Microbial leakage evaluation of warm gutta – percha techniques

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The aim of this study is to compare bacterial leakage of MicroHeat and continuous wave with and without endodontic sealer. Thirty-eight single-rooted extracted mandibular premolars were selected and randomly divided into four experimental groups (n=8) and two control groups (n=3). Teeth were prepared with Mtwo NiTi files and obturated with MicroHeat or System B with or without endodontic sealer. Three teeth were used as positive controls (Ct+) and three intact teeth served as negative controls (Ct-). All samples were tested for bacterial infiltration every day for 60 days. On day 32 overall contamination value was 62.5% for Mseal, 75% for Mnoseal, 75% for SBseal and 37.8% for SBnoseal; after 60 days, the final contamination result was 100% for Mseal, Mnoseal and SBseal and 87.5% for SBnoseal. At the end of the observation period, groups showed no statistically significant differences.

The aim of root filling is to create a bacterial-tight seal, thus minimizing the risk of in infection or reinfection of the root canal system (1) and preventing periradicular pathosis. Obturation of the root canal involves the use of gutta – percha in combination with root canal sealer to provide an adequate seal. Without a sealer, canal obturations exhibit greater leakage. In contrast to gutta-percha, which is chemically and dimensionally stable, the areas filled by sealer are more vulnerable because it can dissolve over time in contact with tissue fluids. Therefore, the amount of sealer should be kept at the lowest, whereas the amount of gutta-percha placed into the canal must be maximized (2). Various root canal filling methods have been developed to increase the success of root canal treatment. Penetration of bacteria and their products from the oral cavity into the obturated root canals put at risk the endodontic treatment success. Therefore, evaluating the quality of root canal obturation as the final stage of root canal treatment is essential. Coronal leakage may occur due to voids or loss of restorative material, allowing the root filling material to come into contact with oral fluids.

Among several methods of evaluation of the sealing ability of endodontic materials, bacterial leakage experiments provide biologically and clinically relevant information (3-10). Studies that use a bacterial tracer derived from specific culture or from human saliva are considered to be more reliable than tests with dye. However, bacterial leakage studies are limited statistic model that do not simulate any condition found in the oral cavity such as temperature changes, dietary influences and salivary flow.

There is a wide range of bacteria found to be responsible for secondary infection of endodontically treated teeth, but one of the most commonly found is Enterococcus faecalis (11-25). For this reason, we selected it for our infiltration test. The null hypothesis was that there is no difference in coronal sealing abilities of two different root warm gutta-percha canal filling

Key words: bacterial leakage, continuous wave, endodontic sealer, filling method, MicroHeat

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techniques with and without endodontic sealer. Our two main purposes were to compare the MicroHeat and continuous wave techniques and to compare the same techniques with and without sealer to evaluate seal ability using a bacterial invasion *ex vivo* test.

MATERIAL AND METHODS

Thirty-eight human mandibular premolars extracted for orthodontic reasons were collected with verbal consent from patients to be used for in vitro studies. No specific consent from Ethical Committee was needed for the present in vitro study. Each tooth was examined by buccal and proximal radiographs and only teeth with single straight canals and a single apical foramen were chosen.

Thirty-five teeth were sectioned to remove the crown and expose the canal leaving root portions of 15 mm in length. The remaining three samples were left intact to be used as negative control. The length of the root canal was established using a #10 K – file (Dentsply/ Maillefer, Baillaigues, Switzerland) up to the apical foramen and subtracting 0,5 mm from this measurement. Each tooth was instrumented with the Mtwo technique (Sweden & Martina, Italy) (5) following basic sequence: 10.04/15.05/20.06/25.06 or 25.07 for System B samples. During the preparation, each canal was irrigated using syringes with 2 mL 5% NaOCL (Niclor Ogna, Italy) and 10 mL 10% EDTA (Tubuliclean Ogna, Italy). Apical gauging was verified using a #25 K-file. An additional step of shaping using a rotating apical 25/40 0.02 taper (Sweden & Martina, Italy) was performed to provide apical stop and complete instrumentation necessary for all 2 techniques. After preparation, a final prolonged irrigation with NaOCL and EDTA was performed to remove the smear layer. During all procedures throughout the experiment, the teeth were kept moist. In order to remove any bacteria or contaminants, every tooth was singularly sterilized through autoclaving for 60 min at 134°C and 2 atm.

After sterilization, the samples were randomly divided as follows:

- **Group 1 (Mseal):** 8 root canals were obturated with MicroHeat technique and endodontic sealer. After the canal drying procedure, a .02 taper size 40 master gutta-percha point (Dentsply Maillefer) was introduced at 1 mm from the WL. Zinc oxide eugenol-based Pulp Canal Sealer (Kerr, Salerno, Italy) was applied on the tip of the master cone. A 25.04 MicroHeat spreader (Sweden & Martina) was set at 300 rpm up to 2 mm from the WL. The Pac – Mac condenser (Sweden & Martina) was coated with warm gutta-percha using a microflow cartridge (EIE Analytic Technology). The Pac – Mac was inserted to 2 mm from the WL and rotated at 6000 rpm. The procedure was repeated at least 3 times per canal to obtain a sufficient filling.
- **Group 2 (Mnoseal):** 8 root canals were obturated with MicroHeat technique using the same procedures in group 1 without endodontic sealer.
- **Group 3 (SBseal):** 8 root canals were obturated with System B and endodontic sealer. All samples were instrumented as in group 1 and 2 with the only difference of using an Mtwo 25.07 as the last shaping instrument. The root canal was thinly coated with Pulp Canal Sealer. For the root filling using System B 25.06 MtwoGutta gutta-percha points (Sweden & Martina, Italy). It was set at 0.35 mm diameter with a caliper. Tug back adaptation was checked. The sealer-coated cone was placed to 0.5 mm of the WL. For the continuous wave of condensation, a Fine Medium System B (EIE Analytic Technology) was set 4 mm of the working length and heated up to 300°C to fill the apical third of the root canal. Once at the proper depth, heat was removed, and the apical pressure was maintained for 10 s. Backfill of the canal was accomplished by condensing the additional gutta-percha cones.
- **Group 4 (SBnoseal):** 8 root canals were obturated with System B without endodontic sealer.

The three specimens in the positive control group were instrumented without performing any root canal filling and leaving the canal empty. Another three teeth with intact crowns served as the negative control group. The filled roots were stored at 37°C and 100% humidity for 15 days to guarantee setting of the sealer. The apparatus used to evaluate bacterial leakage consisted of an upper chamber and a lower chamber. The upper chamber was formed by a glass pipe, obtained by a local anesthetic vial emptied of its content and sterilized. The lower chamber was constituted by a Beta counter tube. The two chambers were assembled through cold-polymerization resin, in order to obtain a hermetic system and to prevent any external access. The testing apparatus was sterilized in autoclave (134°C, 1 h to 2 atm). The external surface of the

bottom of the upper chamber was roughened with a dental burr. The cyanoacrylate (SuperAttack®) was applied on the glass surface and EE-bond (Tokuyama Dental, Italy) was also applied on the coronal dental surface to foster the bond between tooth and glass. A mass of dental composite was used to connect the dental element to the bottom of the upper chamber. Two coats of nail varnish were applied on the external surface of all teeth, except for 1 mm around the apical foramen, in order to prevent bacterial leakage though lateral canals or other discontinuities in the cementum. A standard strain of E. faecalis (ATCC 29212) was used and its initial concentration was 1.5 McFarland. The medium culture used during the test was the Brain Heart Infusion (BHI). One mL of solution containing *E. faecalis* was transferred to the upper chamber contacting the coronal portion of the filling material. The lower chamber was then filled with 5 mL of sterile broth so that about 2 mm of the root apex was immersed in the broth. The whole apparatus was incubated at 37°C and checked daily for the appearance of turbidity in the BHI broth for 60 days. To guarantee the vitality of the bacteria, the refresh of contaminated samples in the upper chamber was performed every 4 days. When turbidity of the medium was observed, confirmation of cell morphology was carried out by Petri dishes with Agar. To estimate the time and probability of coronal infiltration Kaplan-Meier curves were used.

RESULTS

No growth was observed when checking the sterilization of the whole apparatus. All specimens of the positive control group showed broth turbidity within 1 day of incubation. There was no evidence of broth turbidity in the negative control group.

The first positive (Mseal) sample was observed on the fourth day. The other samples showed positive results on the ninth and fifty – seventh day with the

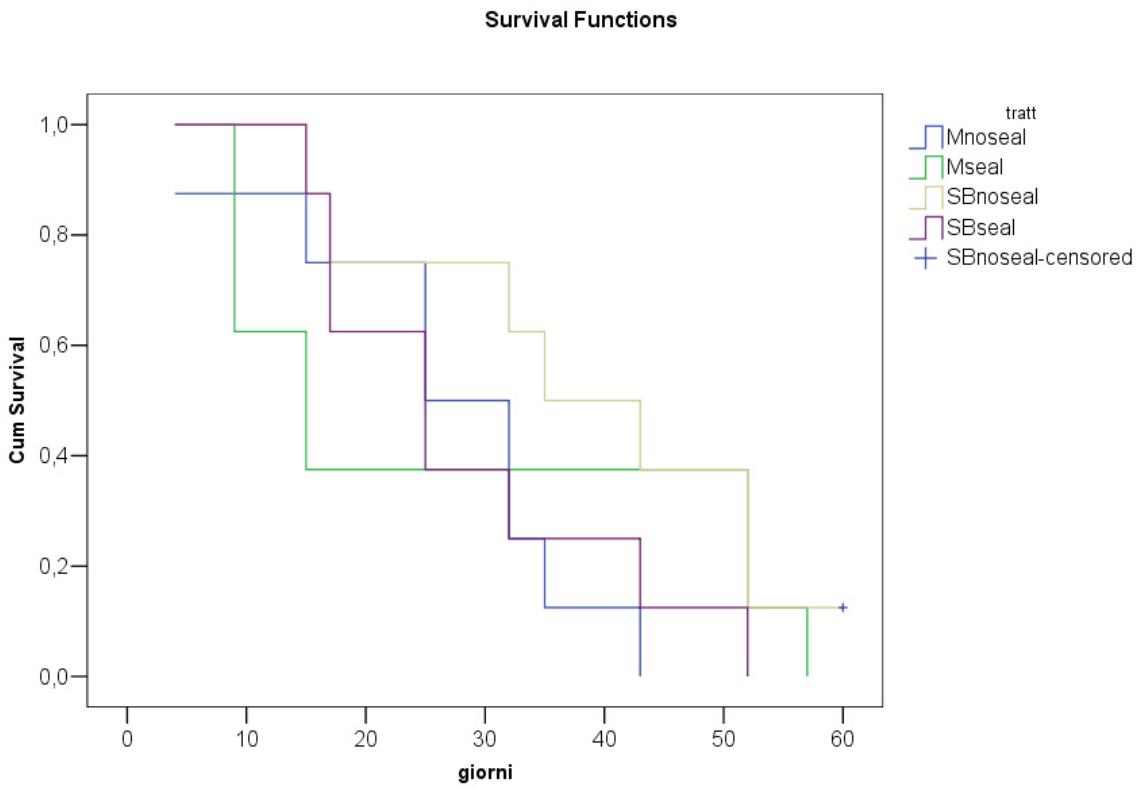

Fig. 1. *Bacterial infiltration curves based on observation time.*

exception of one element (SBnoseal) that did not show signs of contamination (Table I). On day 32, 62.5% of the Mseal samples were contaminated, 75% of the Mnoseal and SBseal samples were contaminated and 37.8% of SBnoseal samples were contaminated. On day 60, 100% of Mseal, Mnoseal, SBseal were contaminated and 87.5% of SBnoseal were contaminated (Table II, Fig. 1).

DISCUSSION

The use of sealer with gutta-percha is necessary to fill the voids and gaps between the filling material and the root walls. Without a sealer, canal obturation exhibit greater leakage (26-33).

Previous study evaluated sealing ability of two warm gutta-percha systems that used injected gutta-percha, Obtura II system, and vertical condensation with and without the use of sealers, because warm gutta–percha has the ability to conform to canal irregularities and radiographically appear dense without sealer. Results showed that obturation groups without sealer demonstrated the highest amount of leakage (34-39). However, Obtura II system was unable to obtain a great sealing root canal filling. Obtura II must be used as backfill in combination with more performing obturation systems (40-49). Although the study did not describe clearly vertical condensation steps, but it is very likely that manual spreader and plugger were used. They did not reach at the apical level the same temperatures as electrical instruments of the System B technique (50-57).

This study investigated the sealing ability of the thermoplastic gutta-percha alone using two

Table I. *Bacterial infiltration test results.*

		GROUP																																	
Days		Mseal								Mnoseal								SBseal								SBnoseal								Ct+			Ct-		
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	1	2	3
TIME	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				-	-	-
	4	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				-	-	-
	9	-	-	-	+	-	+	-	+	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				-	-	-
	15	+	-	+		-		-		+		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-				-	-	-
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	25		-			-		-				+	-	-	+	-	-	-		-	+	-		+		-			-	-	-	-	-				-	-	-
	32		-			-		-					+	-		-	+	-		+		-				-			-	-	-	+	-				-	-	-
	35		-			-		-						+		-		-				-				+			-	-	-		-				-	-	-
	43		-			-		-								+		-				+							-	-	+		-				-	-	-
	45		-			-		-										-											-	-			-				-	-	-
	52		+			+		-										+											+	+			-				-	-	-
	57							+																									-				-	-	-
	60																																-				-	-	-

Table II. *Distribution of teeth exhibiting bacterial leakage after 60 days of evaluation.*

GROUP	TOTAL (n)	NO LEAKAGE	LEAKAGE	%	RANGE DAYS
Mseal	8	0	8	100	9 – 57
Mnoseal	8	0	8	100	4 – 43
SBseal	8	0	8	100	15 – 52
SBnoseal	8	1	7	87,5	15 – 52
Ct +	3	0	3	100	1
Ct -	3	3	0	0	-

modern obturation methods: System B, development of vertical condensation of warm gutta-percha technique, and MicroHeat, development of MicroSeal technique. Our study uses a variant of monomicrobial bacterial leakage (58-61) with *E. faecalis* as infiltrating agent in a two-chamber system to evaluate the microbial leakage through filling material. All root canals of endodontically treated, with were not coronally restored teeth, were recontaminated within 19 and in less than 30 days, respectively (62-65).

By asking the question about the evaluation of short- and long-term root canal filling, the observation has been subdivided in two time periods: from 0 to 30 days, according to other study, and from 30 to 60 days because the results of longer evaluation times can produce more precise data.

Taking into account the results of the System B technique with and without sealer, the samples showed a consistent and uniform pattern of behavior having both an infiltration time between day 15 and 52. Moreover, the behavior of System B without sealer was in accord with the results of previous study where System B without sealer samples were a control group, because it was assumed that techniques without sealer were unreliable. The results of System B with sealer are different from those of tests that evaluate microbial leakage.

In a reference work one half of the samples survived during a period of observation between day 7 and 62, while the results of a test that evaluated saliva leakage showed that 2/3 of samples survived between day 3 and 52. In another coronal infiltration study (8) the samples were contaminated between day 24 and 54. However, by comparing the data of different works it is possible to say that samples always have a different behavior like they are strongly dependent on the kind of experiment.

In a study that evaluated coronal leakage of MicroSeal, Thermafil and System B (8), less than half of the filled roots through MicroSeal and System B techniques resulted positive after a period of observation of 32 days, therefore showing that the techniques are more efficient over time. In our study, Mseal and Mnoseal groups have maintained a uniform behavior to themselves but different to previous work and a similar trend with SBseal in the first 32 days.

A previous study that investigated apical leakage of the System B and MicroSeal (Analytic Endodontics, Orange, CA) obturation techniques showed that there was no significant difference between the apical leakage of the System B obturation and the MicroSeal obturation method.

As already pointed out in other studies, comparing MicroSeal with and without sealer, it is possible to say that the choice to use sealer did not influence the outcomes of this high – pressure technique around the apical control zone. Gutta – percha alone showed better filling at both 3 mm and 1 mm in the MicroSeal and System B techniques. However, there are no other works in the literature investigating coronal leakage of MicroSeal without sealer. Discordant behavior of MicroHeat technique compared to MicroSeal could be associated with different physical and chemical characteristics of gutta – percha. Improvement from earlier works, in this study MicroHeat gutta – percha was used and it was less adhesive, with a higher softening time and with higher viscosity compared to MicroSeal gutta – percha (SybronEndo, CA, USA).

Our study also underlined the bacterial permeability of the barrier gutta – percha/ seal over a period of 60 days, despite the techniques used, confirming the necessity of a coronal restoration to protect the filled endodontic system.

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