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Microbiological Sampling of Interdental Brushes – Importance of Storing Condition and Disinfection

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ABSTRACT

The success of proper oral hygiene depends on optimizing plaque control. Compared to tooth brushing alone, interdental brushes are more effective in reducing plaque and gingivitis. The aim of this pilot study was to find a storage and/or disinfection method to reduce the number of bacteria left on interdental brushes after use, thereby reducing the transfer of bacteria from one interdental space to another. We conducted a microbiological comparison of four different storage and/or disinfection methods for interdental brushes. The results suggest that mouthwash may be a useful and accessible method for patients to disinfect and reduce the bacterial load on interdental brushes. Larger, more thorough studies with a larger sample size are necessary to further validate this.

Keywords: preventive actions, oral care, interdental brushes, disinfection, microbiological sampling

INTRODUCTION

It is crucial that a patient possess the ability to maintain appropriate mechanical plaque control. Subgingival recolonization occurs within 4–8 weeks following scaling and root planing if there is insufficient effective plaque control throughout the healing or maintenance phase.[1] On the other hand, effective supragingival plaque control seems to be adequate to stop disease relapse or recurrence

caused by subgingival recolonization. Inadequate dental hygiene provides a substantial risk for periodontal disease. The technique of brushing is widely used for mechanical plaque control, but it depends on personal skill. It has been demonstrated that a frequency of teeth brushing of twice per day significantly improves gingival health.[2]

An average oral hygiene session may only be able to eliminate around 60% of the overall plaque accumulation. Based on the findings of a recent systematic review, 42% of brushing sessions are effective in removing plaque. In addition, brushing is considered to be more effective in cleaning the buccal surfaces of teeth than their interproximal surfaces. The interproximal areas have the highest risk for plaque accumulation both in the lateral and frontal regions. These areas are therefore more susceptible to periodontal diseases and caries formation, which has a high clinical importance.[3,4]

Different tools are used in plaque control, such as oral irrigators, wooden interdental aids, dental floss, and interdental brushes. The study of Marchesan *et al.* offers proof in favor of interdental cleaning tools as supporters of positive oral health outcomes.[5] A frequency of four to seven times per week of interdental cleaning was also linked to reduced interproximal periodontal disease. The study also indicated that interdental cleaning is connected with less coronal and interproximal caries, fewer missing teeth, and less periodontal disease. These results are in accordance with the results of Crocombe *et al.*, who discovered that the reduction in plaque, calculus, and gingivitis was linked to interdental cleaning.[6]

In 1976, a study was conducted to evaluate the efficacy of interdental brushes (IDBs) in managing plaque 2–2.5 mm below the gingival margin. The results of this study demonstrated the effectiveness of IDBs in managing plaque accumulation at this specific subgingival depth. Several other studies have also stated that the use of IDBs leads to significant improvements in plaque indices, bleeding indices, and probing depth compared to brushing alone. Research conducted by Sälzer *et al.* concluded that interdental brushing is the most effective method of removing interdental plaque compared to other interdental cleaning aids.[7] This result might be due to the ease of use of the IDB and its greater effectiveness in removing plaque compared to the other tools. When choosing an IDB, there are several factors that may affect its effectiveness, such as size. Research conducted by Bourgeois has shown that daily use of appropriately sized IDBs reduces interdental bleeding.[8]

Maintaining good oral health is essential for general health and health-related quality of life. Effective dental hygiene plays a major role in this. For many years, dental floss and tooth brushing have been used to remove dental plaque from between teeth. Nevertheless, IDBs have been developed, and as long as there is enough space between teeth, many individuals find them more accessible to use. Patients highly favor IDB, finding it less time-consuming and easier to use than flossing.[9]

The aim of this study was to find an efficient method for reducing the number of bacteria left on IDBs after use, to prevent the transfer of bacteria from one interdental space to another.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of Scientific Research of the "George Emil Palade" University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, Romania (approval no. 1895/19.10.2022), and all patients provided written informed consent to participate in the study.

We examined four different methods for storage and disinfection of IDBs after use on nine patients and compared the methods microbiologically. We designated the methods as A, B, C, and D (Table 1). We used each method on the same patient but in different quadrants of the oral cavity.

We used the following inclusion criteria: patients without dental plaque or tartar, not using mouthwash, in good general health, without caries, not pregnant or breastfeeding, healthy periodontal condition, not undergoing antibiotic treatment, not having received systemic antimicrobials, and without prosthetic work, implants, or orthodontic devices in the oral cavity.

After carrying out the cleaning procedures and storing the IDBs for 2 h (Table 1), they were inoculated onto four types of culture media: blood agar, lactose agar, Chapman, and Sabouraud (*Candida* spp.). The plates were divided into four groups based on the methods used, and each brush was inoculated into a quarter of the corresponding culture media. In each quarter there was a line indicating the position of the brush (Figure 1), where we added the inhibitory substance to the solid media, thus causing an increase of colonies of desired bacteria. The media were then incubated at 37 °C for 24 h. Colonies not belonging to the normal flora were further identified on ABE medium. The fungi were incubated for 48 h and identified on Candiselect medium.

The blood agar media were analyzed separately and scored based on the size and density of the bacterial cultures growing on them. For size, a score of 0 was given if no

TABLE 1. Description of the methods used for storing and/or disinfection of IDBs

Method	Description	Disinfection and storage protocol
A	The patient's interdental spaces were cleaned in the first quadrant, one by one. The IDB was rinsed with running tap water after each interdental space.	At the end of the cleaning process, the IDB was rinsed with tap water and stored with the cap on.
B	The patient's interdental spaces were cleaned in the second quadrant, one by one. The IDB was dipped in mouthwash* after each interdental space.	After the cleaning procedure, the IDB was dipped in mouthwash and stored with its cap on.
C	The patient's interdental spaces were cleaned in the third quadrant, one by one. The IDB was not rinsed with anything.	After the cleaning procedure, the IDB was not cleaned and was stored with the cap on.
D	The patient's interdental spaces were cleaned in the fourth quadrant, one by one. The IDB was rinsed with running tap water after each interdental space.	After cleaning, the IDB was rinsed with tap water and stored uncapped.

*Listerine Total Care Fluoride Mouthwash

colony was present in the examined quarter, a score of 1 if small colonies growing were slightly visible, a score of 2 if a clearly visible, well-defined colony was present, and 3 if a well-defined, large-diameter colony was present. Density was scored as 0 if no colonies were present in the surveyed area, 1 if colonies covered 1–30% of the surveyed area, 2 if 30–60% of the surveyed area was covered, and 3 if >60% of the area was covered.

RESULTS

During cultivation, we analyzed the blood agar media for the size and density of the bacterial culture growing on them. In terms of size, we obtained an average score of 1.66 for method A, 1.11 for method B, 2.77 for method C, and 2 for method D. As far as density scores are concerned, we obtained an average score of 2.33 for method A, 0.88 for method B, 2.77 for method C, and 2.55 for method D. Method A scored a total of 36 points, method B scored 18 points, method C scored the maximum 50 points, and method D scored a total of 41 points.

Taking into account the average value, size, and density, we determined the effectiveness of the methods. Method A was a moderately effective method as it received an average of 4 points, taking into account size and density. Method B was an effective method as it received an average of 2 points. Methods C and D were the least effective, with C receiving an average of 5.55 and D receiving an average of 4.55 (Table 2).

During the identification of the bacteria, in all cases and methods, bacteria of the alpha-hemolytic *Streptococcus viridans* group grew on the blood agar medium. We did not identify these bacteria further, as they are part of the normal flora of the oral cavity and are not pathogenic. We tried to identify *Enterococcus* with the ABE culture media, given that this bacterium is involved in the processes of pulpitis and periodontitis, but the result was negative. *Candida* spp. were identified using Sabouraud medium. In three of the subjects, *Candida* was present when methods A and C were used, and in only one case when method B was used. Further identification was carried out on the

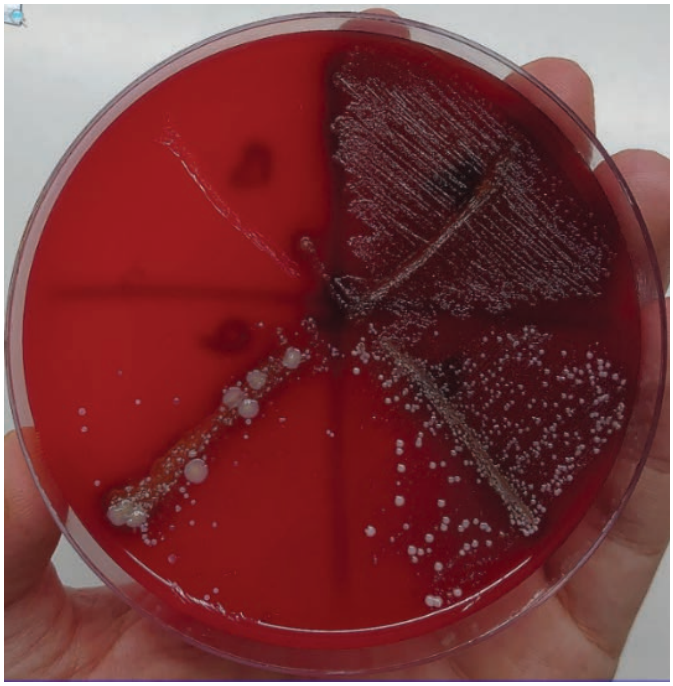

FIGURE 1. Detail of the quarters on a blood agar solid culture medium

TABLE 2. The effectiveness of the methods based on the average values obtained

Average values	Effectiveness	Method
0–2	Effective	B
2.1– 4	Moderately effective	A
4.1– 6	Less effective	C, D

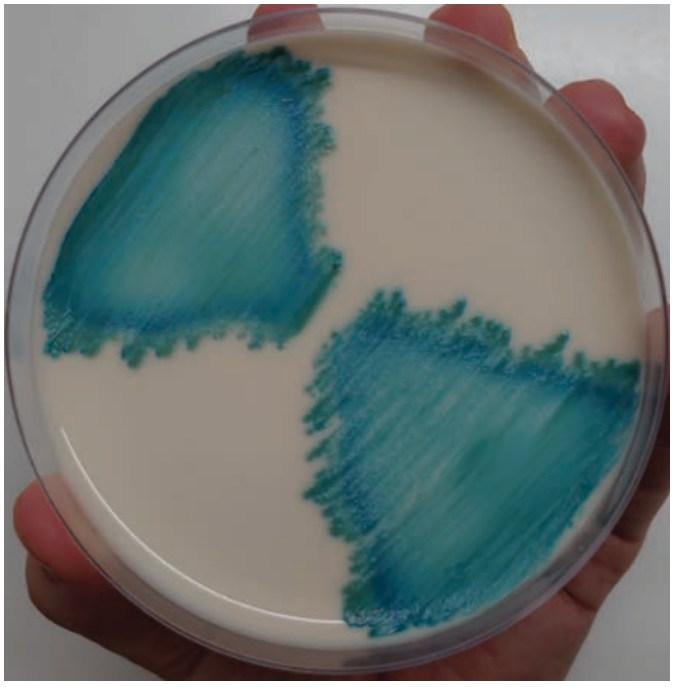

FIGURE 2. *Candida albicans* on Candiselect medium

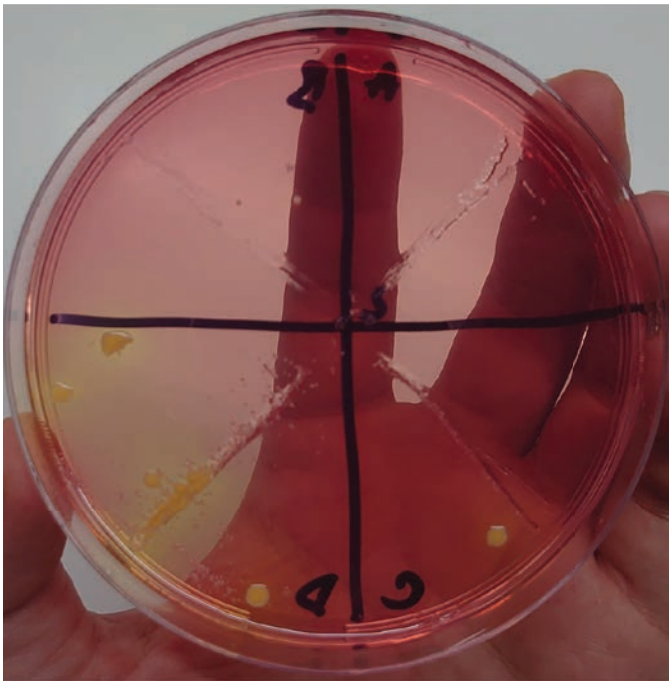

FIGURE 3. Chapman culture showing *Staphylococcus aureus* colonies on the left section

Candiselect medium, and in all cases, we obtained the presence of *Candida albicans*, as the culture media were stained blue (Figure 2).

We also tried to identify *Staphylococcus aureus* on Chapman medium, on which it produces a yellow pigment, discoloring the medium (Figure 3). These colonies grew in the case of four patients. The largest colonies were observed with methods C and D, whereas the smallest colonies were observed using method A.

DISCUSSION

Maintaining periodontal health and oral hygiene is essential not only for the preservation of dentition but also for well-being and general health. The primary cause of periodontal and dental caries is microbial biofilm, a surface-associated, functionally structured multi-species biofilm. However, both diseases are preventable, as their main causes are poor oral hygiene and smoking, which are modifiable risk factors. Therefore, it is possible to successfully avoid these conditions and lower the risk of inflammatory disease by focusing on the numerous physiological factors that can initiate an inflammatory reaction. Maintaining low levels of bacterial reservoirs in the oral cavity is advantageous to lower the risk of infections and chronic periodontal diseases. Limiting the risk of infection from oral pathogenic bacteria should be a top concern, independent of age and medical history.[10,11]

During the inoculation on Chapman and Sabouraud mediums we did not find any significant difference between methods A and D. However, on blood agar, a slight variation was observed between the two methods. Method D proved to be more favorable for the growth of bacteria, as they grew larger in size and density after inoculation. The oral cavity contains more than 700 different types of bacteria, fungi, viruses, and other microorganisms that can lead to various oral diseases. Previous studies found that toothbrushes are typically kept in restrooms, which are highly polluted due to microbes that are disseminated by aerosols. As a result, there is a significant amount of contamination present in these areas. These microorganisms are known to remain viable for up to 7 days. In addition to storage conditions, these factors contribute to the reintroduction of prospective pathogens and contamination into the oral cavity. Therefore, in recent years, the process of eliminating bacteria from toothbrushes and disinfecting them has gained significant attention. The most common techniques for disinfecting toothbrushes include soaking in alcohol and disinfectant solutions, using antimicrobial rinses, cleaning with UV light sources, and storing brushes in closed cabinets that release formaldehyde gas. Typically, toothbrushes and IDBs are rinsed with plain water after use and stored in the bathroom, and there is a high risk of cross-infection through sharing or close proximity. Although there are many publications comparing different toothbrush disinfection techniques, there is limited information about disinfection techniques for IDBs.[12,13]

There are several limitations to our study. We used only one type of mouthwash and relied on the list of components provided by the manufacturer. Research has shown that Listerine contains sufficient antibacterial and anti-inflammatory properties, demonstrating a statistically significant reduction in plaque scores.[14] Although we are aware that mouthwashes were not invented for this purpose, in this pilot study we were looking for storage liquids that are easily accessible to patients. Our aim was to simplify the procedures and make them accessible to everyone because the effort to educate, train, and encourage patients to reduce plaque and bacteria levels is inevitably fraught with problems. The purpose of the study design was to replicate everyday environmental settings, as described previously.[15]

The antibacterial and cariostatic effects of fluorides are widely accepted. They act primarily by forming fluorohydroxyapatite crystals, which are more resistant to organic acids than the hydroxyapatite crystals of tooth enamel. They also reduce the production of organic acids by *Streptococcus mutans* bacteria. The literature describes the use of various compounds such as stannous fluoride, sodium fluoride, sodium monofluorophosphate, acidulated phosphate fluoride, and amine fluoride. Fluoride is bactericidal and bacteriostatic against *Streptococcus mutans*, as well as *Lactobacilli*, *Streptococcus oralis*, *Streptococcus mitis*, and *Streptococcus sanguinis*.[16,17]

A study reported that the combined use of sodium fluoride and cetylpyridinium chloride in mouthwashes resulted in the inactivation of bacteria in the oral cavity to protect tooth enamel. The addition of sodium fluoride did not affect the antibacterial and anti-biofilm efficacy of formulations containing cetylpyridinium chloride. Formulations containing 0.075% cetylpyridinium chloride or combined with 225 ppm sodium fluoride were equally effective in inactivating bacteria in planktonic, ex vivo, and biofilm models, and significantly reduced bacterial viability. Based on these and previous results, the two compounds do not interfere with each other in mouthwashes.[18]

The mouthwash we used also contained zinc chloride. In addition to zinc chloride, zinc can also be found in toothpastes and mouthwashes in the form of zinc oxide, zinc citrate, zinc lactate, and zinc sulfate. Zinc is administered as an antibacterial agent to control plaque and reduce halitosis by inhibiting dilute sulfur compounds and to reduce tartar formation by modifying or inhibiting crystal growth. It has broad-spectrum antibacterial activity, acting mainly on the cytoplasm and glycolytic enzymes of bacterial cells, inhibiting the process of glycolysis. The inhibitory effect of zinc salts on microbial glycolysis depends on the pH of the saliva and the bacteria. Inhibition is greatest at pH 7, as observed for *Streptococcus salivarius* and *Streptococcus sobrinus*. Zinc chloride and cetylpyridinium chloride are effective in inhibiting the growth of seven bacterial strains involved in inflammatory processes around the implant and in the production of halitosis; these bacterial strains are *Streptococcus mutans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Treponema denticola*, and *Tannerella forsythia*.[19]

In a similar study, the effect of green tea extract and alcohol-free mouthwash on the formation of *Candida albicans* biofilm on the surface of synthetic resin was investigated. The authors concluded that both green tea extract and alcohol-free mouthwash reduced Candida albicans biofilm formation and survival on the resin surface. However, it is important to note that more research is needed to understand the efficacy and safety of oral care products.[20]

Our approach may considerably lower the frequency and/or severity of periodontal disorders. The cornerstone of oral health education aimed at preventing periodontal disease is tooth brushing, with a focus on the systematic cleaning of all interdental tooth surfaces.[21] By limiting the growth and colonization of periodontal bacteria and reducing inflammation, this preventive method may have a substantial role in the prevention of periodontitis. Over time, using IDBs correctly on a daily basis and storing them properly should help lower the risk and complications associated with periodontal disorders. However, long-term prospective cohort studies are necessary to strengthen this hypothesis. It is important to note that the majority of studies on IDBs have focused on patients with periodontitis or gingivitis, or on the efficacy of IDBs. The lack of evidence in the literature suggests that there is still room for improvement regarding interdental prophylaxis in clinically healthy individuals. Despite its limitation, our study should open new perspectives for disinfection and oral health.

CONCLUSIONS

Mouthwash effectively reduces the number of bacteria left on interdental brushes after use, reducing the transfer of bacteria from one interdental space to another. A larger, more thorough study with a larger sample size is needed to validate the recommended method for the disinfection of IDBs.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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