Sealing ability of three hydrophilic single-cone obturation systems: An *in vitro* glucose leakage study

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Abstract

Aim: The aim of this study is to compare the corono-apical sealing ability of three single-cone obturation systems using a glucose leakage model. **Materials and Methods:** A total of 90 freshly extracted human maxillary single-rooted teeth was selected, and their crowns were cut. The root canal of each sample was instrumented using a rotary crown down technique and then divided into four experimental (n = 20 each) and two control groups (n = 5 each). Samples in the experimental groups were filled as follows: Group 1, cold lateral condensation using gutta-percha/AH Plus; group 2, single-cone C-points/smart-paste bio-sealer; group 3, single-cone bio-ceramic (BC) impregnated gutta-percha/endo-sequence BC sealer; group 4, single-cone Resilon/RealSeal SE after 7 days, the sealing ability of root canal fillings was tested at different time intervals using glucose leakage model. Glucose leakage values were measured using a spectrophotometer and statistically analyzed. **Results:** The four experimental groups presented significantly different glucose leakage values at all test periods ($P < 0.05$). At the end of the observation period, the cumulative glucose leakage values of groups 2 and 3 were significantly lower than those of groups 1 and 4 ($P < 0.05$). **Conclusion:** C-points/smart-paste Bio and BC impregnated gutta-percha/endo-sequence BC sealer combinations provided the superior sealing ability over the lateral condensation technique.

Keywords: Bio-ceramic sealer, glucose leakage, smart-paste bio

Introduction

Ultimate Goal of the root canal system obturation is to provide a hermetic seal that prevents reinfection of the canal and subsequent leakage of fluid and antigenic agents into or from the periradicular tissues.[1] In today's era, there is increasing demand for prompt, simple and efficient obturation technique, which increases efficient practice and results in negligible stress for patients and clinicians. With the widespread use of rotary NiTi instruments and matched-taper gutta-percha cones, the single-cone obturation technique has become popular.[2] In order to improve the marginal sealing properties of root canal system, hydrophilic root canal obturating systems have been introduced. A Bio-ceramic (BC) root canal sealer has been introduced, commercially known as Endo-sequence BC sealer (Brasseler USA, Savannah, GA). Endo-sequence BC Sealer is a premixed and injectable endodontic sealer, and its nanoparticle size sanctions it to flow into canal irregularities and dentinal tubules. It is hydrophilic and uses moisture in dentinal tubules to initiate and complete its setting reaction. In addition, no shrinkage occurs on setting, resulting in a gap-free interface between the gutta-percha, sealer, and dentin.[3] The most recent obturating system is the Smart-Seal System, which is composed of C-points and Smart-paste Bio-sealer. The C-points have been designed to expand laterally utilizing the inherent moisture present in the instrumented root canal space.[4] The ReaSeal SE system consists of a self-etching methacrylate sealer and Resilon core material. It reduces the application steps of the original epiphany system, thus becoming a more operator friendly and bonds to both the Resilon core and radicular dentin through hybrid layers on both substrates leading to a monoblock unit, which may prevent leakage and improve the root strength.[5-7] The sealing ability is a basic feature that needs to be tested for every root canal filling material or technique. Various test methods have been described to evaluate the quality of the seal by such methods as dye penetration, radioactive isotopes test, bacteria or bacterial metabolites leakage test, electrochemical technique, and fluid filtration.[8] However, the published reports often reach different or even conflicting conclusions. As pointed out by Wu and Wesselink, there was a high level of variation in these results and it was difficult to draw firm conclusions as to which filling technique or material was the best in sealing the root canal system. It was suggested that more studies should be devoted to perfecting

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microleakage methodology.[9] These methods include dye penetration, spectrometry of radioisotopes, fluorometric and electrometric methods, bacterial penetration and fluid transport model.[8] Xu *et al.* discussed a new nondestructive model that measures the leakage of glucose molecules quantitatively using a spectrophotometer. The aim of the present study was to evaluate corono-apical microleakage along root canal fillings using glucose leakage model by comparing three matched-taper single-cone filling systems with cold gutta-percha lateral compaction technique using glucose leakage model at different time intervals.

Materials and Methods

Samples preparation

A total of 90 freshly extracted human maxillary single-rooted teeth with fully formed apices was used. The crowns were cut with the help of diamond disk below the cemento-enamel junction so that the length of roots was standardized at 15 mm. The working length was determined, and the canals were instrumented by pro-Taper NiTi rotary instrument (Dentsply Maillefer) to size F3 using the crown-down technique. The canals were irrigated after using each file with 5 ml of 3% sodium hypochlorite (NaOCl) solution using a syringe and 29-G needle (NaviTip; Ultradent, South Jordan, UT). After finishing the instrumentation, the prepared canals were rinsed with 5 ml of 17% ethylenediaminetetraacetic acid solution for 2 min followed by 10 ml distilled water as final irrigation to remove any traces of NaOCl.

Samples grouping and root canal obturation

After drying all canals with absorbent paper points, the samples were divided according to the obturation technique and materials into four experimental groups of 20 samples each and two control groups of five samples each. Samples in the negative control group did not receive root canal fillings while in the positive control group they were obturated with a single-cone gutta-percha size 30/0.06 but without sealer placement. In group 1, AH Plus sealer was mixed according to the manufacturer's instructions and applied into the prepared root canal using a lentulo spiral size 25. A master gutta-percha cone of size 30/0.06 was coated with sealer and placed into the root canal to the full working length. Lateral condensation was achieved using size 25/0.02 standardized gutta-percha cones and size 25/0.04 finger spreader (Dentsply Maillefer). Excess gutta-percha was cut at the orifice level with a flame-heated hand plugger and vertically compacted. In the other experimental groups (single-cone obturation technique), each sealer was prepared and placed into prepared canals according to manufacturer's instructions. The tip of the matched taper cone (C-points/Bio-ceramic impregnated gutta-percha or RealSeal point) was dipped into the sealer and placed slowly in up and down motion until reaching the full working length. The coronal excess of the master cone was precut to coronal orifice using a scissors at a predetermined length. In the RealSeal group, the coronal surface of the obturation was light cured after 5 min for 40 s. All samples were incubated for 1-week at 37°C and 95% humidity to allow complete setting of sealers.

Microleakage measurement

The roots in the experimental and positive control groups were coated with triple layers of nail varnish, except at the coronal end and apical 1 mm of the root end. The roots in the negative control group were entirely covered with nail varnish. Microleakage along the root canal was evaluated using the glucose leakage model as described by Xu *et al.*[5] The concentrations of leaked glucose (mg/dl) were measured after 1-day and then after 1, 2, 3, 4 and 6 weeks with a glucose kit (glucose liquid, quimica clinica Aplicada S.A) in a spectrophotometer (Beckman Du 520, Coulter, Germany) at a wavelength of 505 nm.

Statistical analysis

The results were statistically analyzed by Kruskal–Wallis and Mann–Whitney tests. To compare the leakage at different times within each group, Freidman and Wilcoxon signed ranks tests were used. All level of statistical significance was set at a $P < 0.05$.

Results

The negative control group showed no detectable glucose leakage throughout the experiment while the positive control group had immediate substantial glucose leakage, which increased over time. This indicates that the seal of the glucose leakage system was effective and reliable. The mean values and statistical comparisons between the experimental groups at each time interval are given in Table 1. After the 1st day onward, there were significant differences between the experimental groups (Kruskal–Wallis test, $P < 0.05$). The results of the Mann-Whitney test indicated that there was no significant difference between groups 2 and 3 throughout the test period. After the 1st day, the highest glucose leakage was observed in group 1. After the 1st week, the lowest glucose leakage was observed in the 3rd group. Starting from the 3rd week onward, the lowest glucose leakage was observed in the Groups 2 and 3. Statistical comparisons between glucose leakage values within each group are presented in Table 2. There was a progressive and significant increase in the glucose leakage values in all experimental groups (Friedman test, $P < 0.05$).

Discussion

In the present study, the leakage along root canal fillings was measured by the glucose penetration method, which is simple and could give reliable quantitative leakage measurements. In the present study, glucose was selected as the tracer because it is of small molecular size (MW = 180 Da) and is a nutrient for bacteria. If the glucose could enter the canal from the oral cavity, bacteria that might survive root canal

Table 1: Glucose leakage at various time intervals

Groups	Glucose concentration, mg/dl (mean±SD)					
	Day 1	Week 1	Week 2	Week 3	Week 4	Week 6
Group 1	0.52±1.31[a]	5.02±1.51[a]	11.02±2.61[a]	13.32±0.62[b]	15.66±4.57[a]	18.43±3.83[a]
Group 2	0.00±0.32[b]	1.34±1.33[b]	5.62±1.53[b]	7.72±0.11[a]	7.98±1.93[b]	10.22±3.51[b]
Group 3	0.00±0.00[b]	1.23±1.21[b]	6.02±2.72[b]	6.99±1.88[a]	7.11±2.32[b]	9.89±2.64[b]
Group 4	0.53±0.67[a]	3.02±0.62[c]	9.13±1.34[c]	9.22±1.73[c]	10.45±2.71[c]	12.44±2.01[c]
Kruskal-Wallis test (*P*)	0.000	0.000	0.000	0.045	0.000	0.000

Mann-Whitney test: Means with the same superscript letters within each column are not significantly different at $P \geq 0.05$. SD: Standard deviation

preparation and obturation could multiply and potentially lead to periapical inflammation. Glucose, therefore, was thought to be more clinically relevant than other tracers used in microleakage tests. Quantitative analysis of leakage was possible by determining the concentration of glucose in the apical reservoir that leaked through the filled root canal.[9] To determine the concentration of glucose, the enzymatic glucose oxidase method was chosen because it provided the ultimate degree of specificity and high sensitivity when compared with other methods, such as copper or ferricyanide methods. With this method, glucose is oxidized by the enzyme glucose oxidase in the presence of oxygen to gluconic acid with the formation of hydrogen peroxide.[10] Then in the presence of a peroxidase enzyme, a chromogenic oxygen acceptor (4-aminoantipyrine and phenol) is oxidized by the hydrogen peroxide, resulting in the formation of a red product (oxidized chromogen). The quantity of this oxidized chromogen is proportional to the glucose present initially in the first reaction, which quantity is determined by spectrophotometry. With this model, it was possible to quantify the endodontic microleakage continuously over time. The amount of microleakage was the cumulative value of leaked glucose.[11] The reactivity of obturating materials with glucose could affect the results of the glucose leakage test. The results Shemesh *et al*. indicated that all materials used in the current study did not show glucose reactivity.[12] The results of this study indicate that all obturation systems allow variable degrees of glucose leakage. The glucose leakage values of AH plus groups either with lateral condensation technique were significantly higher at the end of the experimental period. This might be explained by the fast setting and subsequent polymerization shrinkage of AH plus sealer,[13] the lack of bonding between this sealer and gutta-percha the low penetration ability of this sealer within the dentinal tubules[14] and its hydrophobic property that prevents good adaptation of to the incompletely dried canal.[15] Least leakage is seen in group 2 and 3 attributed to many reasons. The smart-seal system (Prosmart - DRFP Ltd., Stamford, UK). The system consists of obturation points (C-points) containing a polyamide core with an outer bonded hydrophilic polymer coating and an accompanying sealer smart-paste Bio. The endodontic points are designed to expand laterally without expanding axially by absorbing residual water from the instrumented

Table 2: Time interval related glucose leakage

Time	Glucose concentration, mg/dl (mean±SD)			
	Group 1	Group 2	Group 3	Group 4
Day 1	0.52±1.31[a]	0.00±0.32[a]	0.00±0.00[a]	0.53±0.67[a]
Week 1	5.02±1.51[b]	1.34±1.33[a]	1.23±1.21[a]	3.02±0.62[b]
Week 2	11.02±2.61[c]	5.62±1.53[b]	6.02±2.72[b]	9.13±1.34[c]
Week 3	13.32±0.62[d]	7.72±0.11[c]	6.99±1.88[b]	9.22±1.73[c]
Week 4	15.66±4.57[e]	7.98±1.93[c]	7.11±2.32[b]	10.45±2.71[c]
Week 6	18.43±3.83[f]	10.22±3.51[d]	9.89±2.64[c]	12.44±2.01[d]
Friedmann test (*P*)	0.000	0.000	0.000	0.000

Wilcoxon signed ranks test: Means with the same superscript letters within each column are not significantly different at $P \geq 0.05$. SD: Standard deviation

root canal space and the naturally present moisture in the dentinal tubules.[16] The inner core of Pro-points is a mix of two proprietary nylon polymers: Trogamid T and Trogamid CX. The polymer coating is a cross-linked copolymer of acrylonitrile and vinylpyrrolidone which has been polymerized and cross-linked using allyl methacrylate and a thermal initiator. The lateral expansion of pro-points is claimed to occur nonuniformly with the expand-ability depending on the extent to which the hydrophilic polymer is prestressed (i.e., contact with a canal wall will reduce the rate or extent of polymer expansion).[4] BC impregnated gutta-percha and endo-sequence BC sealer, owing to its hydrophilic property and nano-particle composition has also shown comparable microleakage to smart-seal system. BC root canal sealers also exhibit chemical bonding to root canal dentin walls, as well as its corresponding Bio-ceramic particle impregnated gutta-percha.[17] group 4 Resilon/RealSeal was expected to show less leakage in comparison but leakage was significantly higher than groups 2 and 3. Resilon is a polymer-based thermoplastic resin containing bioactive glass, bismuth oxychloride, barium sulfate, and radiopaque fillers.[18]

Many advantages of Resilon (SybronEndo, Orange, CA) have been reported, which include reduction in postendodontic leakage and periapical inflammation and improvement in root strength. These advantages have been attributed to the monoblock concept, whereby the Resilon core is bonded to the sealer, and the resulting complex is bonded to root dentin by a resin-based primer.[3-5] Secondary monoblocks are

those that have two circumferential interfaces, one between the cement and dentin and the other between cement and the core material. In a root canal, the C-factor can be more preponderant than 1000. Hence, any polymerizing endodontic sealer would be subjected to sizably voluminous polymerization stresses during the setting process, resulting in debonding and gap formation along the periphery of the root filling[19] and thus can be a contributing factor for the increased leakage seen in this group. Therefore, in spite of the hydrophilic nature of Resilon, leakage was significantly more than other hydrophilic groups.

Conclusion

Hydrophilic groups have shown significantly lesser leakage as compared to the gold standard hydrophobic gutta-percha obturation system. Furthermore, studies would be required to assess the hydrophilic nature of the recent obturation systems.

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