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The penetration of RealSeal primer and Tubliseal into root canal dentinal tubules: a confocal microscopic study

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Abstract

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Aim To compare penetration depth into dentinal tubules of RealSeal with that of a well-established endodontic sealer (Tubliseal) by means of confocal microscopy.

Methodology Twenty sound extracted, single-rooted premolars were selected. Following completion of root canal instrumentation, the teeth were divided into two groups using a stratified sampling method, ranking teeth according to size. In group 1, 10 teeth were filled with Gutta-percha and Tubliseal using cold lateral condensation. In group 2, 10 teeth were filled with RealSeal. Both sealers were labelled with Rhodamine B dye. The teeth were sectioned parallel to their long axis resulting in 20 specimens per group. Confocal microscopy was used to assess the penetration depths of the sealers at three sites for each specimen (coronal, middle and apical). Data were analysed statistically using Stata Release 9.1.

Results The penetration depth of RealSeal in each one of the thirds of the root canal was found to be higher than that of Tubliseal ($P < 0.05$). The penetration depths of the two sealers was found to be significantly different ($P = 0.001$). The mean penetration value for the RealSeal group was 908.8 μm whereas the mean value for the Tubliseal group was 139.5 μm.

Conclusions The penetration depth of RealSeal into the root dentinal tubules is significantly greater than that of Tubliseal.

Keywords: dentinal tubules, dentine bonding, root reinforcement.

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Introduction

Pulpal and endodontic problems are primarily related to microorganisms or their by-products in the root canal system (Kakehashi *et al.* 1965). The bacteria present in the infected root canal system are mainly obligate anaerobes with similar numbers of facultative anaerobes (Moller *et al.* 1981). This microflora goes on to form a complex biofilm, where penetration into the dentinal tubules may occur (Mannocci *et al.* 2003).

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Gutta-percha has been universally accepted as the 'gold standard' root filling material, and the material against which most others are compared. It is used in a number of forms in practice, with various filling techniques, and associated with different types of sealers.

Sealers are used to attain an impervious seal between the core material and root canal walls. They can be grouped according to their basic components, such as zinc oxide-eugenol, calcium hydroxide, resins, glass ionomers, iodoform or silicone (Gutmann & Witherspoon 2002). Ideally, these materials should seal the canal laterally and apically, and have good adaptation to root canal dentine (Grossman 1982). Even when associated with a sealer, Gutta-percha is

not capable of preventing leakage, and many laboratory studies confirmed the high leakage rate of Gutta-percha and sealer root fillings (Shipper & Trope 2004). Gutta-percha and sealers AH26 and AH Plus allowed leakage of bacteria and fungi (Miletic *et al.* 2002). Some sealers may adhere to the root canal walls (Timpawat *et al.* 2001), but they are unable to bond to the Gutta-percha core material. Upon setting, the sealer pulls away from the Gutta-percha core, leaving a gap through which bacteria may pass (Teixeira *et al.* 2004a). It would thus, be advantageous to replace conventional root canal fillings made with Gutta-percha and sealer by sealers, and core materials with adhesive properties.

The ability of root canal filling materials to penetrate into the dentinal tubules is regarded as a relevant aspect in the prevention of the reinfection of the dentinal tubules and of the root canal itself (Weis *et al.* 2004). New synthetic root canal filling materials, such as Resilon (Resilon Research LLC, Madison, CT, USA) and Epiphany (Pentron Clinical Technologies, Wallingford, CT, USA) based on polymers of polyesters have recently been introduced in the market. Epiphany sealer, when used with the Resilon filling material, forms a bond to the dentine wall and the core material making the filling resistant to bacterial penetration (Shipper & Trope 2004).

Bonding of endodontic sealers may also enhance the fracture resistance of root-filled teeth, for example the use of adhesive sealers has been suggested to reinforce roots (Johnson *et al.* 2000). Teixeira *et al.* (2004b) showed that root canals filled with the Resilon system were more resistant to fracture than roots filled with Gutta-percha and AH26 sealer. It was reported that Epiphany primer conditions the root canal dentinal surface and Epiphany sealer bonds both to the primer and Resilon cones forming a 'monoblock' that has good adaptation (Teixeira *et al.* 2004a). This property is referred to as the Resilon 'Monoblock' system.

The mean penetration into the dentinal tubules of Resilon–Epiphany and conventional sealers has never been compared previously.

A root filling materials, based on polymers of polyester, very similar to Resilon–Epiphany (RealSeal) is available in the market.

The aim of this study was to compare, by means of confocal microscopy, the penetration depth into the dentinal tubules of RealSeal with that of a well-established endodontic sealer (Tubliseal) used in combination with lateral condensation of Gutta-percha.

Materials and methods

Twenty recently extracted, sound human, single-rooted premolars were used (Guy's & St Thomas Hospital Ethical Committee approval 04/Q0704/57). The crowns were removed at the amelo-cemental junction and the pulps extirpated with broaches. A size 15 K-Flexofile was then inserted into the root canal until it was seen at the apical foramen. One millimetre was subtracted from this length to determine the working length. After coronal flaring with Gates-Glidden, the apical preparation was performed with K-flexofiles to a size 20. Instrumentation was completed with Rotary ProTaper files (Dentsply Maillefer, Ballaigues, Switzerland) in a crown-down technique. The F2 ProTaper was taken to length in each of the canals. Irrigation with 1% sodium hypochlorite and patency filing were carried out between each instrument. A rinse was then performed using 5 mL of 17% EDTA, using a size 30 endodontic needle inserted approximately three/quarters into the root canal. A final rinse with water was undertaken. After completion of instrumentation, the teeth were divided into two experimental groups. Teeth were ranked according to size and distributed into the two groups using a stratified sampling method. In this way the experimental groups contained teeth of approximately the same distribution of canal sizes.

Group 1

Ten root canals were filled with Gutta-percha (SPI Dental Mfg, Inchon, Korea) and Tubliseal (Kerr, Scafati, Italy) using cold lateral condensation technique with finger-spreaders. Tubliseal was mixed according to the manufacturer's instructions and placed into the root canal with a lentulo spiral filler.

Group 2

Ten root canals were filled with RealSeal (SybronEndo, Orange, CA, USA). RealSeal primer was inserted into the root canals, excess primer was removed with a paper point and the RealSeal sealant was placed with a lentulo. A master Real Seal cone was placed into the root canal and cold lateral condensation was applied using accessory RealSeal cones.

Both Tubliseal and RealSeal primer were labelled with a few grains of Rhodamine B dye (Sigma-Aldrich, St Louis State, MO, USA).

The filling materials were allowed to set for 48 h. A slow-speed, water-cooled diamond impregnated saw

(Labcut, Extec, Enfield, UK) was then used to section the teeth parallel to their long axis, producing two specimens per tooth.

In each half three notches were made, by a scalpel, 2, 5 and 8 mm apically to the dentine–enamel junction. Observations were performed using confocal microscopy of the dentine–resin-interface of the areas immediately apical and immediately coronal to the notches, and either side of the canal, so that six areas were observed in each root half.

The specimens were examined using a Tandem scanning confocal microscope (Noran Instruments, Middleton, WI, USA) and an oil-immersion lens (×20 NA 0.8; Olympus UK, London, UK), the deepest penetration of the sealer into the dentinal tubules, was measured in μm and was recorded for each one of the thirds (coronal, middle, apical) of each of the samples using the appropriate filters (546 nm excitation; 600 nm barrier).

The null hypothesis was that there was no difference in the penetration depths of RealSeal primer and Tubliseal into the root canal dentinal tubules.

Data were statistically analysed using Stata Release 9.1 (StataCorp, College Station, TX, USA). Significance was pre-determined at $\alpha = 0.05$.

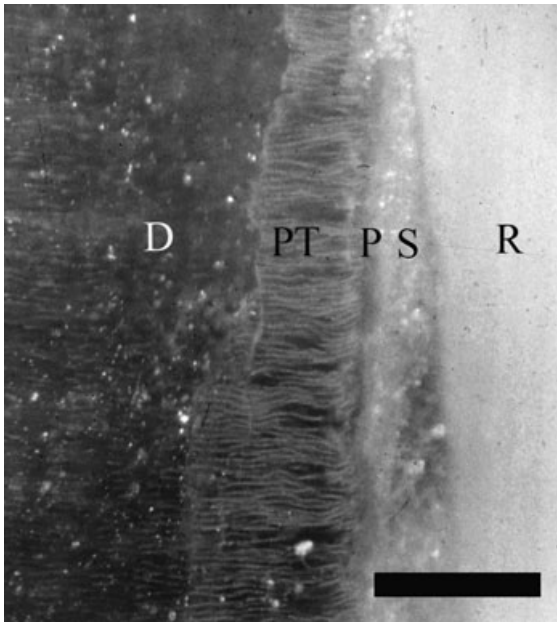

Figure 1 Confocal microscopic image of a specimen filled with RealSeal: extensive primer infiltration of the dentine (bar, 100 μm; D, dentine; PT, primer tags; P, primer layer; S, RealSeal sealant; R, RealSeal core filling material; Reflection image, ×20 NA 0.8 oil immersion lens).

Results

Initial analysis showed no significant effect of the side of the tooth and so the data were pooled. The penetration depths of the two experimental groups are reported in Table 1. As some of the data sets were not normally distributed, the Wilcoxon rank-sum test was used to compare the effect of sealer on the penetration in the different sections. The *P* value associated to the Wilcoxon rank-sum test was of 0.001 in the coronal, middle and apical third of the roots.

In each one of the thirds of the root canal the penetration of RealSeal sealer was found to be higher than that of Tubliseal ($P < 0.05$). Representative pictures of the two experimental groups are shown in Figs 1 and 2.

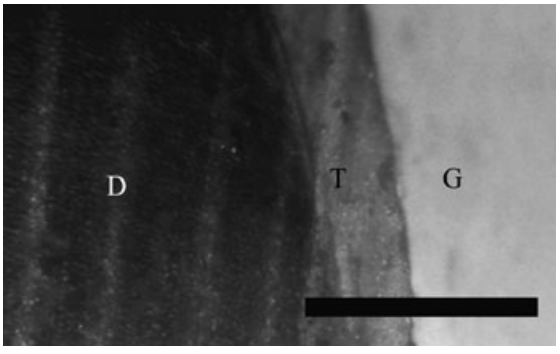

Figure 2 Specimen filled with Tubliseal: no infiltration of root dentine is visible (bar, 100 μm; D, dentine; T, Tubliseal; G, gutta-percha; Reflection image, ×20 NA 0.8 oil immersion lens).

Table 1 Penetration depths of the two experimental groups

	Tubliseal			RealSeal		
Location of the observed sections	Sample size	Mean penetration (μm)	SD	Sample size	Mean penetration (μm)	SD
Coronal	40	190.88	78.07	40	1114.88	291.19
Middle	40	142.25	79.26	40	914.88	229.70
Apical	40	85.50	62.55	40	696.75	313.55
Combined	120	139.54	84.90	120	908.83	326.65

The combined data of the three-thirds of the roots also showed that the penetration values of RealSeal sealer were higher than those of Tubliseal ($P < 0.05$). The mean penetration value for the RealSeal group was 908.8 μm whereas the mean value for the Tubliseal group was 139.5 μm.

Discussion

Confocal microscopy allowed visualization of the sealers within the dentinal tubules without resorting to specimen preparation techniques that would have caused artefacts.

In a pilot test conducted prior to the investigation the penetration of the two root filling materials not labelled with Rhodamine was found to be similar to that of the Rhodamine-labelled sealers. The possibility of false results due to leaching of Rhodamine from the sealers was therefore excluded.

The null hypothesis that there was no difference in the penetration depths of RealSeal primer and Tubliseal into the root canal dentinal tubules was to be rejected. The mean penetration depth of RealSeal sealer into the dentinal tubules was just over 6.5 times greater than the penetration depth of Tubliseal. This greater penetration ability seen with RealSeal sealer may have implications in the final sealing ability of the root canal system with the RealSeal system.

The difference between these two systems is that with RealSeal bonding of the root filling to the root canal walls occurs rather than cementing Gutta-percha points into the root canal. The RealSeal self-etching primer is applied to the dentine walls of the root canals. This is followed by application of RealSeal Sealer, a dual curable, resin-based composite sealer. The penetration into the dentinal tubules of the self-etching primer and composite sealer may prevent shrinkage of the resin filling away from the dentine wall and aid in sealing the roots (Shipper *et al.* 2005). The RealSeal system includes a primer to enhance bonding of the dual curable resin to the dentinal walls and then the sealer bonds to the fully polymerized core material. Tubliseal, on the other hand, does not have this property, hence its increased rate of leakage (Barnett & Trope 2004). Studies by Tay *et al.* (2005), however, have shown that polyester-based root filling materials are susceptible to alkaline hydrolysis, and that enzymatic hydrolysis may occur. This has implications with regard to the biodegradability of RealSeal, suggesting that salivary enzymes and endodontic bacteria may be able to degrade the material and consequently cause re-infection of the root canal system. Further investigations are required to investigate the biodegradation of RealSeal using cultures of endodontic bacteria and human saliva, and also to evaluate the effect of the biodegradability of the material on the strengthening effect of root fillings made with Real Seal. The latest effect has not been demonstrated in clinical studies. A push-out test was performed on thin slices of dental roots filled with either Gutta-percha and sealer or Resilon (Gesi *et al.* 2005). It was found that the fillings performed with Resilon–Epiphany were more easily pushed out of the root slices than the fillings made with Gutta-percha and sealer. The authors concluded that these results challenged the concept of strengthening of the roots obtained with the root fillings made using Epiphany–RealSeal. The 'monoblock concept' on the other hand might not be the only explanation for this potential root strengthening. The removal of the smear layer from the root canal walls is regarded as an essential step of root canal treatment, as it helps in detaching bacterial biofilms from the root canal walls (Torabinejad *et al.* 2002). Once the biofilm is disrupted and the bacteria are floating in the root canal they are more easily killed and flushed away from the root canals by antibacterial agents, such as sodium hypochlorite and chlorhexidine. The removal of the smear layer from the root canal walls is routinely performed irrespectively of the type of root filling material used and results in the opening of the dentinal tubules. In the present study, RealSeal was able to infiltrate the dentinal tubules for almost 1 mm. This ability might in itself contribute to the root strengthening effect of the RealSeal root canal fillings, by simply filling the void spaces created in the dentinal tubules by the etching effect of EDTA. Further laboratory investigations are needed to prove the correlation between root strengthening and penetration into the dentinal tubules of resin-based root fillings.

Conclusions

There was a clear difference in the penetration depths into root dentinal tubules between RealSeal sealer and Tubliseal; RealSeal sealer had a greater penetrative depth than Tubliseal.

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