Significance of Moist Root Canal Dentin with the Use of Methacrylate-based Endodontic Sealers: An *In Vitro* Coronal Dye Leakage Study

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Abstract

This in vitro study compared the effect of different levels of moisture of root canals, from none to wet, on the coronal seal after filling with resin-coated gutta-percha cones/EndoRez [RGPC/ER (groups 1–4)], Resilon/Epiphany [RE/EP (groups 5–8)], and gutta-percha/Grossman's cement [GP/G (groups 9–12)]. The length of 76 single-rooted extracted human teeth was standardized to ~17 mm. After instrumentation with size 10 K-Files, #2 and #3 Gates Glidden burs, and preparation to the working length with K-Type files, the smear layer was removed with 17% ethylenediaminetetraacetic acid followed by flooding with distilled water. On the basis of similarities of canal shape determined by x-rays, roots were assigned to the groups ($n = 5$ per group) and treated according to one of the following protocols. (I) ETOH: excess distilled water was removed with paper points followed by dehydration with 95% ethanol; (II) PAPER POINTS: the canals were blot-dried with paper points with the last one appearing dry; (III) MOIST: the canals were dried with low vacuum by using a luer adapter for 5 seconds followed by 1 paper point for 1 second; and (IV) WET: the canals remained totally flooded. The roots were then filled with one of the obturation systems outlined above. The teeth were coated with 2 layers of nail varnish and 1 layer of sticky wax, except for the coronal access. In addition, positive and negative controls were added. After immersion in 2% methylene blue dye for 7 days, the samples were embedded in clear orthodontic resin and cross-sectioned at 0.5-mm intervals along the length of the roots. Dye penetration was evaluated by an independent investigator with a stereomicroscope at 40× magnification. The results indicated that dye leakage was affected by the degree of moisture. All materials evaluated showed some evidence of dye penetration, however, root canals filled with resin-coated gutta-percha/EndoRez and Resilon/Epiphany demonstrated significantly less leakage ($P < .05$) when moist conditions II and III were present. *(J Endod 2008;34:76–79)*

Key Words

Gutta-percha leakage, methacrylate-based root canal, moisture sealers

It has been established that coronal leakage might contribute to failure after root canal treatment (1, 2). To avoid this problem, a variety of sealers and cements have been tested in combination with gutta-percha for root canal obturation. However, it has been shown that a complete seal of the root canal system is impossible with the currently available materials (3). In recent experiments, a series of methacrylate-based formulations have been tested and have shown promising results (4-6). Preliminary reports have shown that EndoRez (ER) (Ultradent Products Inc, South Jordan, UT), a urethane dimethacrylate resin-based endodontic sealer, provides an effective seal when used with lateral condensation (7, 8). The hydrophilic properties of the sealer allow penetration deep into the root canal walls but not into gutta-percha (8). The lack of adhesion to gutta-percha constituted a weak point because a path for leakage might be created. In an effort to address this issue and to establish a bond between sealer/dentin and sealer gutta-percha, methacrylate resin-coated gutta-percha cones (RGPC) (Ultradent Products Inc) have recently been introduced (9).

A resin-based material for root canal obturation, Resilon (Resilon Research LLC, Madison, CT), has also recently been introduced to the dental profession (10). Resilon is a thermoplastic synthetic polymer-based (polycaprolactone) material that has been positioned as a replacement for gutta-percha as a root canal filling material (10, 11). It has the same handling properties as gutta-percha and is capable of bonding to Epiphany (EP) (Pentron Clinical Technologies, Wallingford, CT), a self-etching primer, and a dual-curing resin-based composite sealer with a filler content of approximately 70% by weight. Both ER and EP sealers set either by means of a chemical cure within the root canal between 15–30 minutes or by radiation for 40 seconds by visible light-curing the coronal portion of the canal to depth of approximately 2–3 mm (Zmener and Pameijer, unpublished data).

Previous investigations have shown that penetration of sealers into dentinal tubules might reduce microleakage (12, 13). The hydrophilic characteristics of ER and EP might improve the penetration of the sealer into moist dentin and dentinal tubules. This might also contribute to substantially reduced microleakage. The manufacturers recommend that the root canal walls be kept moist, not dehydrated, to take maximum advantage of the hydrophilic properties of the sealers, thus allowing for resin tag penetration and/or the formation of a hybrid layer. However, the practitioner is not provided with clear-cut clinical steps on how to accomplish an ideal dentinal root canal surface. Furthermore, until the introduction of methacrylate resin-based sealers (MBRS), root canals had to be dried thoroughly before placement of sealers. Therefore, the significance of residual moisture and the effect it has on the sealing properties of ER and EP are not fully understood or appreciated.

The purpose of this study was to determine the coronal sealing properties of root fillings with RGPC cones and ER or Resilon and EP sealer in instrumented root canals

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that had different levels of moisture, from dry to wet. Control groups were filled with gutta-percha and Grossman's cement. The null hypothesis tested was that neither the type of sealer nor the moist condition affected the coronal seal of the materials used for obturation.

Material and Methods

In this study, 76 extracted human teeth with single, straight root canals were used. They were stored in deionized water with a few crystals of thymol until use. The length of all specimens was standardized by sectioning the roots at ~17 mm from the apex. The working length was established radiographically at approximately 1 mm short from the apex by using a size 10 K-File (SybronEndo, Orange, CA). In all teeth the coronal and middle thirds were flared with #2–#3 Gates Glidden burs. They were then prepared to the working length with K-Type files by using a standard push-pull circumferential filing technique. Biomechanical preparation of the apical part of the canals was considered complete when a size 40-file could easily be inserted to the working length. The remainder of the canal was prepared with a step-back technique coronally to a size 60-file. Throughout preparation, the canals were irrigated with 5.25% NaOCl and 17% ethylenediaminetetraacetic acid (EDTA) followed by rinsing with distilled water. After preparation, they were kept flooded with 17% EDTA for 1 minute. They were then rinsed with 10 mL distilled water to remove all chemicals. Patency was confirmed with a size 10-K file, and the canals were rinsed again, whereas presence of water in the canal was verified visually by the appearance at the opening of the coronal access and extrusion through the apical foramen. All teeth were stored in distilled water of 37°C until used. Buccolingual and mesiodistal radiographs allowed for an assessment of gross similarities in anatomy, which formed the basis for a reasonable equitable distribution between the 12 groups of 5 teeth each ($n = 5$). Specimens were then subjected to 4 different moisture conditions.

Moisture Condition I (ETOH)

Excess distilled water was removed with paper points, and the canals were dried with 95% ethanol administered with a tuberculin syringe with a 30-gauge blunt-tip needle. The needle was carried to the working length. Ethanol was carefully injected into the canal, while at the same time slowly moving the syringe out. Complete flooding was verified visually by observing ethanol coronally and at the apical foramen. It was left in place for 10 seconds followed by removal of excess ethanol with paper points. The roots were stored dry at 37°C to assure completely dry conditions.

Moisture Condition II (PAPER POINTS)

The canals were blot-dried with paper points until the last point appeared dry after removal.

Moisture Condition III (MOIST)

The canals were dried with a luer vacuum adapter operating at low vacuum for 5 seconds and used in an up-and-down motion followed by 1 single paper point for 1 second.

Moisture Condition IV (WET)

The canals remained totally wet (flooded) with distilled water to see whether the moisture would be incorporated into the hydrophilic sealer in conjunction with displacement of excess water.

Groups 1–12

Applying the 4 moisture conditions described above, groups 1–4 were filled with a single RGPC cone and ER (RGPC/ER). Groups 5–8 were filled with a single Resilon cone and EP (RE/EP), whereas groups 9–12 received a single conventional gutta-percha cone and Grossman's cement (GP/G). The cones underwent a trial fit and were trimmed to achieve a friction fit with tug-back at the working length.

The sealers were used according to the manufacturer's recommendations; however, ER and EP were not subjected to light irradiation at the coronal orifice. Immediately after obturation, excess material was removed flush with the coronal surface of the root. The groups that had ER and EP had their access cavity covered with a celluloid matrix strip to prevent the formation of an oxygen-inhibited layer. The obturated roots were stored at 37°C and 100% humidity for 7 days to allow for complete setting. After storage and removal of the celluloid matrix strip, the roots were coated with 2 layers of nail varnish and 1 layer of sticky wax except for the coronal access opening.

For positive controls 4 roots were instrumented and treated according to the 4 conditioning protocols; however, they were neither obturated nor coated with varnish and sticky wax.

An additional 12 teeth (negative controls) were filled with each of the test materials under root dentin conditions I–IV; however, they were coated entirely with nail varnish and sticky wax including the access opening. All control specimens were tested for leakage in a similar fashion as the experimental groups.

The specimens were immersed in a 2% methylene blue dye solution (pH 7.4) for 7 days at 37°C. After removal, they were rinsed with distilled water and stored at 37°C at 100% relative humidity. Measurements of coronal dye penetration were performed according to a technique described by Mann and McWalter (14) with minor modifications. Horizontal cross-sections were made at 0.5-mm intervals along the length of the root in a coronal to apical direction by using a diamond wafering blade, 0.5 mm thick with continuous water lubrication (Isomet; Buehler Ltd, Lake Bluff, IL). All specimens were sectioned to the apex of the roots. They were then treated with 1% hydrofluoric acid for 30 seconds, rinsed with distilled water, and air-dried to remove surface debris caused by sectioning. They were subsequently mounted on microscope slides, and the upper surface of each consecutive section was examined with a calibrated stereoscopic magnifying lens at magnification 40× (Bausch & Lomb Inc, Rochester, NY) under reflected light for the presence of dye between the obturating material and the root canal walls. The extent of dye penetration was measured to the nearest millimeter at which the presence of dye was visible at the interface filling material/dentin wall. The results were analyzed by a two-way analysis of variance, considering the materials as one factor and the moist condition as the other. An analysis between and within groups was performed. Duncan test was performed for post hoc comparisons. Statistical significance was decided at $P < .05$.

Results

The mean coronal leakage and standard deviation for each moisture condition and for each material are shown in Table 1. A statistically significant 2-factor interaction ($P < .05$) between materials (RGPC/ER, RE/EP, and GP/G) and moisture conditions (I–IV) was established, indicating that the sealing ability of the materials tested was affected by the degree of moisture present inside the root canals and depended on the materials used.

There was no statistically significant difference ($P > .05$) between RGPC/ER and RE/EP when moisture conditions I–IV were compared. However, when conditions I-ETOH and IV-WET were compared with conditions II-PAPER POINTS and III-WET, reduced leakage for RGPC/ER and RE/EP was significant for the II-PAPER POINTS and III-MOIST conditions ($P < .05$). Furthermore, dye leakage for RGPC/ER and RE/EP under condition III-MOIST was significantly less than for condition II-PAPER POINTS ($P < .05$).

TABLE 1. Mean values ± standard deviations of coronal dye penetration (mm) under different moisture conditions (groups 1–12)

Material	Total n	I-ETOH (n = 5)	II-PAPER POINTS (n = 5)	III-MOIST (n = 5)	IV-WET (n = 5)
RGPC/ER (groups 1–4)	20	5.20 ± 1.03	2.60 ± 0.65	1.90 ± 0.82	5.70 ± 1.15
RE/EP (groups 5–8)	20	5.00 ± 1.45	2.50 ± 0.93	1.80 ± 0.57	5.50 ± 1.96
GP/C (groups 9–12)	20	3.19 ± 1.19	4.70 ± 1.15	7.20 ± 0.83	7.50 ± 0.79

NOTE. See Results for statistical significance between and within groups.

The performance of GP/G was significantly different (more dye leakage) when compared with all RGPC/ER and RE/EP samples for all moisture conditions ($P < .05$), with exception of the I-ETOH prepared samples. Within the GP/G groups no significant differences ($P > .05$) were found between moist conditions I-ETOH and II-PAPER POINTS and between conditions III-MOIST and IV-WET. However, significantly less dye leakage was observed between I-ETOH and II-PAPER POINTS when compared with III-MOIST and IV-WET ($P > .05$). The negative controls showed no evidence of leakage, whereas the positive controls had leakage along the entire length of the canals.

Discussion

Different assays have been performed to evaluate leakage, including dyes (15, 16), radioisotopes (17), bacteria (18), and an electrochemical technique (19, 20). Dye penetration studies have frequently been used for leakage evaluation (15, 16), in spite of the fact that their clinical significance has been questioned (21, 22). Nevertheless, previous studies revealed a good correlation between dye penetration and other leakage evaluation methods (23, 24). In an effort to standardize the obturating procedures and to reduce the variables related to it (25), a single master cone was used for all groups. It has been demonstrated that a single cone resulted in comparable results to other techniques (26-28), when used in conjunction with low shrinking sealers. In this respect, our findings are in agreement with other reports (29, 30).

The results of this study demonstrated that the moisture condition of root canals at the time of obturation and the type of sealer that was used had a significant effect on microleakage. Therefore, the null hypothesis was rejected. All materials exhibited some evidence of dye penetration, although RGPC/ER and RE/EP performed better than GP/G except for condition I-ETOH, the dehydrated root canal, in which Grossman's sealer performed better. Less leakage was especially evident for RGPC/ER and RE/EP when moist conditions II-PAPER POINTS and III-MOIST were compared with the others. Furthermore, in particular the technique of condition III-MOIST, in which a low vacuum luer tip was used for 5 seconds followed by a paper point for 1 second, demonstrated the best possible seal.

Whether an actual chemical interaction between methacrylate-based sealers and dentin walls takes place is doubtful; most likely, the hydrophilicity allows penetration of resin tags and the formation of a hybrid layer, resulting in micromechanical interlocking (31, 32). Similar results were obtained by Patel et al (33) in root canals filled with RealSeal (Sybron Endo), a methacrylate-based sealer. The hydrophilic propensity of these resin-based materials can thus provide a more thorough seal than the hydrophobic zinc oxide–eugenol (ZOE)–based Grossman's sealer.

Leakage in root canals with moist condition III-MOIST was significantly less in comparison with the other conditions, when using methacrylate-based resin sealers. With the use of a luer adapter under low vacuum for 5 seconds followed by a single paper point for 1 second, the dentin was favorably conditioned for these sealers. In condition II-PAPER POINTS, only paper points were used until the last one, on removal, appeared to be dry, resulting in a greater degree of dryness within the canals. This condition showed significant differences with conditions I-ETOH and IV-WET but demonstrated only slightly more leakage (statistically not significant, $P > .05$) than condition III-MOIST. Wong and Spencer (34) demonstrated that dentinal tubules normally remain filled with water unless the canal is thoroughly dried. This condition was more desirable than a totally dry or totally wet canal but was probably not totally sufficient for maximum effective hydrophilic resin penetration. Whether clinically there is a difference between moist condition II-PAPER POINTS and III-MOIST needs to be determined in a controlled study. In a report by Tay et al (31) the presence of a hybrid layer and resin sealer tags as a result of dentin surface demineralization by EDTA in root canals filled with gutta-percha cones and ER was demonstrated. These observations corresponded well with those of Osorio et al (35), who showed that the collagen network of the dentin is better preserved after the use of EDTA as the final rinse. In the current study as well as in the clinical situation, one might expect a similar effect because EDTA was also used in our irrigation protocol.

Root canals subjected to condition IV-WET showed a higher degree of leakage. In these cases, water can apparently not completely be displaced in spite of the hydrophilic properties of the sealers. Water permeation during the polymerization process might result in the entrapment of water droplets within the sealer-dentin interface. This might result in bond disruption and further increased leakage. Wong and Spencer (34) also reported that excess water can inhibit polymerization of methacrylate-based resins. Therefore, a controlled amount of wetness can help increase the degree of conversion of hydrophilic resin-based sealers and subsequently obtain a better interaction with the root canal walls.

ZOE-based sealers have shown a significant decrease in their physical properties when contaminated with moisture because of their high solubility (36), especially in the early stage of the setting reaction (37). As a result, a reduction of the sealing properties was reported for ZOE-based materials under wet conditions (38, 39). The results of this study also demonstrated that moist canals greatly affect the sealing capacity of Grossman's sealer (which is a ZOE-based material), especially when the moisture content in the canals is high. In contrast, the absence of moisture in the group treated with ethanol resulted in less microleakage. This is in agreement with Kuhre and Kessler (37) and Hosoya et al (38), who showed that optimum sealing conditions were obtained when totally dry canals were filled with a single gutta-percha cone and a ZOE-based sealer.

RGPC/ER and RE/EP groups performed well, but they nevertheless leaked to some extent. An increase in sealer thickness negatively influences the sealing ability of a root canal filling (40, 41); the latter undergoes polymerization shrinkage on setting. This can be offset by inserting multiple RGPC or Resilon cones to reduce the bulk of the sealer in wide flared canals, thus lowering the polymerization shrinkage. In vitro leakage experiments should be regarded as an important technique when analyzing conventional or new endodontic sealers, yet a reliable clinical correlation should be viewed with caution. No evidence exists that demonstrates that if a certain amount of leakage is present, the clinical success will be compromised. Finally, this study reported on short-term (7 days) leakage values, which might change over longer time periods.

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