Longitudinal study on microleakage of three root-end filling materials by the fluid transport method and by capillary flow porometry

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Abstract

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Aim (i) To compare the root-end sealing ability of IRM Caps (IRM), Fuji IX Capsules (Fuji IX) and Pro Root MTA Tooth-Coloured Formula (MTA) in teeth obtained from cadavers. (ii) Further research on leakage study methodology by means of comparison of the fluid transport method (FTM) and capillary flow porometry (CFP).

Methodology Root canal treatment was performed on 33 cadaver teeth *in situ* 2 weeks prior to root resection and ultrasonic retropreparation (S12/90°D-tip on Suni-Max), after which the teeth were retrieved from the cadavers. Two teeth were kept as positive and negative controls. The other teeth were divided in three different groups at random, with each group receiving one of the retrofill materials. Retrofills were exposed to water 5 min after placement. The teeth were stored at 37 °C for 12 h after which the root filling was removed. Microleakage (L in μL day^{-1}) was measured for 24 h under a pressure of 1.2 atm using FTM and recorded as $L = 0$, $0 < L \leq 10$, $L > 10$. The measurements were repeated after 1 and 6 months. After 6 months, leakage was also assessed by CFP in order to measure through pores and their diameters. Results were analysed statistically using nonparametric Kruskal–Wallis and Mann–Whitney *U*-tests, and Spearman correlation coefficients between the results of both methods were calculated. The level of significance was set at 0.05.

Results (i) A statistically significant difference could be demonstrated between Fuji IX and IRM at 1 month with FTM. FTM revealed a significant difference between Fuji IX and the other materials at 6 months, whereas CFP did not. However, using both methods, Fuji IX showed the best result. (ii) When comparing both techniques, CFP demonstrated through pores in all teeth, whereas with FTM in only 14 of the 31 teeth could through pores be demonstrated. A positive correlation between both methods was demonstrated.

Conclusions Under the conditions of this study (i) the conventionally setting glass–ionomer cement Fuji IX showed the best results when used as a root-end material and (ii) CFP appeared to be a useful method for leakage evaluation of through pores in endodontics.

Keywords: capillary flow porometry, fluid transport method, Fuji IX, IRM, MTA, retrofill.

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Introduction

Periradicular surgery includes surgical debridement of pathological periradicular tissue, root-end resection, root-end preparation and the placement of a root-end filling to seal the root canal (Gutmann & Harrison 1994). An ideal root-end filling material would adhere and adapt to the walls of the root-end preparation,

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prevent leakage of microorganisms and their toxins into the periradicular tissues, be biocompatible, be insoluble in tissue fluids and dimensionally stable, and remain unaffected by the presence of moisture (Arens *et al.* 1998).

The objective of placing a root-end filling is to produce a fluid-tight seal that prevents residual irritants and oral contaminants from exiting the root canal system and entering the periradicular tissues (Arens *et al.* 1998). From the literature it appears that some materials may already have proven their clinical efficiency as root-end filling materials but that additional research is needed (Niederman & Theodosopoulou 2003): from the review by Niederman & Theodosopoulou (2003) on *in vivo* root-end obturation materials, it appeared that there was sufficient evidence for the use of amalgam, but additional validating randomized and nonrandomized controlled clinical trials were needed for all other materials. There are few of these studies on glass–ionomer cements (Niederman & Theodosopoulou 2003) and apparently only one on IRM and MTA (Chong *et al.* 2003). IRM was used more frequently in the past but has partly been replaced in favour of other materials, including MTA and glass–ionomer cements. Glass–ionomers already have proved their use in endodontic surgery (De Bruyne & De Moor 2004) but additional research is appropriate.

It is generally accepted that the most fluid-tight apical seal possible is required for successful periapical healing (Hirsch *et al.* 1979). When the seal is not fluid-tight, microleakage may occur. Leakage of various root-end filling materials has widely been investigated mainly by a dye penetration method. Disadvantages of the linear measurement of dye penetration are the destruction of the specimen for evaluation and the lack of reproducible and comparable results (Wu & Wesselink 1993).

Different leakage patterns can be investigated: the fluid transport method first mentioned by Greenhill & Pashley (1981) and adapted by Wu *et al.* (1993) particularly investigates through-and-through voids (Wu *et al.* 2003). With the dye penetration method on the other hand, both coronal and apical leakage are considered when investigating a root canal filling.

An important advantage of the fluid transport model is the nondestructive character of the method, whereas for the tracer methods samples are destroyed after measurement. This advantage ensures the possibility to measure the same samples at different points in time. Fluid transport values indicate mainly the diameter of the void, rather than the length of the void only, as for the dye penetration method (Wu *et al.* 2003).

Capillary flow porometry, which is a well-established method in other domains than dentistry, for example in membrane and filter media testing, measures through pores (Jena & Gupta 2002), i.e. pore size distribution. This method, next to an indication on leakage, also provides information on the pore sizes and pore distribution.

Therefore, the aim of this study was to compare the sealing ability of the reinforced glass–ionomer cement Fuji IX Capsules (GC-Corporation, Tokyo, Japan) (Fuji IX) and Pro Root MTA Tooth-Coloured Formula (Dentsply Tulsa, Tulsa, OK, USA) (MTA) to IRM Caps (Dentsply Caulk, Milford, DE, USA) (IRM) as a reference.

A second aim was further research on leakage study methodology, as suggested by Wu & Wesselink (Wu & Wesselink 1993): a comparison between the well-established fluid transport model and the capillary flow porometry was made. The latter method apparently has not yet been evaluated in both restorative and endodontic leakage research.

To mimic the clinical situation as closely as possible, teeth obtained from cadavers were used in the study. Root canal preparation and filling and endodontic surgery were performed *in situ*.

Materials and methods

Twelve different maxillar and mandibular jaws were retrieved from human cadavers. After diagnostic X-rays were made, 34 single-rooted anterior and premolar teeth with no loss of periodontal ligament were selected.

Root canal treatment

Endodontic access cavities were prepared with a high-speed fissure bur and water spray. After gross removal of pulp tissues, a size 15 Flexofile (Dentsply Maillefer, Ballaigues, Switzerland) was introduced into the canal and a length determination X-ray was made. The working length was determined by subtracting 1 mm from the radiographic length. The root canals were prepared by means of a crown-down/step-back technique (De Moor & De Boever 2000, De Moor & Hommez 2002, De Moor & De Bruyne 2004). The coronal half of the root canals was preflared with Gates Glidden drills (Dentsply Maillefer) in a larger to smaller sequence (numbers 4–3–2) and the canals were copiously irrigated with 2.5% sodium hypochlorite solution with a 27-gauge endodontic needle (Monoject; Sherwood Medical, St Louis, MO, USA). Smear layer was removed using File-Eze (Ultradent Products Inc., South Jordan, UT, USA) during canal preparation. The apical half of the canal was then prepared with the stepback technique. The master file varied between size 30 and 45. The canals were dried with paper points and a standard size gutta-percha cone (Dentsply Maillefer) that matched the master apical file was fitted to the working length with tug back. AH26 sealer (Dentsply De Trey, Konstanz, Germany) was mixed according to the manufacturer's instructions and placed in the canal. The master cone was coated with AH26 and gently seated at the working length. Lateral condensation was then carried out using size 15 and 20 accessory gutta-percha cones with endodontic finger spreaders (Dentsply Maillefer). Following obturation, the gutta-percha was removed from the coronal cavity up to the level of the cemento-enamel junction with a warm instrument (PK Thomas Waxing Instrument, N° PKT-2; Hu Friedy, Chicago, IL, USA) and vertically condensed with Machtou pluggers (Dentsply Maillefer). The access cavities were filled with Ketac-Fil (Espe, Seefeld, Germany) after which the teeth were left undisturbed for at least 48 h until complete set of the sealer.

Root-end procedures

After this period, a full-thickness flap was raised and the entire buccal gingival tissues were excised. Apical access was achieved with a round bur (Komet ISO 500 204-001 001 023; Brasseler GmbH & Co., Lemgo, Germany) in a slow-speed handpiece with water cooling and the apical 3 mm of the exposed roots was resected perpendicular to the long axis of the tooth with a tapered diamond bur in a high-speed handpiece with water cooling (Komet ISO 806 314-199 514 014). Apical root-end cavities were prepared with the S12/90°D tip which is diamond coated (Satelec, Merignac, France) and the Suni-Max ultrasonic unit (Satelec) at the intensity prescribed by the manufacturer (power 7 at power mode S). All root-end cavities were prepared by one operator using a feather-like back and forth motion with slight coronal pressure and water-cooling. In order to standardize the dimension of the retropreparation, the length of the retrotip (3 mm) determined the depth of the preparation and the final diameter was determined by the radius of the tip of the retrofilling instrument (0.85 mm, PLGRF 1, Hu Friedy). The prepared teeth were then carefully retrieved from the jaws at which time one tooth was lost. Two teeth were kept as a positive (no root-end filling) and a negative control and the other teeth were divided in three different groups at random. The retropreparations were dried with paper points and each group was retrofilled with a different material: IRM Caps (Dentsply Caulk, Milford, DE, USA) (IRM) ($n = 10$) (reinforced zinc oxide-eugenol cement), Fuji IX Capsules (GC-Corporation, Tokyo, Japan) (Fuji IX) ($n = 10$) (reinforced glass–ionomer cement) or Pro Root MTA Tooth-Coloured Formula (Dentsply Tulsa, Tulsa, OK, USA) (MTA) ($n = 11$) (mineral trioxide aggregate). After finishing of each root-end filling the material was allowed to set for 5 min. The teeth were then stored at 37 °C for 12 h in water, after which the Ketac-Fil coronal filling and the root canal fillings were removed from the teeth using magnification, except for the negative control. Two layers of nail polish were applied to the external surface of all roots, except for the canal orifice and apical foramen, and allowed to dry. For the negative control the canal orifice and apical foramen were also covered with nail polish and sticky wax.

Evaluation

Fluid transport method

The roots were attached to a fluid transport device, as described by Wu *et al.* (1993). The roots were connected to a plastic tube with Al-Fix Gel (Novatio Belgium n.v., Olen, Belgium) on both sides and additionally sealed with Quick-Bond (Novatio). The plastic tubes on either side of the specimen were filled with distilled water. A standard glass capillary tube was connected to the plastic tube at the outlet side of the specimen. Using a syringe, water was sucked back into the open end of the glass capillary and an air bubble was created. A headspace pressure of 1.2 bar (17 PSI) from the inlet side, which was the coronal side of the tooth, was applied to force the water through the voids along the filling, thus displacing the air bubble in the capillary tube. Microleakage (L in μL day^{-1}) was measured for 24 h and recorded as no leakage $L = 0$, limited leakage $0 < L \leq 10$ and gross leakage $L > 10$. The volume of the fluid transport was measured by observing the movement of the air bubble. The displacement of the air bubble was recorded every 15 min during the first 6 h, at 12 and at 24 h. The samples were removed from the fluid transport device and stored in distilled water and after 1 and 6 months measuring was repeated.

Capillary flow porometry

Apart from the fluid transport method, a second testing method was used after 6 months. Capillary flow porometry (CFP – 1200 – A, PMI, New York, USA) provides fully automated through pores analysis including bubble point pressure, pore size distribution and mean pore size.

A wetting liquid (Galwick, PMI, New York, NY, USA) was applied to all samples to fill the pores and the fully wetted teeth were attached in the sample chamber (Tubepack, Legris connectic, France), with adhesive epoxy (Loctite 3430; Loctite, Kontich, Belgium), after which the sample chamber was sealed. Gas was then allowed to flow into the chamber behind the sample. When the pressure reaches a point that can overcome the capillary action of the fluid within a pore (maximum pore), the equivalent bubble point pressure has been found. After determination of the bubble point pressure, the pressure is increased and the flow is measured until all pores are empty, and the sample is considered dry (Fig. 1). Pressure ranges from 0 to 200 PSI and the pore size range that can be measured lies between 0.035 and 500 microns. The technique is, to our knowledge, used in dentistry for the first time, but has been used in industries worldwide ranging from membrane filtration to nonwovens to battery industries for R & D and quality control. The method has been approved by the American Society of Testing and Materials (ASTM Designation 1999). Measurements were performed at VITO (Vlaamse Instelling voor Technologisch Onderzoek, Mol, Belgium).

Voids responsible for leakage are supposed to be present between the root canal filling or root-end filling and the root itself. To check whether no voids were present within the retrofill materials, four 7-mm diameter discs of each retrofill material were created and submitted to the capillary flow porometry. The height of the discs was 3 mm matching the height of a retrofill in the root-end cavity.

Statistical analysis

Results from both methods were analysed statistically using nonparametric tests: comparisons between the leakage results of the different materials at 24 h, 1 and 6 months were made with Kruskal–Wallis and Mann–Whitney *U*-tests. Spearman correlation coefficients between the results of both methods at 6 months were calculated. The level of significance was set at 0.05.

Results

The leakage of the three materials at the different time intervals and with the different methods is shown in Tables 1–4.

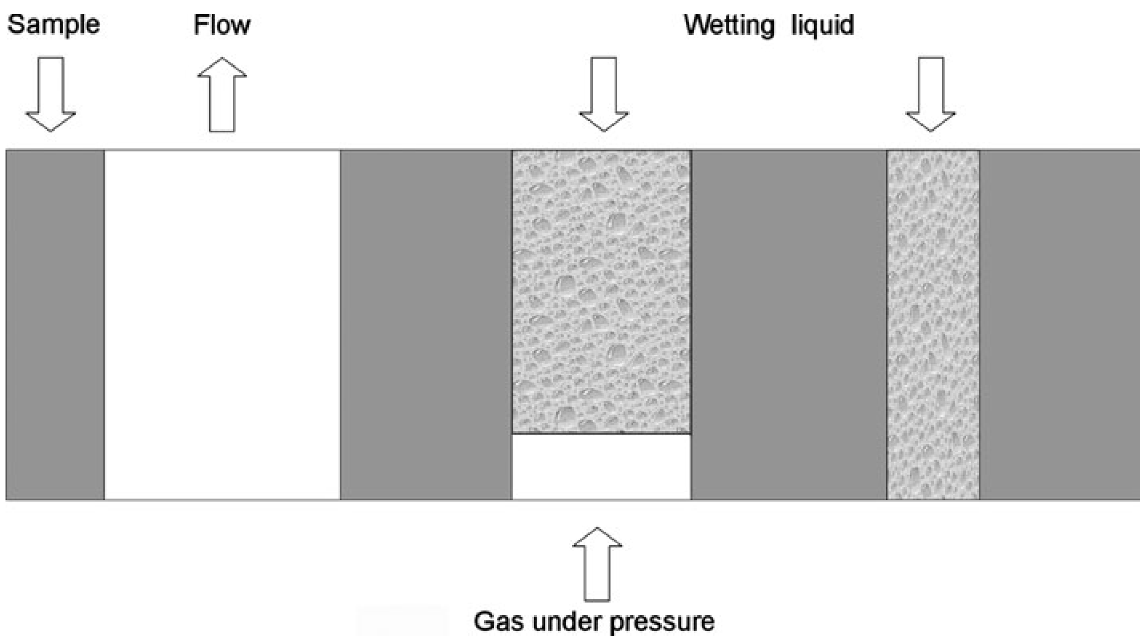

Figure 1 Principle of capillary flow porometry.

Table 1 24 h-results of fluid transport (L in $\mu L\ day^{-1}$)

	Number of samples			
Materials	$L = 0$	$0 < L \leq 10$	$L > 10$	Total
IRM Caps	4	0	6	10
Fuji IX Capsules	7	1	2	10
Pro Root MTA	6	1	4	11

Table 2 One-month-results of fluid transport (L in $\mu L\ day^{-1}$)

	Number of samples			
Materials	$L = 0$	$0 < L \leq 10$	$L > 10$	Total
IRM Caps	6	1	3	10
Fuji IX Capsules	10	0	0	10
Pro Root MTA	10	1	0	11

Table 3 Six-month-results of fluid transport (L in $\mu L\ day^{-1}$)

	Number of samples			
Materials	$L = 0$	$0 < L \leq 10$	$L > 10$	Total
IRM Caps	3	2	5	10
Fuji IX Capsules	9	0	1	10
Pro Root MTA	5	1	5	11

Fluid transport method

At each time interval, no leakage was detected for the negative control, whereas in the positive control, immediate leakage was observed.

The results of the fluid transport method at 24 h are summarized in Table 1. Fuji IX Capsules leaked less than Pro Root MTA which leaked less than IRM Caps, but no statistically significant differences could be demonstrated.

The results of the fluid transport method at 1 month are summarized in Table 2. After 1 month leakage decreased. Statistical analysis by Kruskal–Wallis revealed a significant difference between the groups ($P = 0.035$) and from the two by two Mann–Whitney U analysis a significant difference between IRM and Fuji IX appeared ($P = 0.03$). No statistical difference between the other materials was observed.

The results of the fluid transport method at 6 months are summarized in Table 3. After 6 months leakage had increased. Statistical analysis by Kruskal–Wallis revealed a significant difference between the groups ($P = 0.036$) and from the two by two Mann–Whitney U analysis a significant difference between IRM and Fuji IX ($P = 0.012$) and between MTA and Fuji IX ($P = 0.041$) appeared. No statistical difference between IRM and MTA was observed.

Capillary flow porometry

Determination of through pores in the retrofill material discs

The discs created in the different retrofill materials showed no leakage up till 9 bar (130 PSI), corresponding with no through pores of 0.07 μm or larger; this indicates the absence of voids within the materials.

Table 4 Six-month-results of fluid transport (L in $\mu L\ day^{-1}$) and capillary flow porometry [minimal (=Min), mean (=Mean) and maximal (=Max) pore diameter in μm] per individual sample (S)

IRM					Fuji IX					MTA				
		CFP					CFP					CFP		
ID No.	FTM (L)	Min	Mean	Max	ID No.	FTM (L)	Min	Mean	Max	ID No.	FTM (L)	Min	Mean	Max
Sample leakage results														
S1	0	0.21	0.23	0.26	S11	0	0.07	0.19	0.27	S21	0	0.14	0.19	0.26
S2	$0 < L \leq 10$	0.16	0.23	0.27	S12	0	0.20	0.22	0.39	S22	>10	0.25	0.27	0.46
S3	>10	0.17	0.21	0.45	S13	>10	0.16	1.52	1.54	S23	0	0.17	0.23	0.27
S4	>10	0.17	0.23	0.26	S14	0	0.17	0.22	0.27	S24	$0 < L \leq 10$	0.18	0.22	0.27
S5	>10	0.29	0.71	0.77	S15	0	0.15	0.18	0.27	S25	>10	0.26	0.36	3.91
S6	$0 < L \leq 10$	0.13	0.21	0.27	S16	0	0.46	0.46	4.87	S26	0	0.22	0.23	0.27
S7	>10	0.24	0.34	5.40	S17	0	0.30	0.35	0.50	S27	>10	0.16	1.86	3.04
S8	>10	0.46	0.46	0.46	S18	0	0.16	0.18	0.27	S28	>10	0.50	1.94	3.75
S9	0	0.07	0.27	0.46	S19	0	0.17	0.22	0.27	S29	0	0.21	0.23	0.27
S10	0	4.09	4.43	4.54	S20	0	0.08	0.18	0.27	S30	0	0.16	0.23	0.27
										S31	>10	1.93	20.27	21.50

ID No., identification number; FTM, fluid transport method; CFP, capillary flow porometry.

Determination of through pores in the retrofilled roots

From the capillary flow porometry results of the teeth at 6 months (Table 4) the median of the minimal, mean and maximal pore diameters of Fuji IX was smallest, but no statistical differences could be observed between the three root-end materials.

For each sample a measurement was obtained, confirming the presence of through pores, which was not the case for the fluid transport model where 'no leakage' was observed for 17 samples at 6 months.

Correlation between the fluid transport method and capillary flow porometry

Correlation between the 6 months measurements of the fluid transport method and the capillary flow porometry was calculated. A statistically significant positive correlation was found between the fluid transport measurements and the minimal (Spearman correlation coefficient 0.384, $P < 0.05$), mean (Spearman correlation coefficient 0.621, $P < 0.001$) and maximal (Spearman correlation coefficient 0.617, $P < 0.001$) pore diameters.

Discussion

To mimic the clinical situation as closely as possible, this study was performed on jaws obtained from human cadavers and root canals were first treated *in situ*. Because of the limited number and also to simplify the study, root-end preparation was restricted to teeth with single canals. After the root-end procedures, the teeth were carefully retrieved from the jaws so that cracks in the roots due to extraction procedures could not influence the leakage data. In this respect all teeth were examined for cracks with the microscope (magnification 6×).

An advantage of the fluid transport model in leakage research is the nondestructive character of the method: in this way the quality of the seal can be observed as a function of time. From the study of Wu *et al.* (2003) it appeared that a high value for fluid movement indicates the existence of a wide through-and-through void whilst a low-value fluid movement indicates the existence of a narrow through-and-through void, the results mainly indicating the diameter of the void rather than its length. They concluded that fluid movement suggested pathways between the coronal and apical end of a root filling, i.e. through-and-through voids, rather than apical cul-de-sac-type of voids.

Nevertheless, the fluid transport model has some disadvantages. After working with the system, it appeared that the method is time-consuming (set-up and measuring), technique-sensitive, and, if not automatized, the precision of the measurements is dependent on the precision of the human eye. Apart from this, no exact data on the size of the pores can be obtained (Wu *et al.* 2003).

In capillary flow porometry the through-and-through voids are described as through pores, permitting fluid flow through the sample. Apical cul-de-sac-type of voids are described as blind pores, pores terminating within the sample. Apart from the former types of pores also closed pores can be present, which are not accessible voids within a sample (Jena & Gupta 2002).

The aim of the capillary flow porometry testing in this study was similar to that of the fluid transport model testing. Both methodologies allow for the measurement of through pores in a nondestructive way. In the former technique, a wetting liquid with a low surface tension (Galwick: 15.9 Dynes cm^{-1}) is used to fill the pores of the sample. Pressure of a nonreacting gas on the sample is increased to remove the wetting liquid from the pores and permit gas flow. The largest pores are emptied first and higher pressures are required to empty smaller pores. The flow meters detect the presence of pores by sensing increase in flow rate at a given applied differential pressure due to emptying of pores. Differential pressures and flow rates through wet and dry samples are measured. These measured differential pressures and flow rates are used to evaluate the most constricted through-pore diameters, the largest pore diameter, the mean flow pore diameter and the pore size distribution. Measurement of the volume of displaced liquid due to application of differential pressure on excess liquid maintained on the sample allows computation of liquid permeability. The pore diameter (D) can be derived from the following equation: $D = 4\gamma \cos \theta / p$ (γ = surface tension of the wetting liquid, θ = contact angle of the wetting liquid, p = differential pressure required to displace the wetting liquid from the pore) (Jena & Gupta 2003). Leakage testing based on flow porometry can therefore give highly reproducible and accurate data (Gupta *et al.* 1999, Mayer 2002). More extensive and accurate information is obtained than with the fluid transport model. As described by Pommel & Camps (2001), measurement time and pressure influence the outcome of a fluid filtration test. Several measurement times and pressures have been used with the fluid transport model and the authors emphasized the need for standardization to compare the results from various studies. Capillary flow porometry, which is a standardized method, as such overcomes these problems and provides reproducible data (Gupta *et al.* 1999, Mayer 2002).

The results from this study showed that, although not always significant, Fuji IX seemed to perform better than IRM and MTA over time. What is more, Fuji IX performed most consistently of the three materials over the 6 months. According to Wu *et al.* (1998) Fuji II glass–ionomer cement showed a change in leakage during the first 6 months and no more change afterwards. Measurements at 6 months should as such give an indication about future leakage.

From former fluid transport studies it appeared that MTA (non-Tooth-Coloured formulation) performed better than amalgam which has been used frequently in the past (Bates *et al.* 1996, Wu *et al.* 1998, Yatsushiro *et al.* 1998, Fogel & Peikoff 2001), equal to IRM (Fogel & Peikoff 2001), and equal (Bates *et al.* 1996, Fogel & Peikoff 2001) or superior to Super-EBA (Wu *et al.* 1998) which next to IRM can be considered as a reference in endodontic surgery (Dorn & Gartner 1990). IRM performed equal to or inferior than Super-EBA (Sullivan *et al.* 1999, Greer *et al.* 2001). One study (Wu *et al.* 1998) compared two glass–ionomer cements, i.e. Fuji II and Hi Dense, Tytin amalgam, Super-EBA and MTA (non-Tooth-Coloured formulation). Both glass–ionomer cements and MTA showed less leakage than the conventional amalgam and Super-EBA. These findings were confirmed by the present findings that particularly Fuji IX glass–ionomer and also Tooth-Coloured MTA on the whole performed better than IRM.

The absence of measurements for the separate discs of each retrofill material, confirmed the leakage pattern along the root-end filling and the absence of voids within the material.

With capillary flow porometry measurements were obtained for each sample (31/31), which was not the case with the fluid transport model (14/31): this was an indication for more accurate measurements. The missed measurements concerned pores with small diameters as well as larger pores (Table 4). These results emphasize that more research is needed in order to determine the minimal pressure to measure all voids when using the fluid transport model. The average length of bacteria varies between 0.2 and more than 10 μm, the width between 0.2 and 1.5 μm (Hobot 2002); toxins are even smaller. From Table 4 it can be derived that several fillings which, according to the fluid transport model, do not show leakage, actually could be passed by bacteria or their toxins.

The correlation coefficients between the results at 6 months of both testing materials indicate that the highest correlation existed between the fluid flow over 24 h and the mean and maximum pore diameter, correlation with the minimum pore diameter was apparently lower.

Further research has to indicate whether using higher pressures with the fluid transport method results in a higher correlation between its results and the minimum pore diameter provided by the capillary flow porometry.

Conclusions

Under the conditions of this study it can be concluded that, in spite of the critical handling characteristics, conventionally setting glass–ionomer cements were suitable as a retrofill material and performed at least equal to MTA. More extensive use as a retrofill material in endodontic surgery might be considered, also because of their capacity to bond to dentine, their biocompatibility and antimicrobial activity. Apart from this, compared with MTA, the cost–benefit analysis is in favour of glass–ionomer cements.

It also can be concluded that the capillary flow porometry appeared to be a useful method for leakage evaluation of through-and-through voids or through pores in endodontics. As it provided more extensive and accurate information and seemed to be less technique-sensitive than the fluid transport model, future research with this method is recommended.

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