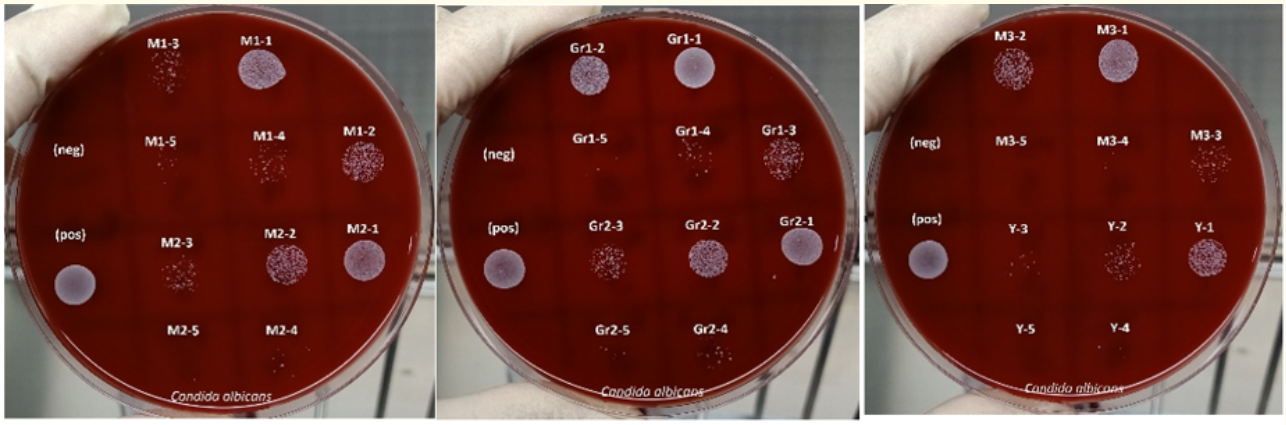

Figure 6: *Miles and Mishra counting of blood agar plates with Candida albicans.*

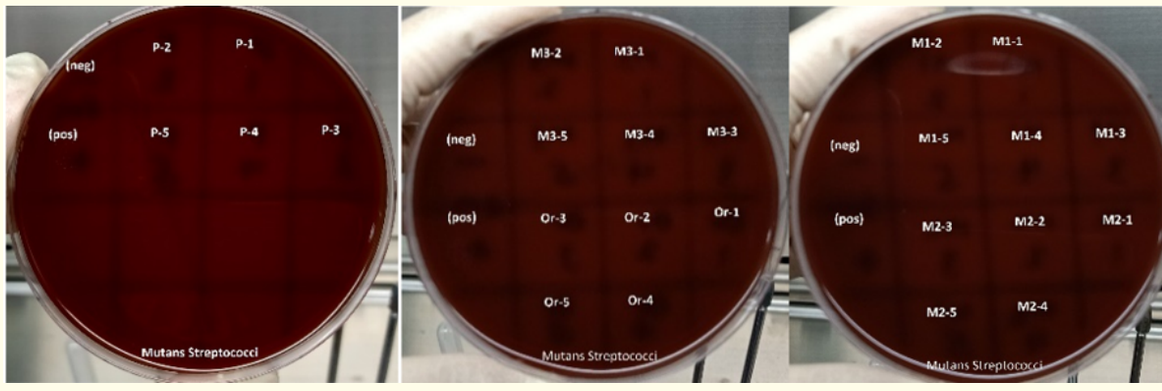

Figure 7: *Miles and Mishra counting of blood agar plates with Streptococcus mutants.*

On average, composite fiber post showed the least adhesion rates as lesser microbial colonies were formed, followed by stainless steel post, having quartz fiber post with the highest rates of adhesion. Statistical Analysis was done by ANOVA and it was observed that there was no statistically significant difference in microbial colonization between the different groups of stainless steel, composite fiber and quartz fiber posts. The mean values of each indicate a slightly increased adhesion of *Staphylococcus aureus* (1.94×10^4) and *Candida albicans* (12.10×10^6) to quartz post. In the case of Stainless steel, the mean values of S aureus (1.05×10^4) showed a slight decrease in adhesion when compared to composite fiber post (1.21×10^4). *C albicans* population adhered more to stainless steel (6.63×10^6) when compared to composite fiber post (6.28×10^6) (Table 1-3).

Sample	Number of colonies	Colony counts	CFU/ml
Stainless Steel			
Stainless steel (1)	2	3	1.5×10^4
Stainless steel (2)	2	3	1.5×10^4
Stainless steel (3)	1	11	5.5×10^3
Stainless steel (4)	2	19	9.5×10^3
Stainless steel (5)	2	15	7.5×10^3
Mean			1.05×10^4
Composite Fiber			
C. Fiber (1)	2	4	2.0×10^4
C. Fiber (2)	2	4	2.0×10^4
C. Fiber (3)	1	13	6.5×10^3
C. Fiber (4)	2	17	8.5×10^3
C. Fiber (5)	2	11	5.5×10^3
Mean			1.21×10^4
Quartz Fiber			
Quartz (1)	2	6	3.0×10^4
Quartz (2)	2	6	3.0×10^4
Quartz (3)	1	6	3.0×10^4
Quartz (4)	2	4	2.0×10^3
Quartz (5)	2	10	5.0×10^3
Mean			1.94×10^4

Table 1: *Colony count (CFU/ml) Staphylococcus aureus with different post materials.*

Sample	Number of colonies	Colony Count	CFU/ml
Stainless Steel			
Stainless steel (1)	4	48	24.0×10^6
Stainless steel (2)	4	12	6.0×10^6
Stainless steel (3)	3	62	3.1×10^6
Stainless steel (4)	3	8	4.0×10^4
Stainless steel (5)	3	5	2.5×10^4
Mean			6.63×10^6
Composite Fiber			
C. Fiber (1)	4	33	16.5×10^6
C. Fiber (2)	4	28	14.0×10^6
C. Fiber (3)	3	11	5.5×10^5
C. Fiber (4)	3	13	6.5×10^4
C. Fiber (5)	3	4	2.0×10^4
Mean			6.28×10^6
Quartz Fiber			
Quartz (1)	4	35	17.5×10^6
Quartz (2)	4	51	25.5×10^6
Quartz (3)	4	35	17.5×10^6
Quartz (4)	3	4	2.0×10^4
Quartz (5)	2	3	1.5×10^3
Mean			12.10×10^6

Table 2: *Colony count (CFU/ml) of Candida albicans with different post materials.*

Material	Stainless steel	Composite Fiber	Quartz fiber	Standard error of mean SE_m
S. aureus (Mean)	1.05×10^4	1.21×10^4	1.94×10^4	0.43
C. albicans (Mean)	6.63×10^6	6.28×10^6	12.10×10^6	4.48

Table 3: *Mean colony values for Staphylococcus aureus and Candida albicans in post materials.*

Discussion

Three widely used posts are metallic, fiber, and ceramic. In this study, we selected a various sample size of posts which are clinically available and have the same predominant characteristics of the three mentioned fabricating materials. The first sample is Stainless Steel post, which represents metal, second is Composite Fiber post which represents the non-metallic fiber-reinforced resins, finally, is the Quartz Fiber post representing the non-metallic glass-reinforced composite. This is to understand if the post material itself is prone to be a likely source of infection in the post and core failures due to inflammation or leakage as seen in the margin of crowns by authors [10]. In our study the probability of adhesion of three different microflora commonly seen in the oral cavity, like *Staphylococcus aureus, Streptococcus mutans, and Candida albicans* to each of the post systems was done. The culturing experiment was done according to the method of Miles and Mishra illustrated in the culturing procedure that was interpreted to the agar plates of microbial colonies [11]. *Staphylococcus*

aureus bacteria are found to be more in cases of Periodontitis, *Streptococcus mutans* in dental caries and tooth decay while Candida albicans in the initiation of oral infections [12]. One of the foremost causes of endodontic failure is persistent microbiological infection [13]. Most studies on this subject have shown that a higher occurrence of gram-positive bacteria (ex: *Streptococci, lactobacilli*, *Enterococcus faecalis*) in both post-instrumentation and post-medication, so the gram-positive bacteria is the most important bacteria that resist the harsh environmental conditions in instrumented and medicated canal, In a study performed on 236 cases of endodontic treatment failures found a correlation between the presence of bacterial infection in the canals and peri-radicular rarefaction in endodontic failures [14].

Improper coronal seal

A well-sealing coronal restoration is important after the completion of obturation because it would prevent the doorway of any microorganisms, which are present within the ambient environment [15]. Coronal seal protects and seals the tooth, preventing percolation of saliva and bacteria apically that results in failed treatment, the coronal access to a root canal treated tooth must be sealed completely for the lifetime. Coronal leakage is a significant etiology in an endodontic failure resulting in saliva exposure, lead to leakage, which can compromise the gutta-percha seal, and the tooth may require retreatment. Endodontics treatment is successful if restoration after the root canal is done properly [12].

Improper obturation

Clinically and radiographically, it is not possible to determine how well the root canal system has been cleaned and obturation, the root filling is judged by its taper, build up and length. Intending to give an all-around condensed root filling finishing only coronal to the apical foramen is desirable and besides significant for periapical health to ensure material isn't expelled into the periapical tissues, A recent systematic review noted that root fillings delivered without voids and which extended to within 2 mm of the radiographic apex, had an essentially improved result [16]. Short root filling or a canal with extruded material could cause delayed healing or treatment failure due to inflammation or outside body reaction [16]. Adequate coronal seal after a properly filled root has positive effects on periapical health. Researchers have published various studies on the comparison of fiber posts to the conventional metallic post [5,17]. Also, articles based on bacterial adherence to the different commercially available dental restorative materials have been reported [10]. Most of these previous *in-vitro* studies favored composite fiber posts to other post materials [18]. The success of a post system also depends on the post space preparation and coronal restoration seal [19]. No evident microbial adhesion studies have been reported on various types of the post though studies on microbial adhesion on restorative material and crown margin have been reported. The limitation of this study was that this was a pilot study and different post materials, various other oral microflora along with a larger sample size are required for further inference.

Conclusion

This study showed the effect of three different dental post material and their adherence to prominent oral pathogens. *Staphylococcus aureus* showed maximum adherence to the quartz post and minimum adherence was seen with the metal post. We also observed that opportunistic fungal pathogen *Candida albicans* which is seen in immunocompromised cases showed maximum adherence to quartz post and minimum to fiber post. Whereas, *Streptococcus mutans,* the most cariogenic oral pathogen did not show adherence to any post. No statistical significance could be ascertained due to the small sample size of dental posts.

The study also brings out the fact that different dental posts can have differences in adherence rates of oral pathogens and can be important in post and core treatment decisions. Nevertheless, more studies with a large sample size are required for further corroboration of results. Based on our results, bacterial adherence on Quartz post was observed to be more and is of clinical significance. Further studies in these regards are warranted and such studies should be made available in the public and scientific domain.

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Conflict of Interest

We do not have any financial interest or any conflict of interest.

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