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MICROBIOLOGICAL ANALYSIS OF ABSORBENT PAPER POINTS EMPLOYED BY UNDERGRADUATE STUDENTS FROM A DENTAL SCHOOL IN SOUTH OF BRAZIL

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ABSTRACT

Aim: The aim of this study was identify contamination on absorbent paper points used by students of Dental Clinic III of the Faculty of Dentistry of Federal University of Rio Grande do Sul in the semester 2015/1, in order to warn students and professionals of the area on the importance to sterilize these materials.

Material and Methods: In a clinical environment, 180 absorbent paper points we collected (80 of them from the first series and 80 from the second one), from 40 students. After the collection, each one was singly immersed in a microtube containing 1.5 ml of the BHI (Brain Heart Infusion) culture. Positive control was composed by one paper point contaminated by saliva, and negative control was composed by a closed microtube, only with BHI. The microtubes were incubated at 37°C in bacteriological incubator during 14 days. The microtubes that presented turbidity were considered positive, and those which did not present turbidity were considered negative.

Results: The results were analyzed by the Fisher Exact Test, which demonstrated that paper points from the second series presented higher agreement contamination between the paper points collected from each box, when compared to the analyzed boxes from the first series (p=0.03). All the samples observed presented growth of *Bacillus spp* in the microorganism identification.

Conclusions: It is possible conclude that absorbing paper points, when exposed to clinical environment suffer contamination, and the autoclave sterilization is necessary before the use, regardless the commercial brand, in order to ensure the aseptic chain maintenance.

KEYWORDS: endodontics, absorbing paper points, contamination

INTRODUCTION

Endodontics treatment has the aim to the sanitization, modelling and elimination of microbial infection of root canals. Then, all the endodontic procedures are addressed to create and maintain the aseptic conditions for root canal filling[1]. The persistence of microorganisms is closely linked to the failures of endodontic treatments, and it often occurs due to failures on the root canal preparation, use of instruments

and/or materials contaminated and infiltration of bacteria through the saliva[2,3].

Nowadays, the use of absorbing paper points constitutes the method more utilized to dry the root canal system. Besides, they are also used to collect microbial samples inside the canals and to content apical bleeding; these materials are important for endodontic treatment[4,5].

According to Leonardo and Leal[6], instruments used for endodontic treatment, when contaminated, may carry microorganisms to inside canals, together other metabolic products, being responsible by development and persistence of endodontic pathologies. Then, absorbing paper points have to be free of microorganisms in the moment of use in endodontic therapy in order to avoid re-contamination of canals[5].

Some commercial brands of paper points are not available sterilized or when in contact to the clinical environment, after being open and close packing, become exposed to microorganisms and contribute to the development of periapical pathologies[6].

Pereira et al.[7] evaluated the contamination of absorbing paper points in sealed packages, sold as sterilized or not, as well as paper points exposed to the dentistry office environment. There was no statistic difference between the groups, and all the absorbing points were contaminated by bacteria and fungus. Therefore, the authors recommend sterilization of absorbing paper points before the clinical use, regardless the commercial presentation.

The sterilization method with autoclave is more widespread in dentistry because it is faster and efficient[6], further it did not change or compromise the function of absorbing paper cones[4,5]. Victorino et al.[8] demonstrated that successive sterilization processes with autoclave do not compromise the function of paper cones, regardless their origination. Besides, microbiological test showed the sterilization process did not provide liberation of any sub-product to cones, which present antimicrobial effect or lysing blood cells.

The use of absorbing paper cones inside the root canal is a routine employed by the endodontist and the sterilization must be indispensable condition for their use during the treatment of root canals[9]. The aim of this study was evaluate the microbiological condition (absence or presence of microorganisms) of absorbing paper cones obtained from students of the subject Dental Clinic III of Faculty of Dentistry of Federal University of Rio Grande do Sul from march to september of 2015.

MATERIAL AND METHODS

This study was sent to the COMPESQ of Faculty of Dentistry of UFRGS, and received approval under the number 28975.

For this study, 160 absorbing paper points were obtained ((80 of them from the first series and 80 from the second one) from 40 students of Dental Clinic III of Faculty of Dentistry of Federal University of Rio Grande do Sul in the semester 2015/1, 4 cones by student. These cones were obtained regardless the commercial brand. Donor students of paper points received the same number of cones from the researchers, already sterilized and packaged. Initially, 160 microtubes (Eppendorfs, Plastbio Labmais Ltda. Curitiba, PR, Brazil) of 2 ml were previously selected and sterilized. The culture medium *Brain Heart Infusion Broth* (BHI, Oxoid Limited, England) was selected for microbiological test, which was carried out as follows: 13.32g of powder were measured and diluted in 360 ml of distilled water. The suspension was honogenized and autoclaved at 120°C during 20 minutes. The 1.5 ml of culture medium was distributed in microtubes under laminar flow chamber. After, the microtubes were numbered from 1 to 40 followed by the letters A, B, C and D. The numbers were used to identify each student and the letters to identify the paper points (A and B - first series; C and D - second series).

The microtubes were numbered from 1 to 40 to identify each student. The paper points were identified by the letters A, B, C and D; A and B were the cones from the first series and C and D from the second one.

To obey the aseptic technique, the absorbing paper points were collected from each student. The first absorbing paper point, of the first serie, was collected with sterilized clinical tweezers from the paper points box belonged to the donor student and transferred to the microtube 1. After take the first paper point out, the tweezers was dived in hydrated ethanol 70%, dismissed in sterile glass container and dried with sterile gauze. Then it could be used to collect the second paper point, which was transferred to the microtube 1B, and successively to the third and fourth paper point. For each student, a previously sterilized tweezers was used with the same protocol described.

Negative control was constituted by a microtube with the culture medium previously distributed in order to prove its sterility. Positive control was constituted by a microtube with culture medium inoculated with salivary microorganisms, and in this case, a paper point was immersed in

saliva for 10 seconds and immediately transferred to the experimental medium.

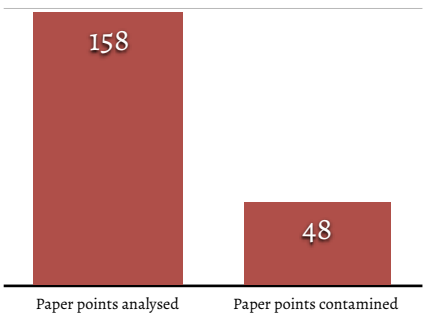
The microtubes were kept under 37°C during 14 days. The observation of experimental microtubes and the controls were carried out daily in order to watch the presence or absence of turbidity, indicating or not the microbial growth. After 24h, 10 microtubes which presented turbidity were selected, and the Gram staining was performed to identify the microorganism. The samples were inoculated on Brain Heart Infusion Agar. The plates with BHI Agar were incubated at 37°C for 48h.

The data were submitted to the Fisher Exact Test that pointed the frequency of contaminated absorbing paper cones, and the comparison between the cones from the first and second series ($p<0.05$).

RESULTS

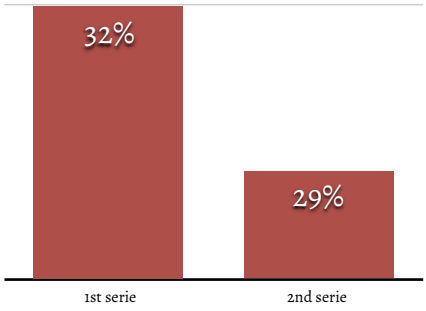
Among the 160 paper points collected, 2 of them were excluded due to the previous contamination of the culture medium. Then, 158 were analyzed and 48 presented contamination (Figure 1), 25 from the first series and 23 from the second one (Figure 2).

Figure 1. Demonstrative graph of relationship between the paper points analyzed and contamined.



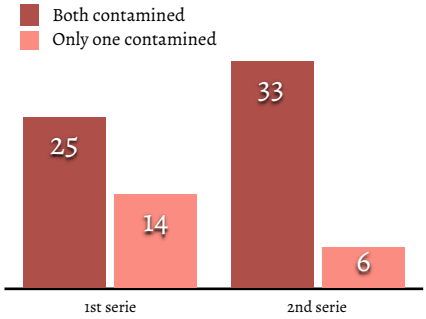
Although the paper points from the first series have presented a higher number of contamination, when compared to the cones from the second series there was no statistically significant difference among the contamination levels of cones ($p=0.72$), according to the figure 2.

Figure 2. Demonstrative graph of contamination incidence of paper points according to the series.



When compared the contamination levels of paper cones inside the same box, there was statistically significant difference ($p=0.03$), the cones from the second series presented higher agreement contamination between the two cones collected from each box, when compared to the boxes from the first series, according demonstrated by the figure 3.

Figure 3. Demonstrative graph of contamination incidence of cones from a same box.

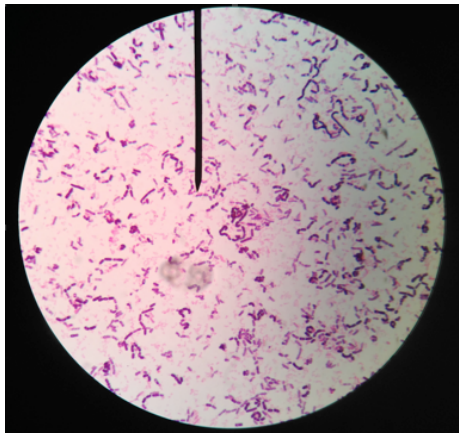


In all the samples analyzed by Gram staining were observed the growth of *Bacillus* spp. (Figure 4).

DISCUSSION

The success of endodontic therapy is directly related to the control of microorganisms inside the root canal systems. Then, the maintenance of the aseptic chain during all the endodontic treatment stages is extremely important. The use of non-sterilized instruments or materials may serve as an entrance door for microorganisms inside the root canal, achieving the apical region. It may be responsible by development or persistence of endodontic pathologies[6].

Figure 4. Gram-positive bacilli, image obtained through microscope analysis.



The aim of the root canal filling is promote a tridimensional sealing of the root canal, and avoid the entrance of new microorganisms, further to promote the burial of bacteria that could resist the chemical-mechanical preparation and could support a periapical inflammation, leading to the failure of the endodontic treatment[10]. Therefore, the drying of root canal constitutes a critical stage of the endodontic treatment, because the humidity inside the root canal, in the moment of the root filling promotes the formation of bubbles, porosity and or empty spaces. It decreases the flow capacity and the adhesiveness of the endodontic sealer, what results in a defective apical sealing[11].

Absorbing paper points are chosen materials to dry the root canal and most professional generally take the cones directly from the packing and

introduce inside the root canals to dry them. Therefore, as they are the last material to be introduced in the canal before the root canal filling they have to be free of contamination. Then, sterilizing it material is necessary.

This study had as aim to identify the presence of contamination on the absorbing paper points used by students of Dental Clinic III, of the Faculty of Dentistry of Federal University of Rio Grande do Sul in the first semester 2015, in order to warn students and professionals of this area on the importance to carry out the sterilization of these materials.

In this study, we observed that from 158 samples, 48 of them were contained, corresponding to 30% of cones used in this research. Verifying the contamination did not lead to consider the commercial brand, chemical composition or standardization of cones, but whether they exposition in an unfavorable clinic environment lead to the contamination or not.

From 10 samples seeded in Agar BHI, the growth of *Bacillus* spp was observed in all of them. This result was also found by other studies[12]. Considering the *Bacillus* spp as an environmental microorganism, found in soil, salt and fresh water and in food kinds, its presence may be justified because it is a surface contaminant. After this study it is clear that sterilize the absorbing paper cones is necessary, because the organisms in them, when in touch with root canal, plays an important role on development and maintenance of pathologies which attack the pulp and the periapical region[13,14].

When compared the contamination level of paper points inside the same box, there was statistically significant difference (p=0.03) among cones from the first and second series, with higher homogeneity among cones of the second series, in other words, one cone from this box was not contaminated, and probably nor the other. Less manipulation of these boxes could explain it, because cones of second series has major diameter (0.45 mm to 0.80 mm), and they are not so used to dry canals than those in smaller diameter for endodontic treatment of poly root teeth; this treatment is performed by students of the subject Dental Clinic III.

Absorbing paper points are commonly marketed in plastic boxes containing about 120 units in standard sizes separated by plastic division. Manufacturers do not ensure the sterility for the product with this commercial presentation. More recently, these manufacturers provided paper points packed in until 5 units, and they ensure their sterility. However, several studies tested the sterility of these cones and the contamination was proved through bacterial growth[7,13,15,16].

CONCLUSIONS

From these results we conclude that absorbing paper points, when exposed to clinical environment, suffer contamination and their sterilization in autoclave is necessary, no matter the commercial brand.

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