Evaluation of Epoxy Resin Sealer After Three Root Canal Filling Techniques by Confocal Laser Scanning Microscopy

DANIELA KOK,[1] MARCO ANTÔNIO HÚNGARO DUARTE,[2] RICARDO ABREU DA ROSA,[1]* MÁRCIA HELENA WAGNER,[1,3] JEFFERSON RICARDO PEREIRA,[4] AND MARCUS VINÍCIUS REIS SÓ[5]
[1]*Rio Grande do Sul Federal University, Porto Alegre, Rio Grande do Sul, Brazil*
[2]*Endodontics Department, São Paulo University, Bauru, São Paulo, Brazil*
[3]*Nursery and Dentistry Department, Santa Cruz do Sul University, Santa Cruz do Sul, Rio Grande do Sul, Brazil*
[4]*Prosthodontic Department, University of Southern Santa Catarina, UNISUL, Tubarão, Santa Catarina, Brazil*
[5]*Conservative Dentistry Department, Rio Grande do Sul Federal University, Porto Alegre, Rio Grande do Sul, Brazil*

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ABSTRACT The aim of this study was to evaluate the penetration of endodontic sealer into the dentin tubules, the integrity of the sealer layer perimeter, and the sealer area at the apical third after different filling techniques by confocal laser scanning microscopy (CLSM). Forty-five mandibular premolars were mechanically prepared with ProTaper files, until F5 file. Thereafter, they were filled with an epoxy-resin sealer (AH Plus) mixed with Rhodamine B dye (0.1% proportion) and allocated in three groups: Group 1, single master cone; Group 2, cold lateral compaction; and Group 3, Thermafil. For confocal laser scanning microscopy analysis, the specimens were transversely sectioned at 4 mm from the apex. The images at $\times 10$ and $\times 40$ were analyzed by Imagetool 3.0 software. Significant differences were not found among the three experimental groups according the dentin-impregnate area by the sealer ($P = 0.68$) and between the sealer and root canal perimeter ($P = 0.18$). However, root canal filling techniques were significantly different when apical sealer areas were compared ($P = 0.001$). Thermafil group showed smaller sealer areas (8.09%) while cold lateral compaction and gutta-percha master cone showed similar areas (17.37 and 21.18%, respectively). The dentin-impregnated area was not dependent on the root canal filling technique. Single master cone, cold lateral condensation and Thermafil techniques presented integrity of the sealer perimeter close to 100% and Thermafil resulted in a significantly thinner sealer layer. *Microsc. Res. Tech. 75:1277–1280, 2012.* © 2012 Wiley Periodicals, Inc.

INTRODUCTION

Endodontic sealer adaptation into the root canal walls plays an important role, due its physical barrier property, hampering microorganisms, and fluids infiltration. These characteristics may contribute to the endodontic therapy failure (De Deus et al., 2002).

Many studies about adhesiveness to dentin have been published. It has been showed that worst adhesiveness at the apical third of the canal can facilitate the marginal leakage (Hovland and Dumsha 1985).

Resin-based sealers present suitable physic-chemical properties, such as appropriated setting time, flow, low viscosity, radiopacity >3 mm of aluminum, appropriate particle size and pH, and low superficial tension (Martin-Bauza et al., 2010). Sealer layer should be thin, homogeneous, and continuous to achieve great sealing ability (De Deus et al., 2003). The sealer layer thickness is important for the treatment success, especially when it appears in thick layers, damaging the sealing capacity of the filling system (Bamiduro et al., 1992). It occurs due to the sealer solubility over time (Bamiduro et al., 1992). However, the presence of the sealer into the filling mass, do not guarantee gap-free root canal fillings (Hammad et al., 2009).

There are no previous reports about the relationship between root canal filling techniques and penetrability of endodontic sealers into dentin tubules. The aim of this study was to evaluate the percentage of impregnated dentin area by resin-based sealer into dentinal tubules after three endodontic filling techniques by using confocal laser scanning microscope. Moreover, the sealer layer was evaluated according its area and integrity at the apical third of the canal.

MATERIALS AND METHODS

The study was approved by Ethical Committee of Rio Grande do Sul University (n^0 19356). Forty-five single rooted premolars, extracted for periodontal reasons, were used for this study. Teeth were disinfected in thymol crystal solution for 48 hours and stored at saline solution at 0.9%.

-Root Canal Preparation

Teeth were transversally sectioned with a diamond disc (KG Sorensen, Cotia, SP, Brazil) under water cooling to standardize the root size at 12 mm. The working

*Correspondence to: Ricardo Abreu da Rosa, DDS, MSD, Rio Grande do Sul Federal University, 408 Gomes Carneiro Street, 97050470 Dores, Santa Maria, RS, Brazil. E-mail: rabreudarosa@yahoo.com.br
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length was established by subtracting 1 mm from the length recorded when the #15 K-files (Dentsply Maillefer, Ballaigues, Switzerland) were visible at the apical foramina. The root canals were prepared by using ProTaper rotary files (Dentsply Maillefer, Ballaigues, Switzerland). Cervical and medium root third were prepared with S1-file. After, S2, F1, F2, F3, F4, and F5-file were used, in this order, in all the work length. To ensure the apical diameter after preparation, # 50 K-files were used at the working length.

The root canals were irrigated after each file with 2.5 mL of 2.5% NaOCl. After the instrumentation, the root canals were irrigated with 5 mL of 17% ethylenediaminetetraacetic acid and ultrasonically activated with a #20 K-file for 20 seconds at the working length. Final rinse was performed with 2 mL of saline solution. Thereafter, root canals were dried with paper points and randomly allocated in three experimental groups according the filling technique ($n = 15$): Group 1, #50 gutta-percha master cone; Group 2, cold lateral compaction; and Group 3, Thermafil (Thermafil, Dentsply-Maillefer, Ballaigues, Switzerland).

Root Canal Filling

Epoxi-resin based sealer AH Plus (Dentsply Maillefer, Ballaigues, Switzerland) was mixed with 0.1% Rhodamine B (RITC; Sigma-Aldrich, St. Louis, MO) (equal parts), for filling five specimens (D'Alpino et al., 2006). Rhodamine B was incorporated to the sealer for confocal laser scanning microscopy (CLSM) improvement (D'Alpino et al., 2006). AH Plus was manipulated according the manufacturer's instructions. A single master cone (Dentsply Maillefer, Ballaigues, Switzerland) was coated with AH Plus sealer and inserted into the root canal (Group 1). Gutta-percha excess and sealer were removed with heated tool until the cement-enamel junction was reached.

Group 2, master cone #50 was coated with the sealer and placed into the root canal. Then, cold lateral compaction was carried out using a finger spreader size B (Dentsply Maillefer, Ballaigues, Switzerland) until 1 mm short the apex. B8 gutta-percha accessory cones (Dentsply Ind. e Com. Ltda, Petrópolis, RJ, Brazil) were inserted until complete root canal filling.

Thermafil System was used at Group 3. Sealer was inserted into the root canal with #50 K-file and a Thermafil cone size 50 (Dentsply Maillefer, Ballaigues, Switzerland) was pre-heated using ThermaPrep Plus (Tulsa Dental, Tulsa, Oklahoma) and placed at the working length carefully. One operator performed all procedures.

- Sample Preparation for CLSM and Images Acquisition

Specimens were stored at 100% humidity and 37°C. After 2 weeks, a cut machine (Extec Labcut 1010, Enfield, CT) under water cooling was used for sectioning transversally the roots at 4 mm from the apex. For CLSM analysis, a 2-mm slice was obtained from the 4 mm apical. Thereafter, surfaces were polished with Arotec paste (Arotec, Cotia, SP, Brazil) for eliminate dentin debris eventually generated during the cut procedures.

Samples were examined from coronal to the apex using an Olympus Fluoview 1000 scanning confocal microscope (Olympus Corporation, Tokyo, Japan).

TABLE 1. Means and standard deviations of the percentage of the dentin-impregnated area; integrity of sealer perimeter; and sealer area at apical portion of canals filled by three techniques

	Dentin-impregnated area*	Sealer perimeter	Sealer area
Cold lateral compaction	52.32[a] (22.15)	98.43[a] (4.55)	17.37[a] (9.13)
Thermafil	59.46[a] (27.83)	100[a] (0.00)	8.09[b] (6.23)
Single master cone	51.71[a] (24.08)	99.54[a] (1.73)	21.18[a] (14.69)

*Columns with the same lowercase letter were not significantly different ($P > 0.05$).

Dentin impregnated area and sealer perimeter were analyzed at ×10. The images recorded were 70 μm depth, with 800 × 800 pixels. Each image was analyzed by using ImageTool 3.0 software (UTHSCSA, Texas). To obtain the sealer area, the lasso tool outlined and measured the canal circumference; thereafter the same tool outlined and measured the gutta-percha area. By subtracting these values, the sealer area was obtained. To analyze endodontic sealer penetration into dentinal tubules, the total dentin area (100%) was measured and, then, it was subtracted from the dentin nonimpregnated area. Finally, freehand line selection tool outlined the root canal and endodontic sealer perimeter and, so, the integrity of sealer perimeter was calculated. Data were statistically analyzed using Kruskal–Wallis and post hoc test, with significance level of 5%.

RESULTS

Table 1 presents the percentage of dentin-impregnated area by the sealer; the integrity of the sealer perimeter; and the sealer area at the apical third of the root canal. Significant differences were not found among the three experimental groups according the dentin-impregnate area ($P = 0.68$) and the integrity of the sealer perimeter ($P = 0.18$). However, root canal filling techniques were significantly different when sealer areas at apical third of the canals were compared ($P = 0.001$). Thermafil group showed thinner sealer areas (8.09%) than cold lateral compaction and gutta-percha single master cone that showed similar areas (17.37 and 21.18%, respectively). Figure 1 presents the CLSM images for the three filling techniques.

DISCUSSION

CLSM works with high contrast points to identify the sealers within the dentinal tubules without resorting to specimen preparation techniques that may cause artifacts (Gharib et al., 2007, Zapata et al., 2008). Compared with scanning electronic microscope, CLSM specimen processing is a nondestructive process and did not dehydrate the tooth (Hammad et al., 2008, Van Meerbeek et al., 2000). In association with epifluorescence, this method allowed analysis of endodontic sealer penetration into the dentin tubules and the relation between sealer and dentin walls. Rhodamine B dye was used with AH Plus with purpose to fluorescence the sealer. The influence on the chemical-mechanical properties of the sealer was discarded due to your small amount (0.1%) (Zapata et al., 2008). Moreover, Rhodamine B did not influence on methacrylate

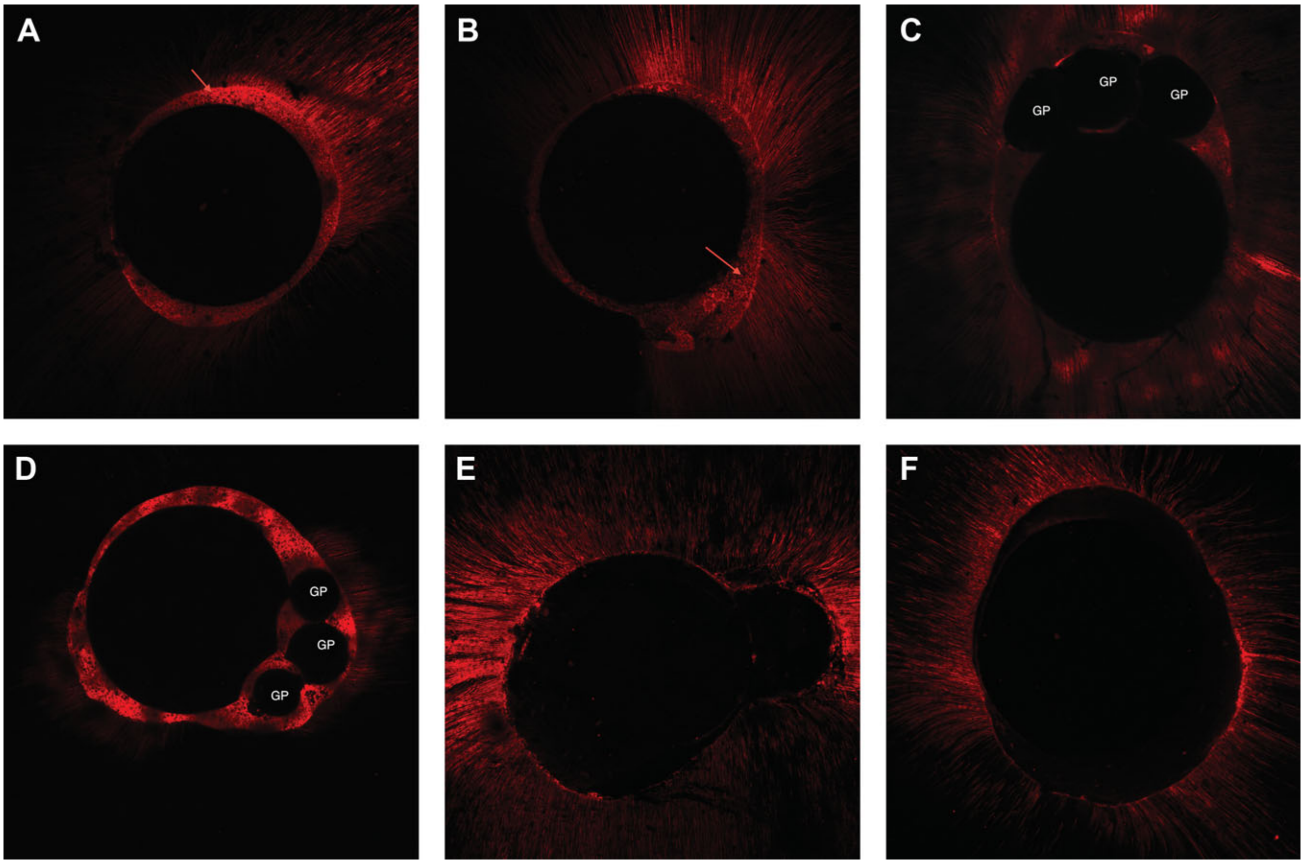

Fig. 1. CLSM (×10) of sealer penetration into dentin tubules, sealer/root canal perimeter and sealer area. **A**, **B**: Gutta-percha single master cone: arrow indicates presence of a thick sealer layer. **C**, **D**: Cold lateral compaction: gutta-percha accessories cones (GP) in the apical area. **E**, **F**: Thermafil technique: presence of a thin sealer layer.

resin and eugenol-based sealers penetration into dentin tubules (Patel et al., 2007).

Using nickel titanium rotary files, the concept of single cone technique is fortified by the availability of master cones that closely match with rotary instruments sizes (Gordon et al., 2005). Thermafil, a core-carrier technique, have been achieving a homogenous and well-adapted root canal filling with a minimum film thickness in the root canals (Gençoglu et al., 1993). Cold lateral compaction is a conventional technique, widely studied and used in several dentistry schools. Regardless of the root canal filling techniques, the integrity of the sealer perimeter at apical third was close to 100%. It means that AH plus covered almost totally the gutta-percha cone at apical third, filling empty spaces between cone and dentin. Previous studies observed 40% (Wu et al., 2000) and 60% (Hall et al., 1996) of perimeter integrity when cold lateral compaction was carried out and 97% (Wu et al., 2000) when single gutta-percha master cone was used. Some studies used optical microscope with ×40 (Wu et al., 2000) and ×1.2 (Hall et al., 1996) for perimeter analyze. On the other hand, other studies present similar results compared with the present study (Pallares et al., 1995, Silver et al., 1999, Weiss et al., 2004).

Ratio between sealer and root canal area is associated with thickness of sealer layer and it may be determinant to apical leakage. Thus, when the sealer layer is thick, the shrinkage can be high after setting time and more gaps would be present. Thermafil technique reaches the lowest sealer area around the gutta-percha carriers (8.09%) when compared with other two techniques (17.37 and 21.18%, cold lateral compaction and single master cone, respectively). Probably it occurred due to the heating of the carrier with gutta-percha which promotes close contact between thermo-plasticized material to dentin and thin sealer layer. Close contact did not occur in cold lateral compaction technique neither with a single master cone as previously described (Bamiduro et al., 1992, Bouillaguet et al., 2008). It's important to notice that root canal morphology may influence the sealer layer. Flattened canals, at transverse section, presented thicker sealer film, especially when roots were filled with a single gutta-percha cone (circular transverse section), thus the endodontic sealer fill empty spaces which gutta-percha did not fill. In Thermafil and cold lateral compaction groups, the empty spaces were filled by heated gutta-percha and accessory cones, respectively. A well-accepted disadvantage of single-cone technique is the inferior adaptation of the master cone to the middle and coronal thirds in irregular-shaped canals (Wesselink et al., 2005) increasing the sealer distribution (Wu et al., 2000), this can explain why only round cross sections from apical third were included in this study. Nevertheless, anatomical variations at apical third contributed for increasing of the standard deviation when the sealer area was evaluated.

Results suggested that different root canal filling techniques promote similar penetration of the root canal sealer into dentinal tubules. However, it should be notice the impossibility to analyze the maximum penetration at the cement-enamel junction direction, since CLSM ×10 did not permit this evaluation. AH Plus' flow into dentinal tubules seems to be associated to dentin permeability and to physic-mechanical properties of the epoxy-resin based sealer (Gutmann, 1993, Martin-Bauza et al., 2010). Moreover, smear layer removal by ethylenediaminetetraacetic acid also contributes to sealer flow into the dentin tubules (Kouvas et al., 1998). In this experimental model, the differences were not related to the root canal filling technique, inferring that sealer penetration is related with the permeability of the dentinal tubules and the chemical and physical characteristics of the sealer (Kouvas et al., 1998). Epoxy resin-based sealer exhibit structural integrity, homogeneity, and tightness of seal within the tubules (White et al., 1987) and maybe have contributed to the depth of tubular penetration.

CONCLUSION

At the apical third, the dentin-impregnated area was not dependent on the root canal filling technique. Single master cone, cold lateral compaction, and Thermafil techniques presented integrity of the sealer perimeter close to 100%. Finally, the Thermafil resulted in a significantly thinner sealer layer.

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