Original Article

In vitro evaluation of microbial contamination of orthodontic brackets as received from the manufacturer using microbiological and molecular tests

Darlene R. dos Santos Gerzson[a]; Daniel Simon[b]; Aline Lima dos Anjos[c]; Maria Perpétua Mota Freitas[d]

ABSTRACT

Objective: To test the null hypothesis that orthodontic brackets as supplied by manufacturers do not have microbial contamination.

Materials and Methods: The sample comprised 140 brackets of four different commercially available brands, used directly from the manufacturer's packaging, divided into 14 groups (n = 10 brackets each). Of the 140 pieces, 60 were full cases and 80 were replacement brackets. Materials were tested to detect bacterial growth, analyze types of bacteria present (biochemical test), and identify bacteria (molecular test with polymerase chain reaction [PCR]).

Results: In two of 12 groups the brackets showed microbial contamination: group 1, Morelli full case brackets, and group 12, Abzil-3M Unitek replacement brackets. *Staphylococcus aureus* and *Staphylococcus epidermidis* were the bacteria identified in groups 1 and 12, respectively (suggested by the biochemical test and confirmed by PCR).

Conclusions: Brackets of two brands (Morelli and Abzil-3M Unitek) were found to be contaminated by bacteria in the original packages supplied by the manufacturers, which suggests a risk for patient contamination. These data suggest that the manufacturers of these materials should improve the quality control of the packaging used, including sterilization, for the security of patient health. (*Angle Orthod.* 2015;85:992–996.)

KEY WORDS: Orthodontic brackets; Product packaging; Microbiology and contamination

INTRODUCTION

The oral microbiota in the human oral cavity contains a number of different habitats and at least 400 to 700 different types of bacteria. This microbiota host a complex and dynamic microbial community that is responsible for two major and highly prevalent infectious diseases: caries and periodontal disease.[1]

When changes occur in the oral cavity, the microbiota also change, causing a loss of balance and increasing the possibility of disease development.[2] Orthodontic treatment, via the use of fixed or removable appliances, causes specific alterations in the oral cavity,[3] including pH reduction, increased accumulation of dental biofilm,[4] and increased levels of microorganisms in saliva and biofilm.[5,6] As a result, diseases can be transmitted through direct contact with contaminated instruments or materials, either when used straight from the manufacturer's packaging or when used in more than one patient without proper disinfection or sterilization.[7]

In orthodontics, several products are available for disinfecting and sterilizing instruments (eg, orthodontic pliers), but data on the microbial contamination of orthodontic brackets as supplied by the manufacturers are lacking in the literature. The brackets that are attached to patients' teeth are usually removed from the manufacturer's original container and transferred straight to the patient's mouth, where they stay for an average of 2 to 3 years.

International regulatory agencies have recommended the use of the Spaulding classification system

[a] PhD candidate, School of Dentistry, Lutheran University of Brazil, Canoas, Rio Grande do Sul, Brazil.

[b] Professor, Department of Genetics, Lutheran University of Brazil, Canoas, Rio Grande do Sul, Brazil.

[c] MSc student, Lutheran University of Brazil, Canoas, Rio Grande do Sul, Brazil.

[d] Professor, Department of Orthodontics, School of Dentistry, Lutheran University of Brazil, Canoas, Rio Grande do Sul, Brazil.

Corresponding author: Dr Maria Perpétua Mota Freitas, Tocantins Street, 1120/26, Agronomia, Porto Alegre, RS 90540-420, Brazil
(e-mail: perpetuamf@hotmail.com)

Accepted: January 2015. Submitted: October 2014.
Published Online: February 27, 2015

Table 1. Characteristics of the Groups of Orthodontic Brackets Assessed

Group	Bracket	Brand	Manufacturer Package
1	Metallic	Morelli (São Paulo, Brazil)	Full case
2	Metallic	Morelli (São Paulo, Brazil)	Replacement brackets
3	Metallic	Uniden (Sorocaba, Brazil)	Full case
4	Metallic	Uniden (Sorocaba, Brazil)	Replacement brackets
5	Metallic	Abzil-3M Unitek (São Paulo, Brazil)	Full case
6	Metallic	Abzil-3M Unitek (São Paulo, Brazil)	Replacement brackets
7	Metallic	American Orthodontics (Sheboygan, Wis)	Full case
8	Metallic	American Orthodontics (Sheboygan, Wis)	Replacement brackets
9	Ceramic	Morelli (São Paulo, Brazil)	Full Case
10	Ceramic	Morelli (São Paulo, Brazil)	Replacement brackets
11	Ceramic	Abzil-3M Unitek (São Paulo, Brazil)	Full case
12	Ceramic	Abzil-3M Unitek (São Paulo, Brazil)	Replacement brackets
13 (Negative control)	Metallic	Morelli (São Paulo, Brazil)	Replacement brackets
14 (Positive control)	Metallic	Uniden (Sorocaba, Brazil)	Replacement brackets
Total	140		

for inanimate objects to evaluate their potential risk of disease transmission and infection.[8,9] This classification system is used by different centers of epidemiologists, microbiologists, and professional medical organizations aiming to determine the degree of disinfection or sterilization required for various medical devices and classifies objects as critical, semicritical, or noncritical.

According to this classification, orthodontic materials, including brackets, are considered semicritical because of the associated risk of infection (direct contact with the oral mucosa), and should therefore be sterilized before use. Semicritical objects are those that have contact with mucous membranes only, preventing the invasion of subepithelial tissues, and should be sterilized.[8,9]

Purmal et al.[1] evaluated four different orthodontic buccal tubes used straight from the manufacturer's packaging and found aerobic bacterial contamination. The microorganisms isolated from the samples were *Micrococcus luteus*, *Acinetobacter calcoaceticus,* and *Staphylococcus haemolyticus*, microorganisms with a contamination potential that can pose risks to patients' health. Those authors recommended that the materials should be sterilized before use.[1]

Based on these findings and the absence of studies, and taking into consideration the patient safety risks involved and the fact that materials that will be used in the oral cavity must be free of contamination,[9] the objective of this study was to test the null hypothesis that orthodontic brackets as supplied by manufacturers do not present microbial contamination. To that end, microbiological and molecular tests (polymerase chain reaction [PCR]) were used to detect the presence of bacterial growth, analyze the types of bacteria present, and identify bacteria.

MATERIAL AND METHODS

The sample comprised 140 brackets of different lots of four commercially available brands, divided into 14 groups of 10 pieces each, used straight from the manufacturer's package. Of the 140 pieces, 60 were full cases and 80 were replacement brackets (Table 1).

A negative control group (group 13) was created to confirm sterility of the brain heart infusion (BHI) medium and allow comparison with the study groups. In this group, a Morelli (São Paulo, Brazil) metal bracket was sterilized in surgical grade paper, in an autoclave, to confirm the absence of bacterial growth. Likewise, a positive control group (group 14) with a Uniden (Sorocaba, Brazil) metal bracket contaminated with *S aureus* was used to determine maximum bacterial growth.

The samples were subjected to qualitative analysis using a microbiological test to determine the presence of bacterial growth as well as biochemical tests to determine the morphology of the microorganisms and to assess the types of bacteria possibly present. By order the method used to identify the bacteria present in the medium was DNA extraction and PCR.

Microbiological Tests

Bacterial growth. For microbiological analysis, 29.6 g of BHI broth (Himedium, Mumbai, India) were prepared and diluted in 800 mL of distilled water. The solution was heated until dissolving and distributed into 200 test tubes (4 mL/tube). Subsequently, the tubes were autoclaved for sterilization at 121°C and 1 atm for 15 minutes.

At this point, orthodontic brackets were distributed into the test tubes containing the sterilized BHI broth and placed in an incubator for 48 hours at 35°C. Analysis of bacterial growth was based on changes in the color of the medium in each tube: tubes showing darkening of the BHI medium were considered positive for bacterial growth and further investigated.

Biochemical analysis. Tubes showing evidence of bacterial growth were subjected to microbiological

Table 2. Sequence of Primers Used to Identify Indicative Cultures of *Staphylococcus*

Species	Sequence Primer (5′–3″)	Amplicon Size	Reference
Staphylococcus aureus	aur-F TCGCTTGCTATGATTGTGG	359 pb	Sasaki et al. 2010[12]
	aur-R GCCAATGTTCTACCATAG		
Staphylococcus epidermidis	epi-F TTGTAAACCATTCTGGACCG	251 pb	Hirotaki et al. 2011[13]
	epi-R ATGCGTGAGATACTTCTTCG		

analysis in petri dishes to determine the types of bacteria present in the medium. These tubes were tested with two dishes, one for streak plate and another for pour plate analysis.

Subsequently, plates were stored in an incubator at 35°C for 48 hours and analyzed. Plates showing growth of colonies were fixed in glass slides or the gram-staining protocol.[10]

Slides were evaluated using an optical microscope (Leica, CME model, Wetzlar, Germany) to determine the morphology of the microorganisms and to assess the types of bacteria possibly present, in preparation for the next step of bacterial identification (molecular test).

Molecular analysis: PCR. The method used to identify the bacteria present in the medium was DNA extraction and PCR. DNA was extracted from culture samples according to the protocol described by Boom et al.[11] Because of the high specificity and sensitivity of this method, the genetic identification of isolated bacteria was performed using the analysis of *nuc* genes, as described by Hirotaki et al.[13] PCR amplification of the *nuc* genes was performed using species-specific primers and a Personal Mastercycler (Eppendorf, Hamburg, Germany) (Table 2).

RESULTS

According to the results of the microbiological tests, none of the samples in group 13 (negative control) showed darkening of the BHI medium, confirming absence of bacterial growth. In contrast, all specimens in group 14 (positive control) showed a darkened medium, suggestive of bacterial growth. These findings attest to the effectiveness of the method used.

Of all groups evaluated only two showed contamination, and it occurred in 100% of the samples: group 1, Morelli, full case brackets, and group 12, Abzil-3M Unitek (São Paulo, Brazil), replacement brackets.

After visual identification of bacterial growth as per darkening of the BHI medium, groups 1 and 12 were subjected to microbiological analysis, which revealed the presence of gram-positive cocci (Figure 1). Biochemical test results suggested the presence of *S aureus* in group 1 and *S epidermidis* in group 12. These suggestions were confirmed by DNA extraction and PCR (Table 2).

DISCUSSION

Infection by microorganisms is a concern for health care professionals in general and for dental practitioners in particular, and the dental literature has long pointed to the need to sterilize or disinfect any material before its use in the oral cavity. However, in orthodontic clinical practice, the use of bands and brackets directly from nonsterile manufacturer's packages is still routine. In this scenario, it becomes extremely important to assess the potential contamination of these materials so as to determine sterilization protocols and maintain the health of patients and dental practitioners working in this field.

In the present study, bacterial contamination was confirmed in orthodontic brackets supplied by two of the manufacturers assessed, namely group 1, Morelli, and group 12, Abzil-3M Unitek. Similarly, Purmal et al.[1] also reported biological contamination of the orthodontic buccal tubes evaluated in their study. Those authors suggested that the presence of the bacteria could result from unhygienic practices during material manufacturing and packaging, justifying the results in packages and in full case and replacement brackets.

According to Anhoury et al.,[3] in a healthy oral environment, the interplay between microorganisms and the host is complex and balanced. However, when orthodontic brackets and bands are placed in the oral

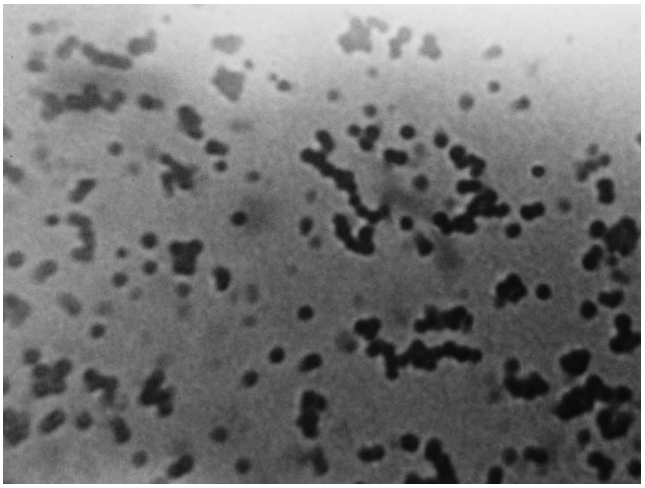

Figure 1. Image showing the presence of gram-positive cocci after biochemical tests.

cavity, they can induce such changes as a decrease in pH and biofilm accumulation, especially when the materials used have not been previously sterilized.[3] The relationship between changes in the oral microbiota and the use of orthodontic materials has been confirmed by Naranjo et al.,[4] who found alterations in subgingival microbiota after the placement of orthodontic bands.[4]

In our study, bacterial contamination of groups 1 and 12 was suggested by biochemical tests and confirmed by DNA extraction and PCR. PCR was chosen because it is a highly specific and sensitive method and because it has been validated for this purpose.[13] Genetic evaluation of our samples using PCR revealed contamination by *S aureus* in group 1 and *S epidermidis* in group 12. Looking at previous studies, Purmal et al.[1] found *M luteus*, *S haemolyticus*, and *A calcoaceticus*,[1] whereas Hong et al.[5] found an increase in *Streptococcus mutans* in the saliva and biofilm after the placement or orthodontic brackets.[5]

With regard to the pathogenicity of microorganisms, Andrucioli et al.[14] had already underscored that high levels of oral microorganisms increase the risk not only of caries and periodontal disease but also of systematic complications.[13] According to Levinson and Jawetz[15] *S epidermidis* may cause endocarditis, a rare but possible complication of dental treatment that will only develop in the presence of bacteremia.[14] McLaughlin et al.[16] and Erverdi et al.[16] have also suggested an association between certain orthodontic procedures, for example, orthodontic banding, and bacteremia.[16,17]

About 75% of bacterial infections caused by coagulase-negative *Staphylococcus* have *S epidermidis* as the causative agent,[16,17] as was also observed in our group 12. These bacteria rarely cause suppuration, but they can infect orthopedic and cardiovascular prostheses and cause diseases in immunosuppressed persons.

About the *S aureus* found in brackets in group 1, their pathogenic capacity is in the combined effect of extracellular factors and toxins, together with their invasive properties. Dissemination of *S aureus* can cause endocarditis, osteomyelitis, meningitis, or lung infection.[18] According to Oliveira et al.,[19] the presence of these respiratory pathogens in the biofilm can serve as a reservoir for microorganisms associated with nosocomial pneumonia.

Finally, considering that not all materials from manufacturers are free of contamination on the packaging, the null hypothesis was rejected. This suggests a concern for the health of patients and the need for better control of contamination in packages of brackets.

CONCLUSION

- In spite of the fact that only two brands in this study (Morelli and Abzil-3M Unitek) showed contaminated by bacteria in the original packages supplied by manufacturers, these data suggest that manufacturers of these materials should improve the quality control of packaging used, including instituting effective protocols, or that clinicians should use a method of disinfection or sterilization before their use in the orthodontic clinic for security of patient health.

REFERENCES

1. Purmal K, Chin S, Pinto J, Yin WF, Chan KG. Microbial contamination of orthodontic buccal tubes from manufacturers. *Int J Mol Sci*. 2010;11:3349–3356.
2. Marsh PD. Dental plaque as a biofilm and microbial community implications for health and disease. *BMC Oral Health*. 2006;6:1–7.
3. Anhoury P, Nathanson D, Hughes CV, Socransky S, Feres M, Chou LL. Microbial profile on metallic and ceramic bracket materials. *Angle Orthod*. 2002;72:338–343.
4. Naranjo AA, Trivino ML, Jaramillo A, Betancourth M, Botero JE. Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement. *Am J Orthod Dentofacial Orthop*. 2006;130: 275.e17–e22.
5. Hong A, Hong-Fei L, Huan-You L, et al. Influences of bracket bonding on Mutans streptococcus in plaque detected by real time fluorescence-quantitative polymerase chain reaction. *Chin Med J*. 2005;118:2005–2010.
6. Leung NM, Chen R, Rudney JD. Oral bacteria in plaque and invading buccal cells of young orthodontic patients. *Am J Orthod Dentofacial Orthop*. 2006;130:698.e11–e18.
7. Morrison A, Conrod S. Dental burs and endodontic files: are routine sterilization procedures effective? *J Can Dent Assoc*. 2009;75(1):39–39d.
8. Brasil Ministério da saúde. *Coordenação de Controle de Infecção Hospitalar. Procedimentos de artigos e superfícies em estabelecimentos de saúde*. Brasília:Brasil Ministério da saúde; 1994:34–67.
9. McDonnell G, Burke P. Disinfection: is it time to reconsider Spaulding? *J Hosp Infect*. 2011;7(3):163–170.
10. Oplustil CP, Zoccoli CM, Tobouti NR, Sinto SI. *Procedimentos Básicos em. Microbiologia Clínica*. Sao Paulo, Brazil:Sarvier; 2010:32–44.
11. Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol*. 1990;28:495–503.
12. Sasaki T, Tsubakishita S, Tanaka Y, Sakusabe A, Ohtsuka M, Hirotaki S, Kawakami T, Fukata T, Hiramatsu K. http://www.ncbi.nlm.nih.gov/pubmed/20053855 Multiplex-PCR method for species identification of coagulase-positive staphylococci. *J Clin Microbiol*. 2010 Mar;48(3):765–769.
13. Hirotaki S, Sasaki T, Kuwahara-Arai K, Hiramatsu K. Rapid and accurate identification of human-associated staphylococci by use of multiplex PCR. *J Clin Microbiol*. 2011;49: 3627–3631.
14. Andrucioli MCD, Nelson-Filho P, Matsumoto MAN, et al. Molecular detection of in-vivo microbial contamination of metallic orthodontic brackets by checkerboard DNA-DNA hybridization. *Am J Orthod Dentofacial Orthop*. 2012;141:24–29.
15. Levinson W, Jawetz E. *Microbiologia médica e Imunologia*. São Paulo:Artmed; 1998:50–76.

16. McLaughlin JO, Coulter WA, Coffey A, Burden DJ. The incidence of bacteremia after orthodontic banding. *Am J Orthod Dentofacial Orthop*. 1996;109:639–644.
17. Erverdi N, Kadir T, Ozkan H, Acar A. Investigation of bacteremia after orthodontic banding. *Am J Orthod Dentofacial Orthop*. 1999;116:687–690.
18. Brooks GF, Butel J, Morse SA. *Microbiologia médica*. Rio de Janeiro:Guanabara Koogan; 2000:12–46.
19. Oliveira LCBS, Carneiro PPM, Fischer RG, Tinoco EMB. A presença de patógenos respiratórios no biofilme bucal de pacientes com pneumonia nosocomial. *Rev Bras Ter Intensiva*. 2007;19:428–433.