ORIGINAL RESEARCH

Sealing Capacity of Bulk-fill Resin in Endodontically Treated Teeth

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ABSTRACT

Aim: The objective of the study is to evaluate the cervical-apical sealing of bulk-fill flow resins associated with a universal adhesive system, in endodontically treated teeth, considering two methods of evaluation.

Materials and methods: The samples used in the bacterial leakage analysis were autoclaved, filled inside a laminar flow chamber, and divided into four experimental groups (n = 8) according to the restorative material: Filtek Bulk-fill flow; Surefil SDR flow; TetricEvoflow and the positive control. The samples were inoculated with the *Enterococcus faecalis* (*E. faecalis),* every 7 days and the microleakage was evaluated on a daily basis for 60 days. Microleakage tested with dye (n = 5) was considered in 2-time intervals of evaluation (24 hours and 72 hours), and the results were obtained in scores from 0–5. Data were submitted to the statistical tests, at a level of significance of 0.05.

Results: About 12.5% of the FBF specimens showed bacterial microleakage as from the thirteenth day, and SDR, from the 20th day. TEF presented the highest percentage of microleakage at 40 days, concluding the 60 days with 37.5% of viable specimens. The Log-rank test revealed that the survival curve of TEF was statistically significant in comparison with those of the other groups. In the test with dye TEF and SDR presented the highest and lowest rate of microleakage, respectively, with statistically significant differences in the time interval of 72h.

Conclusion: The resins FBF, SDR, and TEF were incapable of promoting the hermetic sealing of fillings in the root canal system.

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Clinical significance: No bulk-fill flux studied was able to completely eliminate microleakage, despite the best performance with respect to the microleakage time presented by Filtek Bulk fill, followed by Surefil SDR flow.

Keywords: Bacterial microleakage, Bulk-fill, Composites, Dye microleakage, *Enterococcus faecalis.*

INTRODUCTION

Bacterial microleakage between the tooth and filling material continues to be one of the main causes of endodontic treatment failure. Therefore, hermetical cervical sealing is fundamental for impeding or minimizing the penetration of microorganisms.[1,2] However, in spite of various methods and materials having been suggested to promote this sealing, whether or not the materials used at present are effective, is still a questionable issue.[3,4] Factors such as the lack of bonding to dental tissue; polymerization shrinkage of composites, and the difference in the linear thermal expansion coefficient between the tooth and restorative material have led to inadequate cervical sealing.[5-7]

Seeking to improve the properties of restorative materials, new composite denominated the bulk-fill type have been launched. The characteristic of these materials is that they can be inserted in a single increment of 4–6 mm.[8] According to the manufacturers' recommendations, their adequate polymerization associated with lower contraction stress can be achieved. According to the literature, the lower contraction stress results from the use of specific polymerization modulators and because

they have a low modulus of elasticity. The greater polymerization depth is derived from the use of more potent photoinitiators and their higher translucency that allows more light to penetrate.[9] In addition, the presentation in fluid form allows better adaptation to and filling of the undercut root canal walls.

These composites may be associated with conventional and universal adhesive systems. According to Yoshida et al.,[10] when using an adhesive system that has MDP monomer (10-Methacryloyloxydecyl dihydrogen phosphate) in its composition, with potential for chemical bonding, gap formation may be minimized by increasing the bond strength between the (tooth/material) substrates.

The aim of this study was to evaluate the cervical sealing capacity of the bulk-fill flow resins in endodontically treated teeth, considering two methods of evaluation. Three hypotheses were tested:

- H_0: The association of bulk-fill flow resins with universal adhesive would not impede bacterial microleakage
- H_1: the time interval of evaluation would not influence the microleakage results
- H_2: there would be no difference between the methods of evaluation.

MATERIALS AND METHODS

Selection, Standardization of Samples, and Division of the Groups

Fifty-nine maxillary bovine incisors with closed apices and square roots, and of similar shapes and sizes were included in this study. The roots were a section at 16 mm from the apical foramen, and the real working length (RWL) was determined 1 mm short of the apex (15 mm). Instrumentation was performed with Gates Glidden (Maillefer-Dentsply-Brazil) burs from number 6 to 3 in the cervical and middle thirds, and the apical stop was prepared at the RWL up to 80 k-file (Maillefer, Switzerland). Irrigation was performed with 5 mL (2.5%) sodium hypochlorite at every change of file. On conclusion of instrumentation, the canal was inundated with EDTA (17%), mechanically agitated for 3 minutes with file 20 k, and afterward irrigated with NaOCl (2.5%), aspiration and dry with absorbent paper points (Maillefer–Dentsply). On conclusion of chemical-mechanical preparation, the 120 root remainders were randomly divided into two experimental groups according to the evaluation technique: group 1—evaluated by means of bacterial penetration, and group 2—evaluated with dye, followed by filling the canals. The samples of group 1 were sealed with 02 coats of cosmetic varnish (Colorama, Maybelline, Nova York,

USA) leaving the 2 mm short of the apex and the initial 1 mm of the cervical third free of varnish, and then autoclaved (121°C/20 min), filled in a laminar flow chamber. After filling, 2 mm of the gutta-percha was removed from the canal entrances, for later restoration, according to each experimental material (n = 8). Filtek bulk-fill flow (3M/ESPE St. Paul, MN, USA), Surefil SDR flow (Dentsply Petrópolis, Brazil) and TetricEvoflow (Ivoclar Vivadent AG Schaan, Germany) in accordance with the respective manufacturer's recommendations (Table 1). The positive control (n = 5) considered only the root remainders without endodontic treatment, and the negative (n = 5), only the sterile BHI culture medium. As restorative protocol, the adhesive system All Bond Universal (BISCO–Germany) was used in accordance with the manufacturer's instructions (Table 1), with later insertion and light polymerization of the resin, with the use of a Radii cal (SDI–Australia) Light Polymerizing appliance, at 1200 mW/cm².

Fabrication of Device for Bacterial Infiltration/Leakage Test

For the bacterial leakage test, a device was fabricated, based on the model proposed by Torabinejad et al.[11] with modifications (Fig. 1). Initially, the extremities of sterile Eppendorf tubes were cuts with a no. 15 scalpel blade (Lamedid Solidor) so that when the tooth was inserted, 2 mm of the apical third of the filled roots would remain outside of the tube. The connection between the root and Eppendorf tube was sealed with acrylic resin (Vipiflash, Madespa S/A, Rio de Janeiro, Brazil) to prevent bacterial leakage. Crepe tape (3M) was placed around the edge of the Eppendorf tubes, to seal the space between the tube and flask, for the purpose of preventing external contamination. After sealing the extremities, the Eppendorf/Specimen sets were placed in sterile glass jars, containing 4 mL BHI medium (liquid brain heart infusion – Oxoid Ltda., Basingstoke, Hampshire, England) so that 2 mm of the apical portion would remain immersed in the culture medium.

Obtainment, Inoculation, and Evaluation of Infiltration/Leakage of *Enterococcus faecalis*

The *E. faecalis* strain was obtained from the Microorganism Culture Collection of the Department of Antibiotics of the Federal University of Pernambuco (UFPEDA 138). The *E. faecalis* was cultivated on BHI agar for 24 hours. A sample of the bacterial growth was suspended in 2 Ll sterile saline solution (tube 1), and afterward, the density of the suspension was adjusted according to Tube 05 on the scales of the McFarland turbidity meter (1×10^8 CFU/mL). After this a dilution (1:10) was made by transferring 200
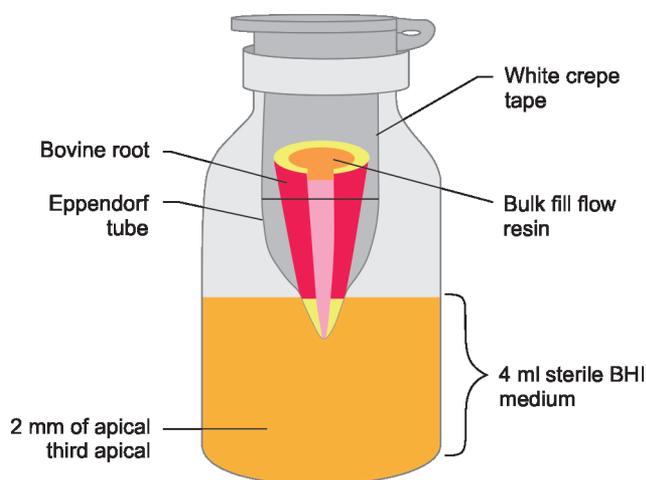
Fig. 1: Illustration of the device used to evaluate bacterial infiltration/leakage

μL of the suspension of Tubes 1 and 2 containing 1.8 mL sterile saline solution, resulting in a bacterial suspension of 1×10^7 CFU/mL. Each sample of the experimental materials was inoculated with 10 μL of the bacterial suspension every 7 days, for 60 days (35°C). Daily evaluations (24 hours) were made to verify the volume and presence, and/or absence of bacterial growth in the culture medium. When turbidity of the culture medium occurred, the sample was removed, and the microbial agent was identified with the purpose of proving the presence of *E. faecalis*. The proofs of identification used

for confirming the bacterial agent were gram staining, Bile–Esculin test and growth in saline BHI (6.5% NaCl).

Evaluation of Microleakage with Dye Solution

Before immersing the specimens in dye, the apical foramina of the roots were sealed with composite resin (Z100, 3M ESPE). Subsequently, the roots were sealed with two coats of nail varnish (Colorama, Maybelline, Nova York, USA) respecting the distance of 1 mm of the most cervical portion,thermal cycled (5000 cycles: 5–55°C/min), immersed in dye (2% methylene blue) for 24 hours and 72 hours, washed in running water, dried with absorbent paper, and longitudinally section in the mesiodistal direction, with the use of a double-faced diamond disc (KG Sorensen), fitted to a (low speed) micromotor. Each hemisection was given a code number that was cataloged according to its experimental group. The microleakage at the tooth/restoration interface in the cervical region was quantitatively evaluated, with the aid of a stereoscopic lens (Coleman Equipamentos para Laboratórios Comércio e Importação Ltda., Santo André SP, Brazil), at 40x magnification, by 03 previously calibrated examiners, using the following criteria: 0–Without leakage at the restoration margins; 1—Leakage at the restoration margins that did not attain the restoration/ filling limit; 2—Leakage at the restoration margins attaining the restoration/filling limit; 3—Leakage attaining the

Table 1: Materials used in the study (Information provided by manufacturers)

	Material and manufactured batch no	Monomers	Filers	Phatoinitiators/ Coiniciators	Shade	Thickness (mm)/curing time and light Intensity
FBFF	Filtek Bulk Fill Flow™ (3M/ESPE St. Paul, MN, USA)–N735392	Bis-GMA UDMA Bis-EMA Procrylat	Triflureto de itérbio Zirconia/ sílica - 64 wt %, 42.5 vol	NA	A2	4 mm/ 20s ≥ 1000 mW/cm² ou 40s 550–1000 mW/cm² (halogen or LED)
SDR	SureFil® SDR Flow ™ (DENTSPLY Petropolis, Brasil)–160613	Modified UDMA EBPADMA TEGDMA	Barium and strontium alumino-fluoro silicate glasses - 68 wt%, 44 vol %	Camphoroquinone (CQ) BHT, UV Stabilizer Titanium dioxide Iron oxide pigments Fluorescing agent	U	4 mm/ 20s 500–1000 mW/cm2 (LED and halogen) ou 10s (high power lights)
TEF	Tetric EvoFlow Bulk Fill® (IVOCLAR VIVADENT Amherst, NY, USA)–U41169	Bis-GMA Bis-EMA UDMA	Prepolymer fillers Barium glass filler, ytterbium fluoride and spherical mixed oxide fillers - 62.5 wt%, 60 vol%	Camphorquinone/ amine Highly reactive, patented Ivocerin light initiator	U	4 mm/ 20s ≥ 600 mW/ cm² 10s ≥ 1000 mW/cm²
ABU	All Bond Universal (BISCO USA)–B-72020K	Bis-GMA, 10-MDP, HEMA, ethanol, initiators, water	Apply first layer, brush for 20s. Dry lightly (30s) Apply second layer, brush for 20s. Dry lightly (30s) Light curing (10s)			

UDMA: urethane dimethacrylate, Bis-GMA: bisphenol A glycidyl methacrylate, Bis-EMA: ethoxylated bisphenol A glycol dimethacrylate, EBPADMA: ethoxylated bisphenol A dimethacrylate, TEGDMA: Tetraethyleneglycol Dimethacrylate,

cervical third of the filling; 4—Leakage attaining the middle third of the filling; 5—Leakage attaining the apical third of the filling.

Statistical Analysis

The bacterial microleakage data were submitted to the Fisher's exact test and Kaplan-Meyer survival curves, and the Long-Rank test. The microleakage data obtained with dye in the time intervals of 24 hours and 72 hours were analyzed using the Mann–Whitney and Kruskal–Wallis tests in the comparison between resins for each time interval of evaluation. All the tests were performed with the use of SPSS version 23 and Medcalc version 14.8.1, considering a level of significance of 0.05.

RESULTS

Table 2 presents the percentage of bacterial microleakage according to the material and time interval of evaluation. By means of the Fisher's exact test, no statistically significant difference was observed among the materials evaluated. When the time interval of the evaluation was considered, the resin TEF was the type with the most samples showing leakage, especially after 40 days, nevertheless, without statistically significant differences. By means of the Log-rank test, a significant

difference was proved between the survival curves ($p = 0.007$) (Graph 1).

Table 3 presents estimates of means and medians and result of the statistical test for comparison between groups. This table shows that the time estimates were higher in groups FBFF (56.12 days) and SDR (54.63 days) when compared with group TEF (35.25 days). The median could only be determined in the TEF group.

The mean and standard deviation values of the microleakage scores with dye, according to the resin and time interval of evaluation are described in Table 4. Group TEF presented the highest microleakage scores in the two-time intervals of evaluation, with significant differences between the other resins in the evaluation at 72 hours. Graph 2 shows the percentage of microleakage between the materials, according to the time intervals of evaluation.

DISCUSSION

To evaluate the cervical-apical sealing capacity of the bulk fill resins associated with the use of a universal adhesive system in endodontically treated teeth, two methodologies were used. Three hypotheses were raised, and the results let to the rejection of the first null hypothesis that reported on the capacity of the association between resins and the adhesive system for impeding the penetration of

Table 2: Perccentage of bacterial microleakage according to the material and time interval of evaluation

Dias de Avaliação	FBFF		SDR		TEF		p value
	N	%	N	%	N	%	
1	8	100,0	8	100,0	8	100,0	p (1) = 1,000
10	8	100,0	8	100,0	7	87,5	p (1) = 1,000
20	8	100,0	7	87,5	6	75,0	p (1) = 0,747
30	7	87,5	7	87,5	4	50,0	p (1) = 0,278
40	7	87,5	7	87,5	3	37,5	p (1) = 0,062
50	7	87,5	7	87,5	3	37,5	p (1) = 0,062
60	7	87,5	7	87,5	3	37,5	p (1) = 0,062

(1) By means of Fisher's exact test. (N) Number of teeth without infiltration
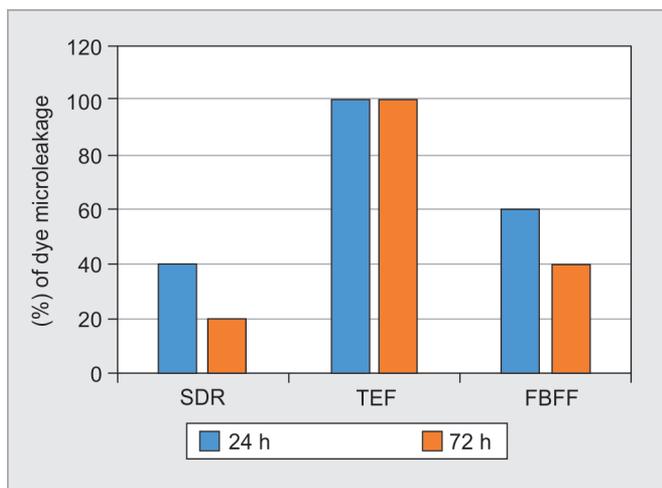
Graph 1: Percentage of micro-leakage on tooth surface with the composite materials
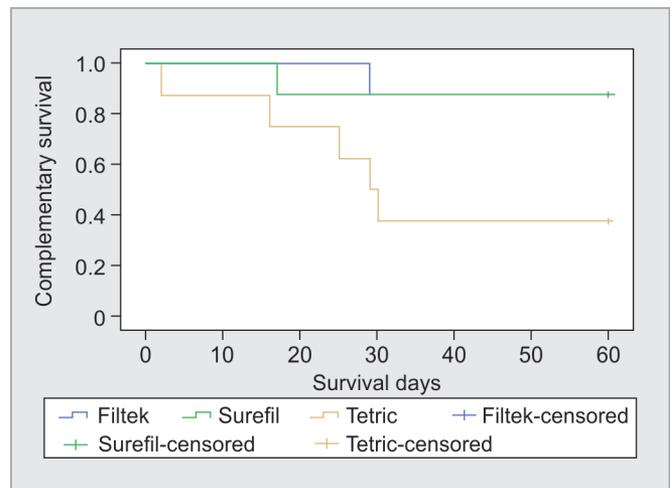
Graph 2: Kaplan–Meier curves of resins relative to bacterial microleakage

microorganisms. All the resins enabled penetration of the microorganism, with consequent contamination of the culture medium. The resins FBFF and SDR had 12.5% of their samples with detectable microleakage in 30 and 20 days, respectively and presented a similar percentage of leakage between them after 30 days. Whereas, tetric had 12.5% of its samples contaminated in 10 days; 25% in 20 days; 50% in 30 days, and 62.5% in 40 days, remaining stable up to the end of the 60 days. The similarity of the results between the FBFF and SDR groups could be justified by the similarity in their chemical composition (organic), both being methacrylate-based; and in the filler content of each of them, which was 64.5% (FBFF) and 68% (SDR).[12,13] Paradoxically, TEF presented a larger filler content (71% by weight) and consequently should have had a lower level of polymerization shrinkage due to the reduction in organic content, resulting in a lower rate of leakage. However, some hypotheses could be related to this result. One of these was the possibility of a restriction in the mobility of the reactive species, and scattered diffusion of the light activator may have occurred.[14] Another explanation would be related to the chemical composition of the new initiator (IVOCERIN) that may have influenced the performance of this resin. On the other hand, considering that all the resins were used in accordance with their manufacturer's recommendations, using the All Bond Universal adhesive system, for analysis of the results presented, the restorative system must be considered as a whole; that is, the "adhesive system + resin composite" set.

With regard to the time interval of evaluation, the authors noted that the longer the period of exposure was, the greater were the chances of contamination, which led to the rejection of the second hypothesis. The results obtained in this study corroborated the findings of Torabinejad et al.,[11] in which over 50% of the root canals were contaminated by *Staphylococcus epidermidis* after 19 days; and 50% by *Proteus vulgaris* in up to 42 days. Whereas, Khayat et al.[15] reported that the contamination of DTE exposed to saliva occurred in fewer than 30 days, a result that was partly similar to that obtained in the TEF group, which showed leakage in 50% of its samples in this time interval. The short period of contamination of this same group agreed with the data of the study of Chailertvanitkul et al.[16] in which the time necessary for leakage with *E. faecalis* to occur ranged from 7 to 86 days. Bacterial tracers have been wisely used to provide data that are biologically and clinically more significant and relevant when compared with other methods.[17]

Dyes and radioactive isotopes are also substances that may be used to evaluate microleakage, and however, due to their reduced sizes, they may not reproduce the true result presented when microorganisms and their by-products are used.[15] In this study, an analysis with dyes (2% methylene blue) was also performed, and the results were shown to be similar to those obtained in the analysis with microorganisms. The samples of all the resins showed microleakage, however TEF present 100% microleakage with dye after thermal cycling in the two-time intervals of evaluation. The results showed that in the two methods of evaluation used, the resins were incapable of promoting hermetical cervical-apical sealing, and that resin TEF presented the highest rates of microleakage in both methods. This led to the acceptance of the third hypothesis that related there would be no difference between the methods of evaluating microleakage. However, according to Barthel et al.,[18] the results obtained in the analysis with dye cannot be extrapolated to comparisons with bacterial microleakage, or with the events that occur in *in vivo* procedures.

The method of evaluation with dyes is influenced by the capacity for dye penetration and by the intra- and inter-examiner reliability, which must not be

Table 3: Estimates of the mean and median of time (days) without infiltration/leakage according to group

Group	Estimates of the mean		Estimates of the median	
	Value	(IC 95%)	Value	(IC 95%)
FBFF	56.12	(49.02 to 63.23)	–	–
SDR	54.63	(44.77 to 64.48)	–	–
TEF	35.25	(20.79 to 49.71)	29.00	(22.07 to 35.93)
p value	p (1) = 0.031*			

(1) By means of the Log-Rank test. (-) could not be determined because the infiltration/leakage occurred in fewer than half of the specimens in the group

Table 4: Mean and standard deviation of microleakage according to the material and time interval of evaluation

	Mean ± SD			
Resin	24 hours	72 hours	Group total	p value
SDR	0.60 ± 0.89	0.20 ± 0.45 (A)	0.40 ± 0.70 (a)	p (1) = 0.722
TEF	2.20 ± 1.10	1.60 ± 0.89 (B)	1.90 ± 0.99 (b)	p (1) = 0.524
FBFF	1.40 ± 1.134	0.40 ± 0.55 (A)	0.90 ± 1.10 (a)	p (1) = 0.286
	p (2) = 0.131*	p (2) = 0.010*	p (2) = 0.005*	

(*) significant difference at 5% (1) By means of the Mann-Whitney test. (2) By means of the Kruskal-Wallis test with paired comparisons of the mentioned test.
Note: If all the capital letters in parenthesis differed, this proved significant difference between the resins in the time interval of 72 hours; and if the lower-case letters differed, there were significant diferences between the resins in the total group

low. This method has been contested because it is an evaluation of a subjective nature, in which the degree of dye penetration is calculated by means of scores.[19] Therefore, to minimize the inter-examiner subjectivity, in this study, the evaluators underwent a process of calibration and the Kappa index was calculated (K> 82%). Other techniques have been used to measure the level of microleakage, among them compressed air, electrochemical investigations, scanning electron microscopy, and more recently micro-CT images.[20] In spite of the advances in technology that allow the use of micro-Ct for the evaluation of microleakage, some authors have reported that the best images were obtained my stereomicroscopy.[21] According to De Santi et al.[22] provided that it is good inter- and intra-examiner agreement, any of the techniques can be used. Furthermore, the authors reported that micro-CT is still not ready to substitute the method of scores as the gold standard in the evaluation of microleakage.

The capacity of microorganisms for penetrating along the filling and for attaining the periapical tissues is favored by ineffective cervical sealing,[23] and by the presence of gaps between the restorative material and dentin wall and/or between the gutta-percha and dentin walls of the root canal.[24] The presence of these gaps may be attributed the diverse causes, such as internal anatomy of the canal; biomechanical preparation; physicochemical properties of endodontic materials; properties of irrigants and filling techniques.[25] Nevertheless, various studies[3,4,26,27] have shown that the materials available for this purpose have not been capable of definitively impeding the penetration of fluids and bacteria, in agreement with the data obtained in the present study. However, the proposal of the materials evaluated in this study was to offer a lower level of polymerization contraction and stress. What the data in the literature does allow us, is to identify which material presents the greatest efficacy relative to cervical sealing, thereby reducing the risk of bacteria penetrating along the canal, and improving the success rates of endodontic treatment.

CONCLUSION

The bulk-fill flow resins evaluated were incapable of promoting hermetic sealing of fillings in the root canal system. Therefore, the binary variable cervical sealing/ root canal filling needs to be performed in an adequate manner; and further researches are required with the purpose of developing a material that is capable of preventing endodontic failures to the maximum extent.

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