RESEARCH

Assessment of the sealing ability of resin based root-canal sealers using glucose leakage model*

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ABSTRACT

Assessment of the sealing ability of resin based root-canal sealers using glucose leakage model

Background: The objective of this study was to asses the sealing ability of resin based sealers.

Methods: Fifty-five extracted human single-rooted teeth were used in this study. After removing the crowns of the teeth at cement-enamel junction, all roots were instrumented with a set of ProTaper Universal rotary instruments. The specimens were divided into three experimental group (n=15), ten roots were used as positive and negative controls (n=5). The root canals were filled by the single-cone technique with MM-SealTM, MetaSEALTM and AH Plus sealers. The sealing ability of the sealers was measured by glucose leakage model and samples were taken weekly four weeks. The glucose concentrations were analyzed using Kruskall-Wallis and Mann-Whitney U tests.

Results: MetaSEALTM showed the most leakage during the test period. Along the three weeks there was no significant difference between MM-SealTM and AH Plus. At forth week MM-SealTM showed the best sealing ability (P< 0 .01).

Conclusion: Under the conditions of this *in-vitro* study, while considering the sealing ability MM-Seal is an alternative material to AH Plus when used with single-cone technique.

KEYWORDS

Glucose leakage method, resin based sealers, sealing ability, single-cone technique

ÖZ

Rezin esaslı kanal dolgu patlarının kapatıcılık özelliklerinin glukoz sızıntısı modeli ile değerlendirilmesi

Amaç: Bu çalışmanın amacı rezin esaslı kanal dolgu patlarının kapatıcılık özelliklerini karşılaştırmaktır.

Gereç ve Yöntemler: Bu çalışmada elli beş adet çekilmiş tek köklü insan dişi kullanılmıştır. Dişlerin kuronları mine-sement hizasından uzaklaştırıldıktan sonra tüm örneklerin kök kanalları ProTaper döner aletleri ile genişletilmiştir. Örneklerden 15 kök içeren üç adet deneysel grup ve beşer kök içeren pozitif venegatif kontrol grupları elde edilmiştir. Kök kanalları deneysel gruplarda açılı tek kon yöntemi kullanılarak AH Plus, MM-Seal ve MetaSEAL kanal dolgu patlarıyla doldurulmuştur. Kanal dolgu patlarının kapatıcılık özelliği glukoz sızıntısı testi ile haftalık olarak bir ay boyunca değerlendirilmiştir. Glukoz konsantrasyon değerleri Kruskall-Wallis ve Mann-Whitney U testleri kullanılarak istatistiksel olarak incelenmiştir.

Bulgular: MetaSEAL test süresi boyunca en fazla sızıntı miktarını göstermiştir. İlk üç hafta MM-Seal ve AH Plus arasında istatistiksel olarak fark gözlenmez iken dördüncü haftada MM-Seal en iyi kapama kabiliyetine sahip olmuştur. (P< 0.01)

Sonuç: Bu *in-vitro* çalışma sonuçlarına göre açılı tek kon yöntemi kullanıldığında MM-Seal kapatıcılık özelliği açısından AH Plus'a alternatif bir materyal olarak görülmektedir.

ANAHTAR KELİMELER

Glikoz sızıntı yöntemi, kapatıcılık özelliği, rezin esaslı kanal patları, tek kon yöntemi

One of the aims of a successful endodontic treatment is to prevent the penetration of microorganisms, their toxins and tissue fluids into the root canal space and periapical tissues.[1-3] This is achieved when the root canal space is obturated in all dimensions and sealed both coronally and apically.[4-6] Because apical leakage of root canal filling is a common cause of endodontic failure.[7, 8]

A variety of in-vitro experimental methods are described to evaluate the leakage of endodontic materials. Dye leakage, fluid filtration and bacterial penetration are the most frequently used methods. Xu *et al* developed and referred glucose leakage model that evaluates the amount of glucose which leaks along the root canal. In this method, glucose is selected as the tracer because it has a low molecular weight. This is a quantitative and nondestructive method assesses the sealing ability of root canal filling materials.[9]

Although a large number of filling materials and techniques are available for the root canal filling, the combination of sealer and gutta-percha is the most common used technique clinically.[10] The use of sealers in endodontic treatment obtains bonding of the core material to the dentin walls and filling the irregularities

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of the root canal system.[11, 12] Properties of an ideal root canal sealers are sealing the root canal system tightly, having good adhesion to dentin walls and being biocompatible, antibacterial, nontoxic and radiopaque.[11, 13] Resin based sealers are one of the most frequently used materials with gutta-percha. Because they provide good physical properties and show adequate biological performance.[6, 14]

MM-SealTM (Micro-Mega, Besançon, France) is a new introduced epoxy-resin based root canal sealer which contains epoxy-oligomer resin, calcium phosphate, ethylene glycol salicylate, bismuth subcarbonate and zirconium oxide. It is produced in a dual syringe package provides accurate dispensing. The manufacturer claims that MM-Seal has good apical tolerance and low solubility in soft tissues.

MetaSEALTM (Parkell Inc, Farmington, NY) is new self-adhesive and dual-curable resin cement which contains 4-methacryloyloxyethyl trimellitate anhydride (4-META). This sealer is also marketed as Hybrid Root Seal in Japan (Sun Medical, Shiga, Japan). MetaSEALTM doesn't need any priming and acid etching protocol because it is a self-etching and dual-cure resin cement.[15] According to manufacturer it is highly biocompatible and well tolerated by periapical tissues. MetaSEALTM bonds to gutta-percha and radicular dentin by creating the hybrid layers.[16] An acidic monomer 4-META allows conditioning of the dentin walls and penetrates into the collagen network.[17] The sealer has hydrophilic characteristics because of 4-META and is suggested with cold compaction or single-cone techniques.[18]

AH Plus (Dentsply DeTrey, Konstanz, Germany) is a commonly used epoxy-resin based sealer which achieves a tight seal in root canal. It has biocompatible, low solubility and antibacterial properties.

The purpose of this in-vitro study is to compare the sealing ability of two new resin based sealers and AH Plus with a single-cone technique using glucose leakage model.

MATERIALS AND METHODS

Fifty-five recently extracted human mandibular premolars with single and straight canal were selected for this study. The teeth had been extracted for periodontic or orthodontic reasons and stored at +4°C in a physiological saline solution before use. Soft tissue remnants and calculus of the teeth were removed and each tooth was placed in 5.25 % sodium hypochlorite (NaOCl) to disinfect the surface. Bucco-lingual and mesio-distal radiographs were taken to verify presence of single canal. The crowns of the teeth were removed at the cemento-enamel junction by using a high speed bur under water cooling so that all roots were approximately 14 mm long.

To determine the working length, #10 K-file (Dentsply, Maillefer) was inserted into the root canal until it was visible at the apical foramen and subtracting 1 mm from this length. The apical patency of the root canals was maintained throughout instrumentation using #15 K-file (Dentsply, Maillefer). All roots were instrumented using a crown-down technique with rotary ProTaper nickel-titanium files (Dentsply, Maillefer) to F3. The root canals were irrigated with 2 ml of a freshly prepared solution of 2.5 % NaOCl between each instrument. After finishing the instrumentation, the smear layer was removed with 10 ml of 17 % EDTA, 10 ml of 5.25 % NaOCl and 5 ml distilled water. The canals were then dried with sterile paper points.

Resin based sealers used in this study are shown in Table 1.

Ten roots were then selected and used as positive and negative controls (n=5). The rest of the roots were divided into three experimental groups 15 teeth each. The root canals were obturated using a single-cone technique, as follows;

Group 1: F3 ProTaper gutta-percha points were fitted to the working length with tug-back. The points were then coated with AH Plus root canal sealer and placed into the root canals. After obturation, excess gutta-percha 1 mm below the coronal surface was removed with heated instrument and vertically compacted with plugger. The cavity was then cleaned with cotton pellets.

Group 2: F3 ProTaper gutta-percha points were fitted to the working length with tug-back. MetaSEALTM (Hybrid Root SEAL) was mixed according to manufacturer's recommendation. As defined, 3 drops of liquid and 1 cup of powder were mixed on the mixing pad with a spatula until having homogeneous paste. The gutta-percha points were then coated with the sealer and introduced into the root canals. After obturation, excess gutta-percha was removed described in group 1.

Group 3: F3 ProTaper gutta-percha points were fitted to the working length with tug-back. According to manufacturer's recommendation, MM-Seal base and catalyst (2:1 wt. ratio) were mixed on the mixing pad with a spatula. The gutta-percha points were then coated with the sealer and placed into the root canals. After obturation, excess gutta-percha was removed in the same manner as in group 1.

In this study, five root canals were filled with F3 ProTaper gutta-percha points without any sealer to serve as positive controls. Five root canals were filled with F3 ProTaper gutta-percha points and AH Plus and were completely covered with nail varnish to serve as negative controls.

Coronal surfaces of the roots were filled with temporary filling material Fermin (Detax, Ettlingen-Germany) and all of the specimens were stored for 1 month at 37 °C and 100% humidity for sealers' setting.

Table 1.

Resin based sealers used in this study

Sealer	Ingredients	Manufacturer
AH Plus	Paste A: Bisphenol-A epoxy resin, Bisphenol-F epoxy resin, Calcium tungstate, Zirconium oxide, Silica, Iron oxide pigments, Aerosil Paste B: N-Dibenziyl-5-oxanonane, TCD-Diamine, Aminoadamantane, Tricylodecane-diamine, Calcium tungstate, aerosil Zirconium oxide, Silica, Silicone oil	Dentsply DeTrey, Konstanz, Germany
MM-Seal	Base: epoxy-oligomer resin, calcium phosphate, ethylene glycol salicylate, bismuth subcarbonate, zirconium oxide Catalyst: poly aminobenzoate, triethanolamine, calcium phosphate, bismuth subcarbonate, zirconium oxide, calcium oxide	Micro-Mega, Besançon, France
MetaSEALTM (Hybrid Root SEAL)	Powder: mixture of zirconium oxide filler, SiO2 filler, and polymerization initiators Liquid: 4-META, monofunctionalmethacrylate monomer, multifunctional macrylate monomers and photo-initiators	Parkell Inc, Farmington, NY

Measurement of microleakage

All samples were placed into a glucose leakage model designed to evaluate the leakage. This method firstly introduced by Xu *et al* and used by others.[1, 9, 19] The coronal 7 mm of the roots were embedded in acrylic resin to form a cylinder around the samples. The acrylic resin block around the root was individually connected to a 16-cm-long plastic pipette with a silicone tube of which diameter was 5 mm. The assembly was then placed in a sterile glass bottle with a screw cap. 3 ml of 0.2% NaN_3 was injected into the glass bottle so that the root samples were immersed in the solution. NaN_3 was used to prevent the growth of the microorganisms that might influence the glucose readings through the decomposition of glucose.[9, 19] Cyanoacrylate adhesive was used to seal all of the interfaces. The tracer used in the present study was a 1 mol L^{-1} glucose solution. Glucose has a low molecular weight and is hydrophilic and chemically stable. About 5 ml of 1 mol L^{-1} glucose solution containing 0.2% NaN_3 was injected into the plastic pipette a until the level of the solution was 14 cm higher than the coronal surface of the root which created a hydrostatic pressure of 1.5 kPa or 15 cm H_2O.[9] To assure an open system 22-gauge needle was used in the screw cap (Figure 1). The specimens were placed into an incubator at 37 °C temperature for the duration of observation periods.

A total of 150-μL of the solution was drawn from the glass bottle by using a micropipette at 7, 14, 21 and 28 days. The same amount of 0.2% NaN^3 was added to the glass bottle reservoir to maintain a constant volume of 3 ml. The samples were then analyzed with a Glucose kit (Flex, Siemens) in a spectrophotometer at a wavelength 340 nm. Concentration of glucose in the lower chamber was calculated in mg/ml.

Figure 1.
Glucose leakage model

Statistical analysis

Data were analyzed with the Kruskal-Wallis and the Mann-Whitney U tests. Differences between the groups were analyzed with the Kruskal-Wallis test and the Mann-Whitney U test was used to make pair-wise comparisons. We considered values of $P < 0.01$ as significant.

RESULTS

In the negative control group there was no detectable glucose in the glass bottle and the positive control group showed the excessive amount of glucose leakage for 4 weeks.

The mean (standard deviation [SD]) and median (range) of glucose leakage values for each group are shown in Table 2. The difference between the glucose concentration of the experimental groups were significant at week 1, 2, 3, and 4 (P<0.01) (Figure 2).

Table 2.

Mean (standard deviation [SD]) and Median (range) of glucose penetration (mg/ml) along the root canal filling

Group		Week			
		1	2	3	4
AH Plus	Mean (SD)	0,26 (0,08)	0,32 (0,09)	0,33 (0,05)	0,38 (0,06)
	Median (range)	0,28(0-0,36)	0,36(0-0,38)	0,34(0-0,43	0,36(0-0,52)
MetaSeal	Mean (SD)	0,42 (0,19)	0,84 (0,33)	0,90 (0,23)	0,62 (0,27)
	Median (range)	0,46(0-0,64)	0,85(0-1,73)	0,86(0-1,58)	0,68(0-0,93)
MM-Seal	Mean (SD)	0,29 (0,10)	0,34 (0,05)	0,40 (0,12)	0,30 (0,11)
	Median (range)	0,30(0-0,52)	0,35(0-0,43)	0,36(0-0,72)	0,29(0-0,53)

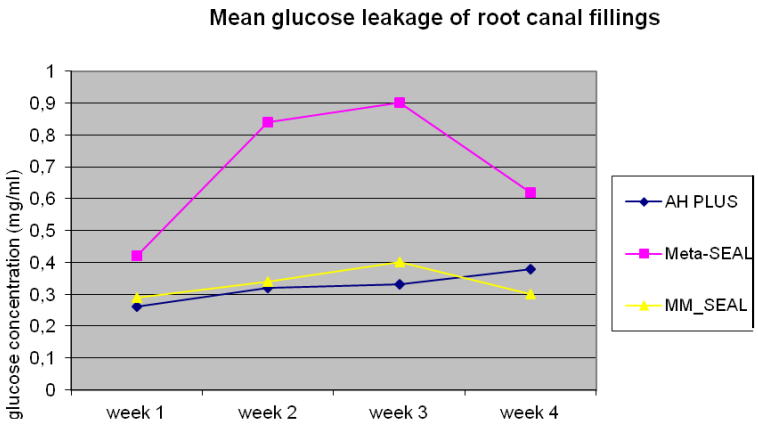

Figure 2.

Mean glucose leakage of root canal fillings per group over time

There were no significant differences in glucose penetration between the groups filled with AH Plus and MM-Seal during 3 weeks, apical sealing abilities of these materials were better than MetaSEALTM. At forth week, the glucose penetrations between the root canals filled with MetaSEALTM, AH Plus and MM-Seal were statistically different. MM-Seal showed less leakage than AH Plus and MetaSEALTM. The sealing ability of AH Plus was better than MetaSEALTM MetaSEALTM showed the greatest amount of glucose leakage during the test period (P<0.01).

DISCUSSION

Several in-vitro methods have been used to asses the sealing ability of root canal sealers, such as radiolabeled isotopes,[20] dye leakage,[21] bacterial penetration,[22] electromechanical tests[23] and fluid filtration.[24] Dye, radioisotope, fluid, bacteria and their products have been used as a tracer in leakage tests. A large variation of the result was produced with dye leakage method and to reproduce and compare this method is difficult.[9, 25] In bacterial penetration method to maintain aseptic conditions during the experiment was difficult,[9] antibacterial effects of the sealers might change the test results.[26] The fluid filtration method which is popular recently has no standardization.[9] The measurement time, the applied pressure, the diameter and the length of the bubble might influence the results.[27] In this present study glucose leakage method in which glucose solution was chosen as the tracer was used to evaluate the leakage of the sealers. Glucose solution was selected in this method because it has low molecular weight, is hydrophilic, chemically stable and an important nutrient for bacteria and biofilm. Xu *et al* stated that if glucose could leak into the root canal from the oral cavity, bacteria that might survive after root canal preparation and filling could proliferate and cause periapical inflammation. Therefore choice of the glucose solution was thought to be more clinically relevant than other tracers used in microleakage tests.[9] Shemesh *et al* reported that glucose test might be more sensitive than the measurement of the fluid transport. Although in glucose penetration method, glucose concentration is determined by a sensitive enzymatic reaction measured by the spectrophotometer, measurement of the bubble movement is carried out by eye in fluid filtration method.[28, 29] Furthermore glucose leakage method which is practical has an advantage that the specimens are not destroyed. The amount of glucose that leaked through the root canal filling could be determined with glucose oxidase method quantitatively.

Shemesh *et al* stated that glucose react with $Ca(OH)_2$ containing products. According to results of their study Portland cement, MTA, $Ca(OH)_2$ and sealer 26 reduced the concentration of glucose significantly after a week.[30] But there are a lot of studies used glucose leakage method with $Ca(OH)_2$ containing materials.[9, 31, 32] Xu *et al*[9] used Pulp Canal Sealer EWT, AH Plus and Sealapex with glucose leakage method. Bailón-Sánchez *et al*[31] used CavitTM G, Tetric EvoFlow and ProRoot TM MTA as intraorifice barriers and evaluated the sealing ability of these materials with glucose leakage method. They stated that they didn't find lower ProRoot TM MTA values with this method. Zou *et al*[32] used calcium sulphate barrier to repair the perforations and evaluated the sealing ability of resin based material with glucose leakage method. They reported that they did not take the reaction between MTA and glucose into consideration when the glucose leakage model was chosen in their study. This reaction may be affected by several factors, such as the concentration of the glucose solution, the volume of MTA, and the solubility of MTA.[33] In this study MM-Seal showed the same sealing ability with AH Plus during three weeks. At the forth week sealing ability of MM-Seal observed the lowest amount of leakage. That might be owing to the calcium oxide or calcium phosphate contents of MM-Seal.

The results of this present study showed that the sealing ability of MM-Seal is better than AH Plus and MetaSEALTM after a month when used with single-cone gutta-percha technique. Bodrumlu *et al* compared the sealing ability of resin based sealers after radiotherapy application.[8] They used AH Plus, AH 26 and MM-Seal sealers with lateral condensation technique. They immersed the samples in 5 % methylene blue dye and centrifuged them at 30 gauss for 4 minutes to measure the leakage. The results showed that MM-seal and AH Plus showed similar apical sealing abilities. Onay *et al* reported that apical leakage values of RealSeal and MM-Seal were higher than MetaSEALTM when used with resilon.[3] Sealing ability of MM-Seal and gutta-percha (Herofill) combination is superior to Hybrid Root Seal and Resilon combination.[3] The core material used with sealer might affects the test results. Resin based sealers (AH Plus and MM-Seal) show high bond strength to root dentin. They chemically bonds to root dentin by reacting with exposed amino groups in collagen to form covalent bonds between the resin and collagen.[34, 35]

The fourth generation methacrylate resin based sealer MetaSEALTM contains the combination of an etchant, a primer and a sealer into all-in-one. This self adhesive sealer reduces the treatment time. Manufacturers recommend the removal of the smear layer with EDTA as the final irrigation to reduce the leakage and improve the sealing ability. Kim *et al* reported that after applying EDTA to solve the smear layer, MetaSEALTM created a thin hybrid layer and penetrated dentinal tubules to produce filler-containing resin tags.[36] In our previous study, MetaSEALTM showed less leakage with vertical and cold lateral condensation techniques when compared with Thermafil and Ultrafil techniques.[37] Belli *et al*[5] evaluated the sealing ability of MetaSEALTM comparing with Real SEAL and AH Plus sealers at different time intervals with fluid filtration method. They used these sealers both resilon and tapered gutta-percha points. According to their findings; leakage of AH Plus was more than MetaSEALTM/Gutta Percha group at first week, MetaSEALTM showed statistically similar sealing ability with AH Plus and Real SEAL at 24 week. Leakage of MetaSEALTM / gutta-percha combination didn't change until 24th weeks. In the present study, the sealing ability of AH Plus was better than MetaSEALTM during the test period. The reason of these different test results may be leakage methods and time intervals. Hybrid Root Seal (MetaSEAL) shows less bonding strength than resin- and bioceramic-based sealers when used with gutta-percha.[38] Using Hybrid Root Seal (MetaSEAL) with traditional gutta-percha cones prevents the adhesive bonding between the core material and sealer.[38] Methacrylate based sealers show polymerization shrinkage inside the root canals which can cause to de-bonding of sealer.[39]

There are a lot of studies with different results shows the sealing ability of AH Plus.[6, 10, 40, 41] In this study AH Plus showed same sealing ability with MM-Seal until the fourth week. Zmener *et al*[40] compared the sealing abilities of AH Plus and AH 26 by dye penetration method. They stated that AH 26 less leaked than AH Plus at ten days. Yücel *et al*[10] showed that specimens filled with AH Plus exhibited same leakage values with AH 26 with bacterial penetration method after 60 days. Da Silva Neto *et al*[6] evaluated the sealing abilities of AH Plus, EndoREZ and AH 26 by fluid filtration method. They stated that AH 26 showed more leakage than AH Plus. In a study of Onay *et al*[41] AH Plus and gutta-percha combination demonstrated better sealing ability than AH Plus and Resilon combination.

CONCLUSION

There isn't enough study about the sealing abilities of MM-Seal and MetaSEALTM (Hybrid Root SEAL) when used with single-cone technique. Further investigations and long term evaluations should be done to determine the effectiveness of these new resin based sealers.

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