ORIGINAL ARTICLE

Differential efficacy of endodontic obturation procedures: an ex vivo study

Andrea Ardizzoni · Luigi Generali · Elena Righi · Maria C. Baschieri · Francesco Cavani · Lidia Manca · Eleonora Lugli · Luigi Migliarese · Elisabetta Blasi · Rachele G. Neglia

Abstract By means of a double-chamber model, different root canal filling materials and procedures were compared. Briefly, the root canals of single-rooted human teeth, extracted for periodontal reasons, were instrumented and obturated by gutta-percha/Pulp Canal Sealer EWT (PCS) or by Resilon, in association with different sealers (Real Seal, RelyX Unicem or Meta). Obturation was achieved by traditional continuous wave of condensation technique (TCWCT), a modified version of it (MCWCT), or single cone technique (SCT). The obturated roots, inserted in a double-chamber model, were sterilized by gamma irradiation. Next, *Enterococcus faecalis* was added to the upper chamber and the specimens were incubated at 37 °C for up to 120 days; the development of turbidity in the lower chambers' broths indicated bacterial leakage through the obturated root canals. The kinetics of leakage were analyzed in different groups by means of Kaplan–Meier statistics and compared by log-rank test. The results showed that root canals obturated with either gutta-percha/PCS using the MCWCT, Resilon/Real Seal SCT or Resilon/RelyX Unicem using the TCWCT displayed significantly better performance than the remaining groups ($p < 0.01$). Histological evaluation, performed to investigate microbial localization inside specimens, confirmed that this parameter varied according to the obturation procedures and materials employed. This ex vivo study indicates that gutta-percha/PCS, if used with the MCWCT, is as effective as Resilon when coupled to Real Seal with the SCT or, interestingly, to RelyX Unicem with the TCWCT. These data suggest that further improvement of the currently employed root canal filling procedures is achievable, depending on both the filling materials and the technique employed, thus encouraging clinical studies in this direction.

Keywords Bacterial leakage · *Enterococcus faecalis* · Gutta-percha · Resilon · Root canal filling

A. Ardizzoni · E. Righi · M. C. Baschieri · L. Manca · E. Blasi (✉) · R. G. Neglia
Dipartimento di Medicina Diagnostica, Clinica e di Sanità Pubblica, Università di Modena e Reggio Emilia, Istituti Biologici (2° piano), Via Campi, 287, 41125 Modena, Italy
e-mail: elisabetta.blasi@unimore.it

L. Generali · E. Lugli · L. Migliarese
Dipartimento Chirurgico, Medico, Odontoiatrico e di Scienze Morfologiche con interesse Trapiantologico, Oncologico e di Medicina Rigenerativa, Università di Modena e Reggio Emilia, Modena, Italy

F. Cavani
Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Università di Modena e Reggio Emilia, Modena, Italy

Introduction

Apical periodontitis of treated teeth is associated with the presence of bacteria in the root canal system (RCS) [1]; such bacteria are either remains of a primary infection that resisted treatment procedures, or representative of post-obturation reinfection, via coronal leakage. Among the species that are able to survive treatment, *Enterococcus faecalis* is the one most commonly isolated from endodontic failures [2–6]. This species survives for long periods, until changes in root canal conditions will allow re-growth [7–12].

A combination of strategies, aimed at preventing microbial persistence and/or reinfection, is routinely applied for a better prognosis of root canal filling [13]. The

chemo-mechanical disinfection of the RCS is undoubtedly a critical step [14, 15]: canals that had been disinfected to a high level display a better clinical outcome than those with residual bacteria [16–18]. Coronal restoration has also been shown to be important to prevent microbial leakage and endodontic treatment failure [13]. Also, the obturation process is acknowledged as critical, since the development and maintenance of a seal of the root system is the major prerequisite for success in endodontic treatment [19–21]. Notwithstanding the large number of filling materials available, the combination of gutta-percha and a root canal sealer is still the most commonly employed method. Indeed, gutta-percha has a good adaptation to root canal walls, a satisfactory dimensional stability and radio-opacity; furthermore, it can be easily removed [1, 13–15, 22]. On the other hand, gutta-percha is known to undergo shrinkage during its cooling down; therefore, endodontic sealers are used in combinations to enhance the efficacy of obturation [23].

Progress in dentine bonding has led to the development of resin root filling materials, such as Resilon (Real Seal, SybronEndo, Orange, CA, USA), a thermoplastic synthetic polymer with the same handling properties as gutta-percha [20, 21]. The Resilon system (consisting of Resilon with a primer and sealer [22]) is gaining popularity because of its ability to bind to the canal wall and to create a long-term radicular seal.

Furthermore, the obturation technique employed may influence the success of the endodontic treatment as well. Torabinejad and co-workers [16] have previously shown that bacterial penetration occurs in about 50 % of the teeth, whose root canals had been obturated with a combination of gutta-percha and a sealer, using the lateral condensation technique. In addition, when lateral and vertical condensation techniques were compared, all the root canals were recontaminated within 30 days, irrespective of the technique employed [19].

The aim of this paper is to evaluate the possibility of improving the performance of two common fillers, namely gutta-percha and Resilon, the former being employed by the continuous wave of condensation technique (TCWCT) or a modification of the same method (MCWCT) and the latter used in association with different materials as sealers [Real Seal (Real Seal, SybronEndo, Orange, CA, USA), Hybrid Root SEAL (MetaSEAL in the USA; Sun Medical Co Ltd., Shiga, Japan; Parkell Inc., Farmingdale, Edgewood, NY, USA), RelyX Unicem (3M ESPE, Seefeld, Germany], employing the TCWCT or the single cone technique (SCT). In particular, RelyX Unicem, normally employed to cement fiber posts, has been used as a sealer for the Resilon system. Histological analysis was performed to assess microbial localization and trace the routes of microbial leakage.

Materials and methods

Selection and preparation of samples

The research was conducted in accordance with ethical principles, including the World Medical Association (WMA) Declaration of Helsinki (2008). A total of 84 single-rooted human teeth with a fully formed apex, extracted for periodontal reasons at the Dental, Oral and Maxillofacial Surgery Unit of Modena University Hospital, were selected for the present study. The teeth were placed in 5 % sodium hypochlorite (NaOCl) (Niclor 5; Ogna, Muggiò, Italy) for 20 min and then stored in sterile saline solution. A coronectomy was performed from the cement–enamel junction using a cylindrical diamond bur (Komet no.5856) to obtain 13-mm-long roots. All subsequent phases were performed using a surgical microscope (OPMI Pico; Carl Zeiss Meditec Inc., Jena, Germany). A #10K-file (Dentsply Maillefer, Baillagues, Switzerland) was inserted to probe each canal and make it patent. The canals were shaped using #8 to #20 stainless steel K-files (Dentsply Maillefer, Baillagues, Switzerland) and nickel–titanium rotary instruments 0.06 tapered (Endowave, J. Morita, Dietzenbach Germany), in a crown-down method using a dedicated motor (ATR Tecnika Vision, Pistoia, Italy) at 500 rpm according to the manufacturer's setup configuration in the following sequence: #35 0.08 to enlarge the coronal portion of the canal, followed by #30 0.06 and #25 0.06 until 2-mm short to the working length. Then, size #25 0.06 and #30 0.06 were used at the working length.

Irrigation was performed on each passage of instruments, alternating 17 % EDTA (Ogna, Muggiò, Italy) with 5 % NaOCl. Once the shaping was complete, the canal was irrigated with 5 % NaOCl, with the exclusion of groups III, IV, V and VI (see below), which were to have monoblock adhesive obturation, to avoid resin-curing alterations; in such groups, final irrigation was performed with sterile saline solution. The canals were dried using sterile paper points.

Root canal obturation

Teeth were randomly divided into 3 groups, according to the type of obturation material (Fig. 1), namely gutta-percha (Group A: 36 teeth), Resilon (Group B: 40 teeth) and composite (Group C: 8 teeth). Then, teeth from Group A and Group B were further divided into 6 experimental
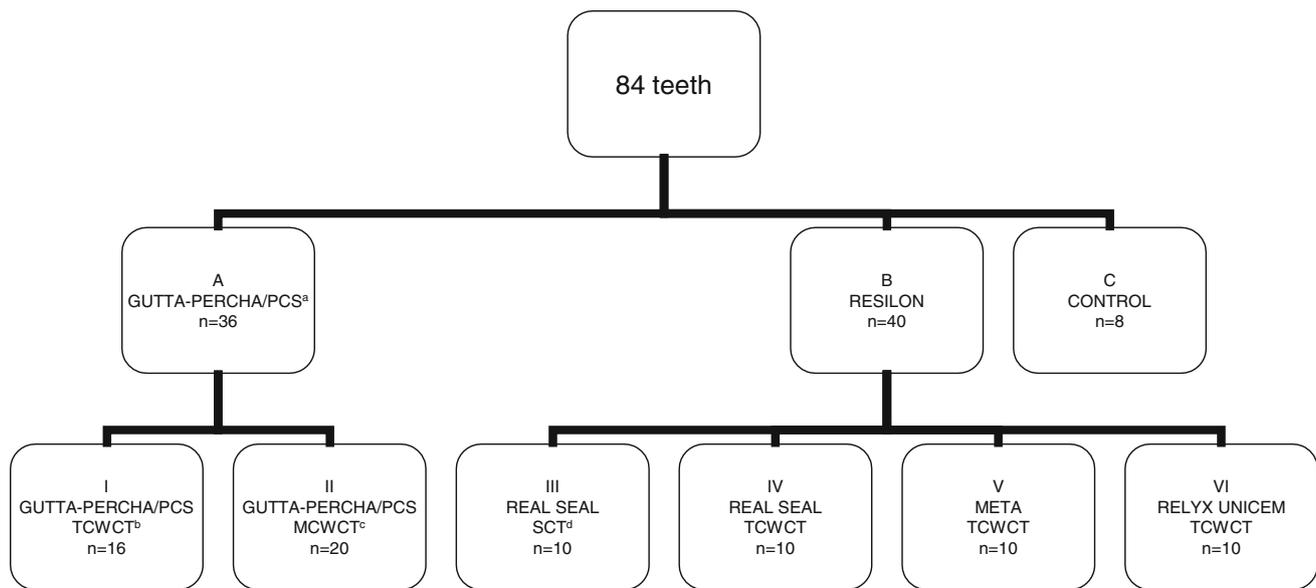
Fig. 1 Study groups. The 84 teeth were randomly divided into different groups according to the obturation materials and the techniques employed. The number of teeth in each group is also shown. [a]Gutta-percha/PCS, Pulp Canal Sealer EWT; [b]TCWCT, traditional continuous wave of condensation technique; [c]MCWCT, modified continuous wave of condensation technique; [d]SCT, single cone technique

groups and root canal obturations were performed according to different procedures, as detailed below.

Group I (16 teeth): gutta-percha/Pulp Canal Sealer (PCS) EWT–traditional continuous wave of condensation technique (TCWCT)

We used a #30 0.06 taper gutta-percha cone (Dentsply, Maillefer, USA), fitted 0.5 mm from the working length (WL), and Pulp Canal Sealer (PCS) EWT (SybronEndo, Orange, CA, USA). All procedures were performed as described by Buchanan using an Obturation Unit's fine tip (SybronEndo, Orange, CA, USA) at 150 °C [24].

Group II (20 teeth): gutta-percha/PCS EWT–modified continuous wave of condensation technique (MCWCT)

To prepare this group, a #30 0.06 taper gutta-percha cone (Dentsply, Maillefer, USA), fitted 0.5 mm from the WL, and PCS EWT were used. A fine-medium tip was fitted to the Obturation Unit at 250 °C. When the silicone stopper on the plugger was 3-mm shy of the binding point, the heat was deactivated and apical pressure was maintained until the silicone stopper reached the binding point. The heat was reactivated for 1 s to remove the coronal part of the gutta-percha cone and 28 s later gutta-percha compaction began, lasting for an additional 1.5 min. The Obturation

Unit extruder (SybronEndo, Orange, CA, USA) was used for backfilling at 250 °C.

Group III (10 teeth): Resilon/Real Seal–single cone technique (SCT)

A size #30 0.06 taper Resilon Seal™ (SybronEndo, Orange, CA, USA) cone, coated with Real Seal sealer (SybronEndo, Orange, CA, USA) and inserted into the canal to the WL, was used. The canal was previously filled with Real Seal sealer using a paper point to the WL. The cone excess was removed with the plugger of the Obturation Unit and the specimens were immediately light cured for 40 s with a B Max LED curing light (Tecnogaz, Parma, Italy) to create a coronal seal.

Group IV (10 teeth): Resilon/Real Seal–TCWCT

A size #30 0.06 taper Resilon-Real Seal™ cone, fitted 0.5 mm from the WL, and self-etch Real Seal sealer were used. All procedures were performed as described by using an Obturation Unit's fine tip at 150 °C [24].

Group V (10 teeth): Resilon/Meta–TCWCT

A size #30 0.06 taper Resilon-Real Seal™ cone, fitted 0.5 mm from the WL, and 4-Meta self-etched sealer

(Hybrid Root Seal; Sun Medical Co Ltd., Shiga, Japan) were used.

Group VI (10 teeth): Resilon/RelyX Unicem–TCWCT

A size #30 0.06 taper Resilon-Real Seal™ cone, fitted 0.5 mm from the WL, and RelyX Unicem self-adhesive cement (3M ESPE, Seefeld, Germany) were used. The RelyX Unicem was mixed in a capsule in a triturating device and directly extruded through the tip of the capsule.

Control group

The 8 teeth from Group C, hereafter named as control group, were treated with composite, to obtain complete sealing of the specimens. Each root canal was etched using 37 % orthophosphoric acid (Scotchbond™ Etchant-3M ESPE, Seefeld, Germany) for 30 s and then rinsed using sterile saline solution. Sterile paper points were used to dry the canal. An adhesive system (Adper Scotchbond MP Plus Adhesive System, 3M ESPE, Seefeld, Germany) was applied along the whole length of the canal and cured for 30 s. The canal was filled with flowable composite (Tetric Evoflow, Ivoclar Vivadent, Schaan, Liechtenstein) and then cured for 30 s until full canal obturation was obtained. All the specimens were entirely covered with two coats of red nail varnish.

At the end of the obturation procedures, all 84 specimens were individually sealed in bags, sterilized by gamma irradiation and placed in incubator (Cultura Ivoclar Vivadent, Schaan, Liechtenstein) for 21 days at 37 ± 1 °C and 100 % humidity to allow the cements to set completely.

Microorganisms

E. faecalis (ATCC 29212), stored at -80 °C, were thawed and cultured in Bile Esculin (BE) agar (Difco/Becton–Dickinson, Sparks, MD, USA); isolated colonies were then subcultured in Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke, Hampshire, UK) for 18 h at 37 °C. The bacterial suspensions were properly adjusted to a working concentration of 10^4 CFU/mL. To confirm the concentration, viable counts were performed by plating serial dilutions onto Brain Heart Agar (BHA) (Oxoid, Basingstoke, Hampshire, UK).

Assessment of bacterial leakage

To evaluate the bacterial leakage in each specimen, a two-chamber system was employed [25, 26], with minor modifications. In detail, to avoid bacterial leakage through lateral canals, two coats of red nail varnish were applied on the external surface of each tooth, except on the apices and coronal ends. Each filled root was tightly set in a 1.5-mL Eppendorf tube, whose bottom had been cut off to accommodate the root apex. The small gap occurring at the root external surface–Eppendorf tube junction was filled with a drop of cyanoacrylate gel. The Eppendorf tube was fitted inside a 15-mL polycarbonate centrifuge tube (Corning Incorporated, New York, NY, USA) (outer chamber). Then, specimens were sterilized by ethylene oxide and subsequently handled under aseptic conditions.

To test the seal between the tooth and the inner chamber, the following control steps were performed: (1) the outer chambers were filled with ~ 8 mL of sterile BHI broth, so as to cover the root tip; (2) to the inner chamber, ≤ 40 μL of E. faecalis suspension (10^4 CFU/mL) was added to fill the space between the root's external surface and the Eppendorf tube, carefully leaving about 1 mm below the edge. Subsequently, the samples were incubated at 37 °C for as long as 7 days, and 16 samples that displayed turbidity of the outer chamber broth were excluded from the study.

The assessment of bacterial leakage was performed employing 68 samples that were incubated at 37 °C in a humidified chamber and checked daily for development of turbidity. For each positive sample, the presence of E. faecalis in the outer chamber was confirmed, by plating onto BE agar plates. At the end of the experiment, the broth from the inner chambers of the non-infiltrated teeth was also controlled; in all cases, bacteria were viable, colonies exhibited the expected morphology and there was no evidence of contamination by other microbial species.

Histological analysis of specimens

When a sample became positive (i.e., when it developed turbidity in the lower chamber's broth) or at 120 days, it was fixed in 4 % paraformaldehyde, pH = 7.4, in phosphate-buffered saline for at least 72 h and microscopic evaluation was performed in 36 samples, covering at least 50 % of each group. Briefly, the roots were recovered, the nail varnish removed by a few minutes of immersion in acetone and demineralization was performed using Morse solution, composed of equal parts of solution A [500 mL formic acid (Sigma Chemical Company, St. Louis, MO, USA) + 500 mL distilled water] and B [400 g sodium citrate (Sigma Chemical Company, St. Louis, MO, USA) + 2,000 mL distilled water]. The demineralization was achieved when the root exhibited a rubber-like consistency. Then, roots were dehydrated in a graded series of ethanol and embedded in methyl methacrylate. Methacrylate blocks were placed in a microtome (Reichert-Jung 1150 Autocut, Nussloch, Germany) and the roots were sectioned into 5-μm-thick sections along their longitudinal axis. Sections intersecting the root canal were mounted on numbered glass slabs and stained using a modified Gram

Fig. 2 Kinetics of bacterial leakage in the 6 experimental groups. For each group, the percentage of roots with leakage is expressed as a function of time. The kinetics of leakage was analyzed by Kaplan–Meier statistics and compared by log-rank test. Group I: Gutta-percha/PCS EWT–TCWCT. Group II: Gutta-percha/PCS EWT–MCWCT. Group III: Resilon/Real Seal-SCT. Group IV: Resilon/Real Seal–TCWCT. Group V: Resilon/Meta–TCWCT. Group VI: Resilon/RelyX Unicem–TCWCT
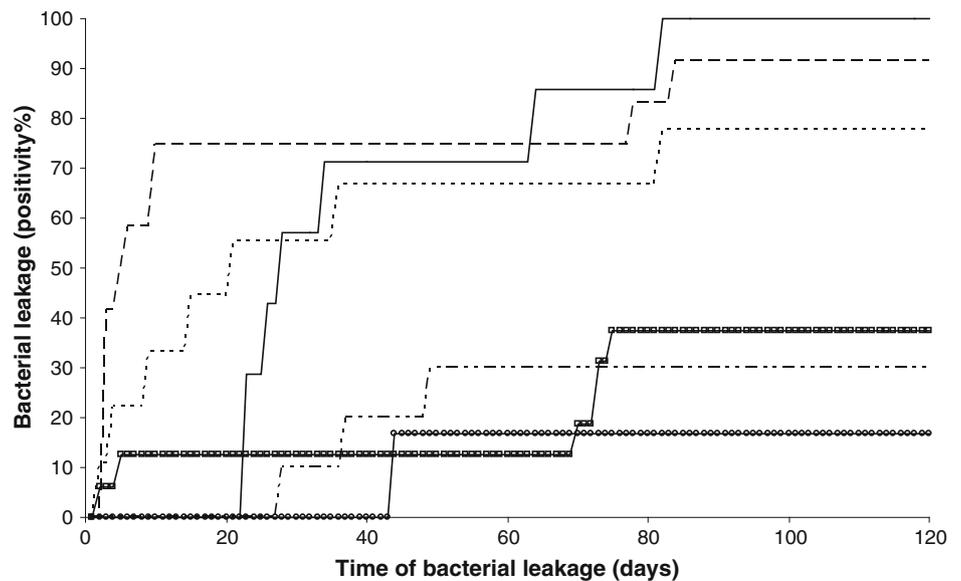
Table 1 Resistance to leakage observed in the different study groups

Groups	Days of resistance to leakage[a] Mean ± SD	Comparison between paired groups[b]					
		I	II	III	IV	V	VI
I	34.6 ± 47.0		p < 0.01	p < 0.01	n.s.	n.s.	p < 0.01
II	93.6 ± 40.8	p < 0.01		n.s.	p < 0.01	p < 0.01	n.s.
III	95.4 ± 40.0	p < 0.01	n.s.		p < 0.01	p < 0.01	n.s.
IV	45.1 ± 48.2	n.s.	p < 0.01	p < 0.01		n.s.	p < 0.01
V	40.0 ± 23.4	n.s.	p < 0.01	p < 0.01	n.s.		p < 0.01
VI	107.3 ± 31.0	p < 0.01	n.s.	n.s.	p < 0.01	p < 0.01	

n.s not significant

[a] Times of resistance to bacterial leakage were established by the turbidity test. The test was terminated at day 120

[b] Comparison between pair groups were performed by log-rank test

procedure (basic fuchsin staining not performed). Slices allowing visualization of the root canal were analyzed by a light microscope (NIKON Instruments Europe, Amsterdam, Netherlands, MOD. 80i) with × 40 and × 100 objectives.

Statistics

For each tooth, the days of resistance to leakage were recorded; mean times, standard deviations (SD), range and relative frequency of leakage within each group were then calculated. Kinetics of leakage in different groups was analyzed using Kaplan–Meier statistics and compared using log-rank test.

Statistical analyses were carried out using SPSS (Statistical Package for Social Sciences) version 18.0 for Windows. For all tests, a p value of 0.05 was set as limit for the significance, while a p value < 0.01 indicated high significance.

Results

As shown in Fig. 2, in group I (gutta-percha TCWCT), many specimens showed early leakage (55 % returned turbidity within the first 20 days) and all but one became positive by day 120 (83 %). In group II (gutta-percha MCWCT), two samples were infiltrated by day 5, but then, up to day 70, no additional leakage occurred and, at the end of the experiment (day 120), only 37 % of samples were infiltrated. Specimens of group III showed the first positivity at day 28; at days 37 and 49, two other samples showed leakage, while no further positivity was observed when the experiment was terminated (totally, only 30 % infiltrated teeth at day 120). Group IV displayed 44 % of infiltrated teeth at day 21; then, the leakage rate reached 89 % at the end of the experiment. Specimens from group V failed to leak up to day 23 and then leakage occurred in many samples: 25–70 % of the samples were infiltrated in the following 8 days; at day 82, all the specimens were
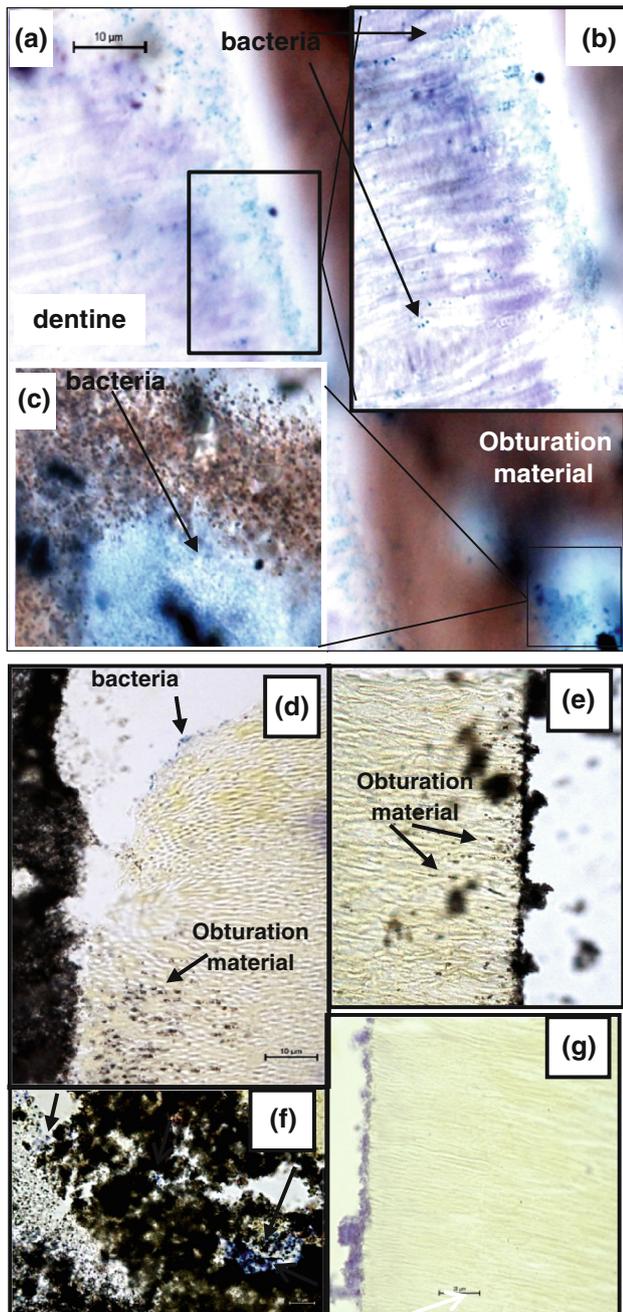
infiltrated. Group VI had only one sample positive at day 44 and no other leakages were ever observed. Concerning the control group (completely sealed teeth), none of the 8 teeth showed turbidity up to 120 days; when the experiment was terminated, all the controls remained negative to the leakage test (data not shown). Overall, the data in Fig. 2 indicate the occurrence of two major clusters of profiles: the first one, consisting of groups I, IV and V, and the second one, consisting of groups II, III and VI.

Table 1 shows the mean times (days) of bacterial resistance to leakage in each of the six experimental groups and the relative statistical analyses, aimed at comparing pairs of groups (achieved by log-rank test). As detailed, the mean days ranged from 34.6 to 45.1 in groups I, IV and V, while it was above 90 days in groups II, III and VI. Statistical differences occurred ($p < 0.01$) only when comparing pairs belonging to the two sets: groups I, IV and V versus groups II, III and VI. These findings confirm the existence of two distinct profiles in terms of days of bacterial leakage.

To trace the routes of bacterial leakage through the obturated roots, histological analysis was performed in more than 50 % of the samples from each group. In all specimens, bacteria were detectable, yet in different amounts and with different localizations. Selected images of the different microscopical features are given. Figure 3 shows roots from group II (gutta-percha/PCS MCWCT) that were characterized by late (day 73) leakage. As detailed, bacteria had heavily occupied dentinal tubules; in particular, the invasion was maximal at the coronal area (a) and gradually decreased toward the middle and apical parts (b and c). In contrast, as shown in Fig. 4, a specimen representative of an early leakage (day 2) showed bacteria present on the root canal surface–obturation interface (a) with minor tubule invasion (b). Bacteria were also detected within the obturation material (c). A specimen from group V (obturated with Resilon/Meta), which had become positive on day 23, is shown in panels d, e and f. In particular, bacteria were present at the upper level (d), where a gap between filler and dentinal surface occurred; conversely, in the middle portion of the root (e), the obturation material had tightly adhered to the canal wall, partially penetrating tubules, within which bacteria were not detected. Furthermore, in the apical area (f), the filler appeared poorly compacted and was heavily loaded with bacteria (indicated by the arrow). Finally, a specimen from group VI (Resilon/RelyX Unicem) that had not developed leakage throughout the 120 days is shown in panel g; in this case, a tight adhesion between obturation and canal surface was evident, dentinal tubules were plugged and bacteria were not detected in the dental tissue.
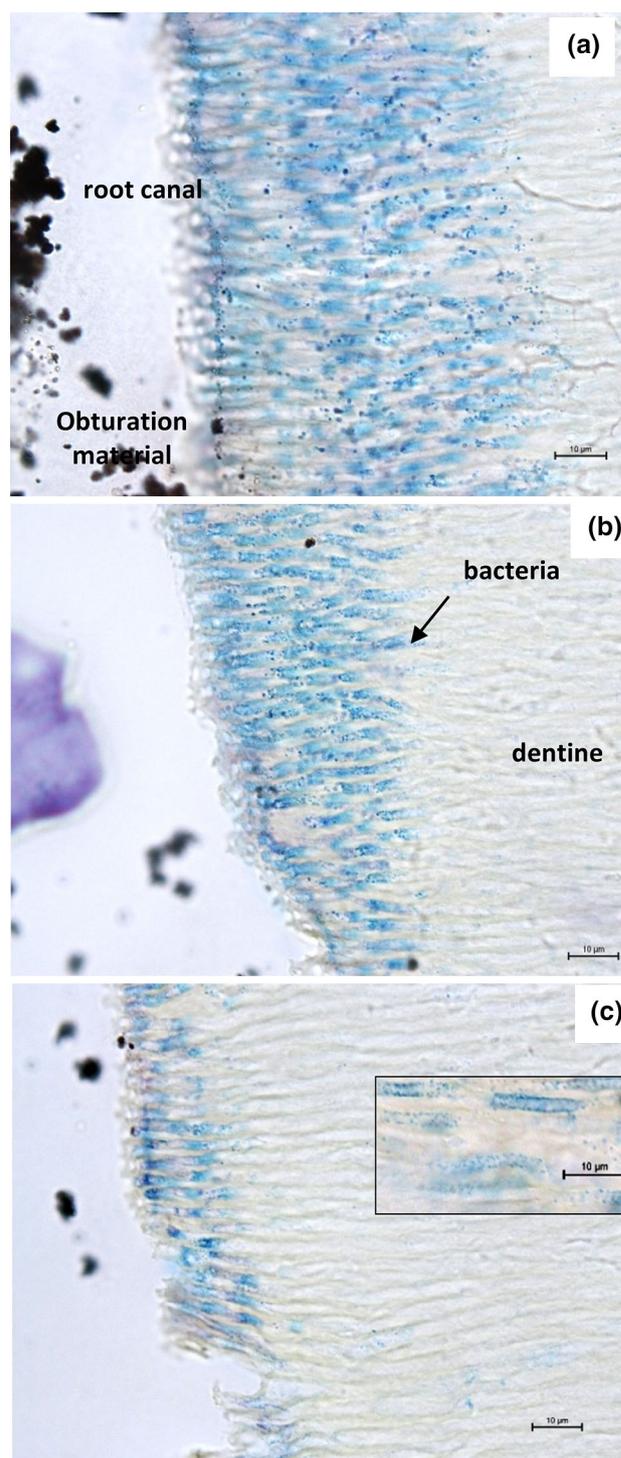
Fig. 3 Photomicrographs of bacterial occurrence in the radicular dentine. The specimen, belonging to group II (gutta-percha/PCS MCWCT), was positive on day 73 at the turbidity test. Selected portions of coronal **a**, middle **b** and apical **c** areas are shown. All the tubules appear heavily invaded by *E. faecalis* (see the inset for details); the depth of bacterial penetration within the dentinal tubules is higher in **a** (60 μm average) and progressively decreases toward the apex. The images also show the lack of filler adhesion to the root canal walls (magnification ×1,000; *bar* represents 10 μm)

to assess the long-term efficacy of different filling/sealing systems.

Gutta-percha has been universally accepted as a "gold standard" filling material, because it is inert, usefully plastic when heated, stable and tissue tolerated [27]. Traditional obturation techniques rely on cold or warm compaction of gutta-percha into the canal system, in the presence of a sealer. Yet, clinical studies demonstrate that gutta-percha/sealer combinations partially fail to prevent coronal leakage [25]. In line with those reports, by using a conventional technique (TCWCT), an average leakage time of 34.6 ± 47.0 days can be observed, with all samples but one becoming positive within the 120 days (Table 1, group I). Interestingly, the gutta-percha/PCS, applied by the recently described MCWCT [28], shows as many as 62.5 % non-infiltrated teeth up to the end of the observation period, while the positive specimens show a long average leakage time (93.6 ± 40.8 days; Table 1, group II). The main difference between the two obturation techniques employed consists in the compaction of gutta-percha, which starts 28 s later when using the modified technique [28]. Thus, this delay may likely allow compensating for the shrinkage effect occurring along the cooling phase. In any case, this is the first evidence that MCWCT obturation technique can be highly efficient, providing the best results, at least in our ex vivo model.

In recent years, the use of Resilon and its employment in combination with different sealers has been proposed [21, 22], with the aim of creating a monoblock and, in turn, to obtain a successful obturation; yet, Lin and co-workers [20] claim that the resin system allows levels of leakage comparable to those of the gutta-percha system, irrespective of the technique employed. When comparing groups where Resilon has been investigated in combination with Real Seal (groups III and IV), Meta (group V) or RelyX Unicem (group VI), heterogeneous results have been obtained, depending on the sealer and the condensation technique employed. In particular, 70 % of teeth, whose root canals had been obturated with Resilon/Real Seal, by using SCT

Discussion

By means of a bacterial leakage assay, performed in a double-chamber model, a microbiological assessment of the root canal filling procedures has been possible. The lack of coronal restoration allowed evaluating the time of bacterial leakage solely depending on the filling materials and/or the obturation techniques. Moreover, the employment of *E. faecalis,* well known for its ability to survive under unfavorable conditions [10–12, 17, 18], has allowed

(group III), remain negative to the leakage test, up to the experiment's truncation; in contrast, 77.7 % of the teeth, whose root canals had been obturated with Resilon/Real Seal, by applying TCWCT (group IV), show leakage with an average time of 45.1 ± 48.2 days, strongly arguing on the relevance of the obturation technique in the efficacy of root filling. Furthermore, all the teeth whose root canals had been obturated with Resilon/Meta (group V) are infiltrated by 80 days (average leakage time: 40.0 ± 23.4 days), while the combination of Resilon with RelyX Unicem (group VI), a self-adhesive cement, here employed as an endodontic sealer, successfully prevents bacterial leakage in all the samples but one; interestingly, this group showed the longest average leakage time (107.3 ± 31.0 days). RelyX Unicem, because of its self-adhesive properties, is commonly employed to cement the fiber posts. The drawback of employing RelyX Unicem as an endodontic sealer is the difficulty in removing it in case of re-treatment, thus limiting its use in daily clinical practice. Taken together, the present findings emphasize the relevance of sealers when employing Resilon; namely, the capability of a specific sealer to promote tight connections between Resilon and dentine appears critical to prevent bacterial leakage and, as a consequence, to determine the outcome of the endodontic treatment.

As confirmed by the statistical analysis, the observed profiles of teeth leakage can be aggregated into two clusters: the first, which includes groups I, IV and V, shows early development of turbidity in the lower chambers' broths (approximately, 50 % in 20 days), followed by maximal percentage of leakage after around 80 days; the second, including groups II, III and VI, is characterized by a very low percentage of infiltrated teeth throughout the time. As expected, no statistically significant differences were detected within the same cluster, in terms of days of resistance to bacterial leakage or percentages of infiltrated teeth. Overall, these findings strengthen the efficacy of the Resilon/Real Seal SCT and add new light to gutta-percha becoming as effective as Resilon, provided that MCWCT is used.

As further suggested in a recent editorial [29], bacterial leakage studies, carried out by two-chamber models, must be complemented with histological analysis to trace the route of leakage. Accordingly, in the present study, the occurrence and localization of bacteria were investigated. Control specimens (completely sealed teeth) were consistently negative to histological investigations (data not shown). Conversely, experimental teeth showed the presence of bacteria, yet in different amounts and localization; in particular, in the case of leakage occurring at late times, a consistent and gradual bacterial penetration of most dentinal tubules occurs (deepest invasion in the coronal portion and minor invasion of lateral tubules in the apical area). On the other hand, a rapid leakage (within a few days) allows *E. faecalis* to largely overwhelm the root canal surface with little or no penetration of the dentinal tubules and/or to remain within the filler gaps. Interestingly, when the obturation materials are compact, tightly adhere to the canal wall surface and even penetrate in the dentinal tubules, bacterial leakage is hampered and intra-tissue localization prevented; this condition commonly occurs in experimental groups II (gutta-percha/PCS MCWCT), III (Resilon/Real Seal SCT) and VI (Resilon/RelyX Unicem), further strengthening the good results obtained by coupling certain filling/sealing materials with certain obturation techniques.

Taken together, the leakage data and the histological pictures underline a critical contribution of the sealer and/or the technique employed, even when materials such as Resilon are employed. It is worth noting that Resilon is known for its ability to bind to canal wall and to create a long-term radicular seal [22].

Although with its intrinsic limitations, in particular the lack of coronal restoration which implies the direct and long-term exposure to an *E. faecalis* suspension, this model has allowed mimicking what happens within root-filled teeth, thus establishing bacterial survival/allocation with respect to different materials/endodontic procedures. The overall findings indicate that both types of materials, gutta-percha and Resilon, as well as the obturation procedures applied contribute to the success of the root filling, by providing efficacious resistance to microbial leakage; yet, in this model, the role of coronal restoration remains unexplored.

In conclusion, this study demonstrates the best performance of gutta-percha and Resilon, provided that the condensation technique and RelyX Unicem, or Real Seal in combination with SCT are used, respectively. Clinical investigations are needed to establish the in vivo counterpart of these findings and to definitely prove their relevance in endodontic practice.

References

1. De-Deus G, Audi C, Murad C, Fidel S, Fidel RA. Sealing ability of oval-shaped canals filled using the System B heat source with either gutta-percha or Resilon: an ex vivo study using a polymicrobial leakage model. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;104:114–9.

2. Love RM. *Enterococcus faecalis*—a mechanism for its role in endodontic failure. Int Endod J. 2001;34:399–405.

3. Pinheiro ET, Gomes BP, Ferraz CC, Sousa EL, Teixeira FB, Souza-Filho FJ. Microorganisms from canals of root-filled teeth with periapical lesions. Int Endod J. 2003;36:1–11.

4. Rôças N, Jung IY, Lee CY, Siqueira JF Jr. Polymerase chain reaction identification of microorganisms in previously root-filled teeth in a South Korean population. J Endod. 2004;30:504–8.

5. Siqueira JF Jr, Rôças IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;97:85–94.

6. Gomes BP, Pinheiro ET, Jacinto RC, Zaia AA, Ferraz CC, Souza-Filho FJ. Microbial analysis of canals of root-filled teeth with periapical lesions using polymerase chain reaction. J Endod. 2008;34:537–40.

7. Lleò MM, Bonato B, Tafi MC, Signoretto C, Boaretti M, Canepari P. Resuscitation rate in different Enterococcal species in the viable but non-culturable state. J Appl Microbiol. 2008;91:1095–102.

8. Figdor D, Davies JK, Sundqvist G. Starvation survival, growth and recovery of *Enterococcus faecalis* in human serum. Oral Microbiol Immun. 2003;18:234–9.

9. Sedgley CM, Lennan SL, Appelbe OK. Survival of *Enterococcus faecalis* in root canals ex vivo. Int Endod J. 2005;38:735–42.

10. Portenier I, Waltimo TMT, Ørstavik D, Haapasalo M. The susceptibility of starved, stationary phase and growing cells of *Enterococcus faecalis* to endodontic medicaments. J Endod. 2005;31:380–6.

11. McHugh CP, Zhang P, Michalek S, Eleazer PD. pH required to kill *Enterococcus faecalis* in vitro. J Endod. 2004;30:218–9.

12. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006;32:93–8.

13. Michelotto AL, Moura-Netto C, Araki AT, Akisue E, Moura AA, Sydney GB. In vitro analysis of thermo compaction time and gutta-percha type on quality of main canal and laterals canal filling. Braz Oral Res. 2010;24:290–5.

14. Brosco VH, Bernardineli N, Torres SA, Consolaro A, Bramante CM, de Moraes IG, Garcia RB. Bacterial leakage in root canals obturated by different techniques. Part 1: microbiologic evaluation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;105:e48–53.

15. Williamson AE, Marker KL, Drake DR, Dawson DV, Walton RE. Resin-based versus gutta-percha-based root canal obturation: influence on bacterial leakage in an in vitro model system. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;108:292–6.

16. Torabinejad M, Ung B, Kettering JD. *In vitro* bacterial penetration of coronally unsealed endodontically treated teeth. J Endod. 1990;16:566–9.

17. Neglia R, Ardizzoni A, Giardino L, Ambu E, Grazi S, Calignano S, Rimoldi C, Righi E, Blasi E. Comparative in vitro and ex vivo studies on the bactericidal activity of Tetraclean, a new generation endodontic irrigant, and sodium hypochlorite. New Microbiol. 2008;31:57–65.

18. Ardizzoni A, Blasi E, Rimoldi C, Giardino L, Ambu E, Righi E, Neglia R. An in vitro and ex vivo study on two antibiotic-based endodontic irrigants: a challenge to sodium hypochlorite. New Microbiol. 2009;32:57–66.

19. Khayat A, Lee SJ, Torabinejad M. Human saliva penetration of coronally unsealed obturated root canals. J Endod. 1993;19:458–61.

20. Lin ZM, Jhugroo A, Ling JQ. An evaluation of the sealing ability of a polycaprolactone-based root canal filling material (Resilon) after retreatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;104:846–51.

21. Attam K, Talwar S. A laboratory comparison of apical leakage between immediate versus delayed post space preparation in root canals filled with Resilon. Int Endod J. 2010;43:775–81.

22. Shipper G, Teixeira FB, Arnold RR, Trope M. Periapical inflammation after coronal microbial inoculation of dog roots filled with gutta-percha or Resilon. J Endod. 2005;31:91–6.

23. Lee KW, Williams MC, Camps JJ, Pashley DH. Adhesion of endodontic sealers to dentine and gutta-percha. J Endod. 2002;28:684–8.

24. Buchanan LS. Continuous wave of condensation technique. Endod Prac. 1998;1:7–10.

25. Siqueira JF Jr, Rôças IN, Favieri A, Abad EC, Castro AJ, Gahyva SM. Bacterial leakage in coronally unsealed root canals obturated with three different techniques. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;90:647–50.

26. De-Deus G, Coutinho-Filho T, Reis C, Murad C, Paciornik S. Polymicrobial leakage of four root canal sealers at two different thicknesses. J Endod. 2006;32:998–1001.

27. Stratton RK, Apicella MJ, Mines P. A fluid filtration comparison of gutta-percha versus Resilon, a new soft resin endodontic obturation system. J Endod. 2006;32:642–5.

28. Generali L, Mancuso R, Travaglini D, Ardizzoni A, Gianetti L, Bertoldi C, Ambu E. Sealing ability of gutta-percha in root canals obturated by a modified continuous wave of condensation technique (Abstract). Minerva Stomatol. 2010;59(Suppl. 1):448.

29. De-Deus G. Research that matters—root canal filling and leakage studies (Editorial). Int Endod J. 2012;45:1063–4.