Influence of different disinfection protocols on gutta-percha cones surface roughness assessed by two different methods

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The objectives of this study were to evaluate how different disinfection protocols affect the surface roughness of gutta-percha (GP) cones used for the dental root canal filling using DIN 4768 standard and another alternative process for assessing roughness of small surface area (multiple profile), comparing both methods in order to identify similarities. The GP cones used were the conventional (C) and a new one impregnated with zirconia oxide, known as the coated cone (CC). Samples were distributed for each group and they were immersed in the correspondent chemical solution as follows: Group 1 (G1), sodium hypochlorite (NaOCl) at 5.25% for 1 min; Group 2 (G2), sodium hypochlorite at 2.5% for 10 min; and Group 3 (G3), chlorhexidine gluconate (CHX) at 2% for 5 min, as recommended by dentistry protocols. The averages and standard deviations of the surface roughness parameters—average roughness (R_a) and root mean square deviation roughness (R_q)—were calculated. Statistical analysis was made before and after immersion by paired t-test. Results showed a statistically significant difference for C GP cones after immersion in 2% CHX and 2.5% NaOCl (p < 0.01). No difference was found in CC GP cones. DIN 4768 standard and multiple profile measurements showed similar trends and behavior.

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1. Introduction

Endodontic treatment is intended to prevent contamination and/or sufficiently remove micro-organisms from within the dental root canal to ensure clinical success [1].

The root obturation is the filling of the dentin canal in all its extension, completely sealing all the space previously occupied by the dental pulp. The material used for the filling of the root canal is gutta-percha (a solid material) associated with the endodontic sealer (a fluid material) that must fill the
entire internal region three-dimensionally, in order to form a monobloc between GP, sealer and dentin wall [2].

The GP cones must be disinfected by a chemical method due to its thermolability before its insertion into the root canal space. Therefore, heat is not appropriate and then chemical substances can be indicated for this purpose such as chlorhexidine, iodized alcohol, peracetic acid and sodium hypochlorite, which is the most commonly used substance in daily clinics [3].

Efficient protocols for disinfection of contaminated GP cones were proposed by Gomes et al. [4] with NaOCl solutions. Some studies show that sodium hypochlorite can induce morphological changes to the surface of the GP cones, by causing surface corrosion [5–8] and as a consequence roughness change [5,8–12]. Some authors [3,5–7,13–16] assume that physical changes in the GP cones may compromise its adaptation to the root canal walls.

Cardoso et al. [17] proposed the use of CHX solutions as an alternative to NaOCl solutions. CHX is a chemical compound with great antibacterial, anti-fungal and anti-viral activity and is not corrosive.

Assessing roughness is a way to study these physical changes, and it is already established in the dentistry literature as a resource for verifying material loss or material external surface alteration provoked by disinfection with oxidative products [5,8–12,14].

Nowadays two GP cones capture the attention of dentists: the conventional one, which is mainly constituted by zinc oxide and gutta-percha (a vegetal resin similar to latex). The other type of GP cone is also constituted by zinc oxide and gutta-percha, but it is coated with a layer of zirconium oxide. This GP cone is known as coated GP cone or bioceramic cone [18,19]. Both cones were developed to be used with calcium silicate sealers in order to enhance its root filling properties [1,19].

Coated GP cones are a new endodontic material. Few studies have been done about coated GP cones, regarding fracture resistance [18,19], adhesion [20], micro-infiltration [21], without considering the effect of disinfection on coated GP cones surface roughness. So, as disinfection of this material is an important step for the clinical use, it is important to evaluate the influence of these chemical protocols.

Surface roughness can be assessed in a variety of ways, commonly using a contact profilometer, optical profilometer, confocal microscope and atomic force microscopy, for instance. With confocal microscope the measurements can be accomplished following DIN 4768 standard or a method named multiple profile, which is more appropriate for roughness assessment of small samples like dental materials.

The objectives of this paper were to evaluate the effect of different disinfection protocols on surface roughness of GP conventional cones and coated GP cones, using a confocal microscope, and compare the measures of DIN 4768 standard and multiple profile method in order to identify similarities.

## 2. Materials and methods

### 2.1. Materials

The materials used were the conventional GP cone (Tanari, Brazil) and the cone impregnated with zirconia oxide (FKG Dentaire, Switzerland). A total of 12 conventional cone samples as well as 12 coated cone samples were made.

The chemical disinfectants used were 5.25% sodium hypochlorite (NaOCl), 2.5% NaOCl and 2% chlorhexidine gluconate solution (CHX) (Fórmula e Ação, Brazil).

#### 2.1.1. DIN 4768

For this analysis, it was created a homemade acrylic resin holder to cope with the conic shape of the samples. Thus, this holder increases stability and decreases the influence of the slope of the cone in the value of the roughness, illustrated in Fig. 1. The whole cone is used for this technique, due to the total length required for the test, which is 5.6 mm considering a contact profilometer, only possible in the longitudinal direction.

#### 2.1.2. Multiple profile method

For this analysis, axial cuts were performed on the GP cone. Each cone was cut with a number 15 scalpel blade measuring 3 mm long. All 3 pieces of each sample were fixed with SuperBond (Loctite, Brazil) at a metal bracket with 3 cm in diameter according to the type of disinfectant used. These samples can be seen in Fig. 2.

### 2.2. Methods

#### 2.2.1. Chemical disinfection

The cones were sampled in three groups according to the chemical used for their disinfection:

- Group 1: immersed for 1 min in 5.25% NaOCl;
- Group 2: immersed for 3 min in 5.25% NaOCl;
- Group 3: immersed for 5 min in 5.25% NaOCl;
- Group 4: immersed for 3 min in 2% CHX;
- Group 5: immersed for 3 min in 2.5% NaOCl.

Fig. 1 – Schematic design of the homemade GP holder: (A) GP position indicated in pink, (B) acrylic resin holder and (C) image of the homemade sample holder with GP.
Group 2: immersed for 10 min in 2.5% NaOCl; Group 3: immersed for 5 min in 2% CHX.

In this study, it was used the concentrations and time protocols for NaOCl solutions described by Gomes et al. [4]. They found that this protocol was enough for disinfecting resistant and more common microorganisms found in contaminated cones. For each group, it is the minimal time needed to kill 100% of the microorganisms with the used concentrations. For the chlorhexidine gluconate solution, the concentration and time proposed by Cardoso et al. [17] were used.

After disinfection process, samples were rinsed with distilled water (Fórmula and Ação, Brazil) for residual removal and dried with absorbent paper [7,22,23]. For all samples, roughness analysis was performed before and after disinfection.

2.2.2. Confocal microscope measures
The confocal microscope has been widely used to characterize rough surfaces, mainly due to the fact that a high precision measurement can be achieved and non-contact measurements can be made without destruction of the sample. In addition, images can be readily taken with quality [24].

For a better understanding of the surface analyzed in the experiments, the Interferometric Leica DCM 3D confocal microscope (Leica Microsystems, Germany) was used.

The measures were carried out according to DIN 4768 and multiple profile method, a technique for small area samples roughness measurement. Both topographic and roughness analysis used 10× magnification lens and blue LED (460 nm).

2.2.2.1. Measuring roughness with DIN 4768. For surfaces with a non-periodic roughness profile, the procedure described in the DIN 4768 was performed.

It started with a topographic analysis of all the GP cones with 10× magnification lens, obtaining an estimate of $R_a$ and $R_q$, to check the cut off it must be used. In all samples of this work, measures were coherent with a cut off of 0.8 mm. Then, five distinct measurements with a total length of 5.6 mm along the axis of the GP cone, before and after disinfection with the substances, were performed. In order to obtain the $R_s$, of each line, an evaluation length of five cut off is considered (4.0 mm), once when using a contact profilometer, the first and last sampling length are discarded due to the acceleration and braking of the contact of the stylus. In order to do this, the cones were rotated around their longitudinal axes. All data are stored, so that a comparison between an optical microscope and a contact profilometer is possible.

Then, the average of the five values of each parameter $R_a$ and $R_q$ as well as their respective standard deviation were obtained. All data of before and after immersion were tabulated and statistically analyzed by paired t-test.

2.2.2.2. Measuring roughness with multiple profile method. Firstly, topographical analysis of the surface of the GP cone was carried out with a confocal microscope. In this topographical analysis, the start values of $R_a$ and $R_q$ were obtained along the sample and it is possible to choose the region of interest. Then the roughness analysis was carried out with the multiple profile method, with the field of view (FOV) of 210.82 × 210.82 μm, which resulted in eight profiles of roughness. Regardless of the values of $R_a$ and $R_q$, in this procedure, cut off was set at 0.8 mm. This area was chosen because it is the largest possible area where the value of $R_a$ and $R_q$ had similar values on the X-axis compared to the Y-axis of the topographical analysis of the samples. This suggests that the tapering of the sample was not significantly influenced by the value of the roughness at these dimensions.

The average and standard deviation of the results of $R_a$ and $R_q$ of the eight lines of each sample was performed and subsequently the same was done with the average and standard deviation of all samples. All measures were tabulated and also statistically analyzed by paired t-test.

3. Results and discussions

Fig. 3 shows the 2D and 3D topographical images of the coated GP cone before and after immersion in 5.25% NaOCl. Fig. 4 shows 2D and 3D topographical images of the conventional GP cone before and after immersion in 5.25% NaOCl. Comparing Figs. 3 and 4, coated GP cones present a homogeneous surface, whereas conventional GP cones present a more irregular surface, with apparent scratches and holes.

Considering conventional GP cones, the obtained values for $R_a$ and $R_q$ for both methods, i.e., DIN 4768 and multiple profile methods, are shown in Tables 1 and 3. It can be noticed, that there was a decrease in roughness ($R_a$ and $R_q$) after immersion.
in 2.5% NaOCl and 5.25% NaOCl. However, immersed cones at 2% CHX showed an increase of roughness (Ra and Rq) after immersion.

For the coated GP cones, the results in Tables 2 and 4 show that after immersion in solutions of 2.5% NaOCl and 2% CHX, there was a reduction in the value of both Ra and Rq for both methods (DIN 4768 and multiple profile) whilst after immersion in 5.25% NaOCl there was an increase.

Quantitatively there was a difference between the values of the Ra and Rq parameters in the roughness analysis, using either DIN 4768 or multiple profile methods. Nevertheless, as both methods showed similar behavior regarding qualitative measurement, a validation of the multiple profile method may be considered to be used in small samples measurement as recommended for dental materials analysis.

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**Table 1 – Ra values (average, standard deviation and percentage) before and after disinfection of the conventional GP cone.**

<table>
<thead>
<tr>
<th></th>
<th>DIN 4768</th>
<th>Multiple profile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ra before (µm)</td>
<td>Ra after (µm)</td>
</tr>
<tr>
<td>NaOCl 5.25%</td>
<td>1.074 ± 0.064</td>
<td>1.037 ± 0.12</td>
</tr>
<tr>
<td>NaOCl 2.5%</td>
<td>1.044 ± 0.093</td>
<td>1.034 ± 0.135</td>
</tr>
<tr>
<td>CHX 2%</td>
<td>0.885 ± 0.079</td>
<td>0.918 ± 0.124</td>
</tr>
</tbody>
</table>

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**Table 2 – Ra value (average, standard deviation and percentage) before and after disinfection of the coated GP cone.**

<table>
<thead>
<tr>
<th></th>
<th>DIN 4768</th>
<th>Multiple profile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ra before (µm)</td>
<td>Ra after (µm)</td>
</tr>
<tr>
<td>NaOCl 5.25%</td>
<td>1.259 ± 0.068</td>
<td>1.262 ± 0.119</td>
</tr>
<tr>
<td>NaOCl 2.5%</td>
<td>1.317 ± 0.084</td>
<td>1.239 ± 0.201</td>
</tr>
<tr>
<td>CHX 2%</td>
<td>1.598 ± 0.134</td>
<td>1.455 ± 0.226</td>
</tr>
</tbody>
</table>
In order to obtain a statistically significant result, a paired t-test was performed, considering both surface roughness methods, using parameters $R_s$ and $R_q$, taking into account the effect of disinfection protocols on both GP cones (conventional and coated), before and after disinfection protocols.

For conventional GP cones, the results showed that for Group 2, using multiple profile method, both parameters $R_s$ and $R_q$ presented a statistically significant decrease after immersion ($p < 0.01$). This behavior may have occurred due to the immersion time and it could cause more loss of surface material than the greater concentration used in Group 1, but in a smaller time. Group 2 data showed that surface became less rough than before the immersion, and these results agree with those found by John et al. [10]. Indeed, they reported in conclusion that a decrease was observed in surface roughness of conventional GP surface in 2.5% NaOCl even at 10 min.

**Table 3 – $R_q$ values (average, standard deviation and percentage) before and after disinfection of the conventional GP cone.**

<table>
<thead>
<tr>
<th>Conventional GP Cone (Average and Standard deviation)</th>
<th>DIN 4768</th>
<th>multiple profile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_q$ before ($\mu m$)</td>
<td>$R_q$ after ($\mu m$)</td>
</tr>
<tr>
<td>NaOCl 5.25%</td>
<td>1.822 ± 0.122</td>
<td>1.676 ± 0.224</td>
</tr>
<tr>
<td>NaOCl 2.5%</td>
<td>1.558 ± 0.227</td>
<td>1.547 ± 0.196</td>
</tr>
<tr>
<td>CHX 2%</td>
<td>1.366 ± 0.135</td>
<td>1.451 ± 0.268</td>
</tr>
</tbody>
</table>

**Table 4 – $R_q$ values (average, standard deviation and percentage) before and after disinfection of the coated GP cone.**

<table>
<thead>
<tr>
<th>Coated GP Cone (Average and Standard deviation)</th>
<th>DIN 4768</th>
<th>Multiple profile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_q$ before ($\mu m$)</td>
<td>$R_q$ after ($\mu m$)</td>
</tr>
<tr>
<td>NaOCl 5.25%</td>
<td>1.654 ± 0.082</td>
<td>1.655 ± 0.165</td>
</tr>
<tr>
<td>NaOCl 2.5%</td>
<td>2.017 ± 0.12</td>
<td>1.766 ± 0.352</td>
</tr>
<tr>
<td>CHX 2%</td>
<td>2.193 ± 0.155</td>
<td>1.996 ± 0.352</td>
</tr>
</tbody>
</table>

Fig. 4 – 2D and 3D representative topographical image of the conventional GP cone before (A) and (B), and after (C) and (D) immersion in 5.25% NaOCl.
Group 3 also presented statistically significant differences (p < 0.01), using multiple profile method for parameter Rq, but not for Rs. In this case, this result can be attributed to the definition of parameter Rs, which further highlights the values of peaks and valleys of the surface heights in the roughness calculation.

Considering coated GP cones, both methods and parameters did not show any statistically significant differences (p > 0.05) for the used immersion times and chemical solutions.

Once that disinfection protocols are ultimately needed, the desired result is the null hypothesis, which yields that there are no differences regardless of the used disinfection protocol.

The study of Prado et al. [11] evaluated, by atomic force microscopy (AFM), the same area of conventional GP cones before and after disinfection and concluded that there was no significant change in the values of RMS surface roughness. Otherwise, authors such as Valois et al. [5], Mishra and Tyagi [8], John et al. [10] and Tilakchand et al. [12], who analyzed smaller and different areas before and after disinfection, found significant changes in the values of roughness. Such divergent results may be related to the methodological differences in relation to the analytical techniques like sample area, different submersion times, concentrations, size of the analyzed area and the equipment used.

In the present study it was found that GP cones of the same brand, size and lot presented different surface roughness values, which can be noticed inspecting the first and third columns of Tables 1, 2, 3 and 4. These values were obtained by different parameters (Rq and Rs) and methods (DIN 4768 and multiple profile) before disinfection procedures. This result is in accordance with others authors [10, 11 e 13], and this dispersion can also influence the results.

4. Conclusion

Under the conditions and circumstances of this study, it was possible to conclude that regarding the time and concentrations of the chemical disinfection protocols there was no statistically significant change in the surface roughness of coated GP cones. Moreover, there was no qualitative difference between the two methods used for surface roughness assessment as per DIN 4768 and multiple profile.

Conventional GP cones showed a statistical difference on surface roughness after immersion in CHX 2% (p<0.001) and NaOCl 2.5% (p<0.01) when measured by multiple profile. DIN 4768 measurements showed no statistical difference. Nevertheless, both methods showed similar trends of measures, before and after the immersion in all groups.

Conflicts of interest

The authors declare no conflicts of interest.

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