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ORIGINAL RESEARCH

Evaluation of the root filling quality with experimental carrier-based obturators: a CLSM and FEG-SEM analysis

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Keywords
carrier-based obturators, confocal laser scanning microscopy, dentinal tubules, GuttaFlow Bioseal, sealer penetration.

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Abstract

This study evaluated tubule penetration of GuttaFlow Bioseal with cold single cone or carrier-based technique, under confocal laser scanning microscopy (CLSM). Twenty straight single-rooted teeth were instrumented with Hyflex CM and divided in two groups (n = 10) according to the obturation method: single cold gutta-percha cones; experimental carrier-based obturators. GuttaFlow Bioseal, labelled with Rhodamine B dye, was used as sealer in both groups. Teeth transversally sectioned were observed under CLSM. Percentage of sealer penetration and integrity of sealer layer perimeter were measured. Surface and microstructural characteristics of obturators and gutta-percha cones were compared by FEG-SEM and EDX analysis. No significant differences were found between groups for each examined parameter. Significant differences ($P < 0.05$) were reported mainly within groups. Integrity was similar among and within groups. FEG-SEM/EDX analysis of obturators revealed the presence of Ba and Zn. Carrier-based obturation technique associated with GuttaFlow Bioseal does not seem to affect sealer penetration into dentinal tubules.

Introduction

Adequate obturation of the root canal space is considered as one of the fundamental gearwheels of an extremely complex system that is the root canal treatment (1). Over time, several techniques and materials have been advocated to achieve complete canal filling (2) and different root canal sealers have been used. Newly developed calcium silicate-based (CS) sealers present the main advantage to possess low cytotoxicity (3) and high biocompatibility, inducing an osteogenic and bioactive response (4–6). Moreover, they were reported with comparable or superior sealing ability when compared to conventional resin-based sealers (7). Among many bioceramic sealers today available, GuttaFlow Bioseal (Coltène/Whaledent, Altstätten, Switzerland) is defined as a hybrid cement consisting of a formulation of polydimethyl-siloxane–gutta-percha doped with calcium silicate-bioglass-containing particles. Its aim is to provide both intrinsic biointeractivity-related and extrinsic chemo/physisorption-related apatite-forming ability (8) promoting the apical tissues regeneration (5). On the other side, consisting the endodontic obturation both of a sealer and of a semi-solid core material (1), carrier-based systems are gaining popularity due to ease of use and possibility to obtain consistently adequate technical results (9,10). Recently, carrier-based systems were proven to satisfy root filling quality and consequently the long-term periapical healing and survival (11,12).

In 2020, new experimental carrier-based obturators (Coltène/Whaledent, Altstätten, Switzerland) were designed with the claimed possibility to be used in combination with GuttaFlow Bioseal. To the best of our knowledge, to date, no study assessed the effectiveness of the new obturators in association with GuttaFlow Bioseal.

This *in vitro* study aimed to evaluate tubule penetration of GuttaFlow Bioseal sealer labelled with Rhodamine B by means of confocal laser scanning microscopy (CLSM), used in association with cold single cone or carrier-based technique. The null hypothesis tested was that there are no significant differences in terms of sealer penetration between the two experimental groups.

Materials and Methods

Root canal preparation

The study was approved by Ethical Committee, University of Bologna (n. 0000832).

Twenty straight single-rooted teeth of similar morphologic parameters were stored in distilled water at 4°C and cut to obtain roots of 14 ± 1 mm length. Radiographs in mesiodistal and buccolingual direction verified each canal shape.

Coronal thirds were enlarged with 25.08 orifice opener, 20.04, 25.04, 20.06, 30.04 Hyflex CM (Coltène/Whaledent, Altstatten, Switzerland) at working length (WL), following manufacturers' recommendations at 500 rpm and 2.5 Ncm in continuous rotation. Canals were irrigated by alternating 5 mL of 5% NaOCl (Niclor, Ogna, Muggiò, Italy) and 1 mL of 10% EDTA (Tubuliclean, Ogna) with 27-gauge side-vended needles (12) and final irrigation was performed with sterile water. Sterile paper points (Mynol, Milwaukee, USA) were used to dry canals.

Samples were randomly divided in two groups (n = 10).

Group 1: 25.04 single gutta-percha cones (Roeko Coltène/Whaledent, Altstatten, Switzerland) customised for each sample to obtain adequate tug-back at WL;

Group 2: 25.04 carrier-based obturators (Coltène/Whaledent, Altstatten, Switzerland).

For both groups, GuttaFlow Bioseal sealer was homogeneously mixed according to the manufacturer's instructions with a syringe mixing tip (Coltène/Whaledent, Altstatten, Switzerland) and used as sealer. Rhodamine B dye (Carlo Erba Reagenti, Arese, Italy) was added to the GuttaFlow Bioseal sealer in approx. 0.1 wt % (13) to mark the sealer and make it visible as red colour under CLSM observation (Leica TCS SP8 AOBS, Mannheim, Germany).

Sealer was inserted in the canals with sterile k-file of the same dimension of last file used apically in both groups. In Group 1, gutta-percha point was moved in a pumping motion for 5 s till WL was reached and in Group 2, excess of sealer were removed with sterile matching paper point according to the manufacturers' recommendations. Each experimental carrier-based obturator was heated using Herofill Oven (Micro-Mega, Besançon, France) for the recommended time and slowly inserted into the canal root until it reached the WL, with firm pressure. Obturators were separated after 120 s and a heated instrument was used to compact the material at the canal entrance.

All root canals were filled by two trained operators. Coronal seal was obtained with a temporary filling (Coltosol F, Coltène/Whaledent, Altstatten, Switzerland). Mesiodistal and buccolingual radiographs were immediately taken to evaluate the placement of root filling material. Samples were stored in an incubator in simulated body fluid (Hank's Balanced Salt Solution HBSS) at 37°C in 100% relativity humidity for 2 weeks.

CLSM analysis

Teeth embedded in room temperature-setting epoxy resin (Hard Rock 554, Remet, Bologna, Italy) were transversally sectioned at 2, 5 and 8 mm from the apex with a saw microtome (Leica SP 1600, Nussloch, Germany), under continuous water irrigation, to obtain 200 µm thick sections. These were examined with Leica TCS SP8 AOBS confocal microscope equipped with a White Light Laser (Mannheim, Germany) using specific wavelengths for Rhodamine B and images were acquired using a 10× objective. Image mosaics were merged using the Navigator tool. Each image was obtained performing 20 µm z-stacks with 1 µm step size (14). Evaluated parameters are summarised in Table 1.

Table 1 Summary of evaluated parameters and calculation methods

Parameter	Calculation
Depth of sealer penetration	Average penetration measured, by using straight-line tool of Fiji software (National Institutes of Health, Bethesda, MD), at 8 standardised points starting from the inner side of canal wall at 2, 5 and 8 mm from the apex
Point of deepest penetration	Measured from the canal wall to the point of maximum depth of sealer penetration
Percentage of sealer penetration	Calculated by measuring rhodamine B-stained surfaces of canal wall where sealer penetrated inside dentinal tubules (sealer tags) and dividing values by the circumference of root canal itself and multiplying result by 100
Integrity of sealer layer perimeter	Evaluated on each image acquired by measuring the rhodamine-stained perimeter of canal wall and dividing this value for the root canal circumference and expressed as percentage

Scanning electron microscopy

Four additional obturators were gold-sputtered and evaluated under a scanning electron microscope (SEM) (JSM-5200, JEOL, Tokyo, Japan) at middle (8–10 mm from the tip) and apical third (1–5 mm from the tip) for superficial features.

FEG-SEM/EDX analysis

A field emission gun scanning electron microscope (SEM: Nova NanoSEM 450–FEG–source: FEI–ThermoFisher Scientific, Hillsboro, Oregon, USA) equipped with energy-dispersive X-ray (EDX) detector (Quantax-200 system with XFlash 6/10 Si-drift detector: Bruker Corp., Billerica, MA, USA) was used to analyse obturators and gutta-percha cones. Micrographs were taken at increasing magnification (200×–4000×) on the surface of gutta-percha cones and obturators and on axial section of obturators. EDX analysis verified the overall chemical composition of obturators and gutta-percha cones and differences between inner/outer surfaces. EDX analysis was conducted in a selected window where the spectra were acquired.

Statistical analysis

After verifying normal distribution of values measured with Shapiro–Wilk test, comparison between groups was performed with Student's *t*-test for each level. Differences within each group were analysed with ANOVA test. When data were not normally distributed, the Mann–Whitney test was used to compare groups and the Kruskal–Wallis test for comparison within group. STATA 11 (College Station, TX: StataCorp LP) software was used. P values < 0.05 were considered significant.

Results

No voids were detected in both groups with bidimensional radiographs acquired after root canals obturation.

Confocal laser scanning microscope analysis

Sealer penetration into dentinal tubules: Mean and standard deviations of dentinal tubule penetration are summarised in Table 2. Medium depth of sealer penetration into dentinal tubules decreased in both obturation groups, significantly from 8 to 2 mm level from the apex (Table 2) in single-cone group, while in Obturator group, the decrease was significant among the three levels. Maximum sealer penetration into dentinal tubules decreased in both obturation groups (Fig. 1), significantly from 8 to 2 mm level from the apex in both groups and from 5 to 2 only in the Obturator group (Table 2). No statistically significant differences were found between obturation groups at 8, 5 and 2 mm levels from the apex concerning mean and maximum penetration depth (Figs. 2 and 3).

Table 2 Medium and maximum sealer penetration depths (Mean±SD) expressed in μm into dentinal tubules at 2, 5 and 8 mm levels from the apex, in Single-Cone Group and Obturator Group

Level from the apex	Medium penetration depth (μm)		Maximum penetration depth (μm)	
	Single cone	Obturator	Single cone	Obturator
8 mm	614 ± 301*	628 ± 193*,**	1296 ± 506*	1608 ± 446*
5 mm	360 ± 252	389 ± 211*	865 ± 447	1193 ± 338*
2 mm	148 ± 112	123 ± 118	431 ± 264	358 ± 257
Total	374 ± 299	380 ± 271	864 ± 540	1053 ± 630

*P < 0.05 versus 2 mm.
**P < 0.05 versus 5 mm.

Percentage of integrity of the sealer layer perimeter: A high percentage of integrity was found in both groups and in all examined levels. No significant differences were found both within and between groups (Table 3).

Percentage of sealer penetration decreases, significantly from the 8 mm to the 2 mm level in both groups (Table 3). No significant differences were found between groups at each level.

Scanning electron microscopy analysis

Obturators revealed a homogeneous and smooth external surface free from porosities and irregularities (Figs. 4 and 5). No microcracks were detected neither on gutta-percha coating nor in correspondence of inner carrier.

FEG-SEM/EDX analysis

Superficial analysis conducted by FEG-SEM on obturators confirmed the presence of a regular surface texture and a uniform structure with small wave-like depressions. Micrographs of gutta-percha cones showed a flat and uniform surface texture. EDX spectrum revealed the presence of Barium (Ba) and Zinc (Zn) on the outer obturator coating and in the inner obturators core. EDX survey on gutta-percha cones indicated the presence of Silicon (Si) and Zirconium (Zr) on the surface (Fig. 6).

Discussion

Mechanical interlocking between endodontic sealer and dentinal tubules is considered an essential requirement

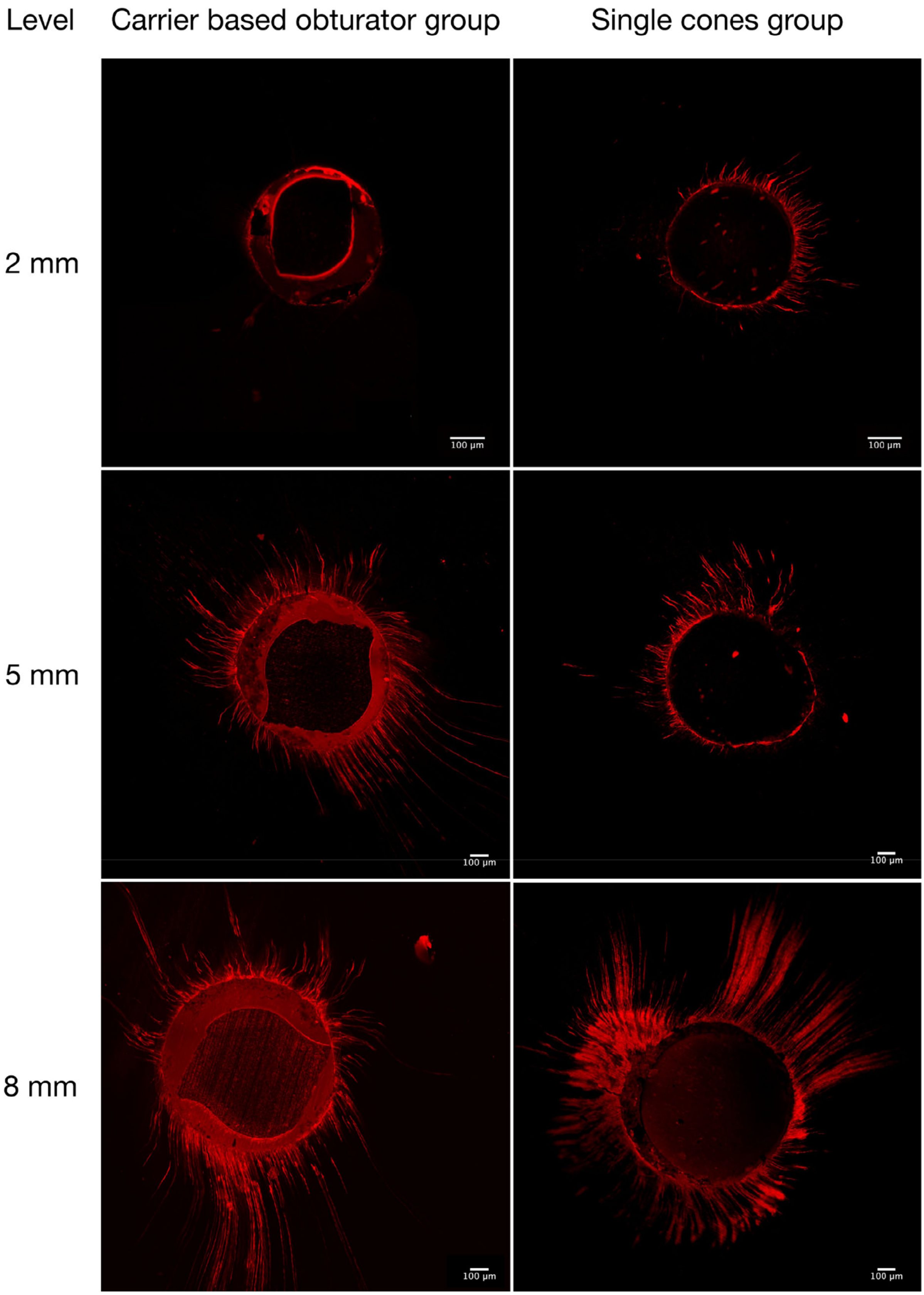
Level
Carrier based obturator group
Single cones group
2 mm
5 mm
8 mm
100 µm

Figure 1 Representative CLSM images from each experimental group at three standardised points. Depth of sealer penetration at 2, 5 and 8 mm from the apex is depicted. The outline of the tested carrier-based obturators is represented. A S-italic cross-section was noted with an asymmetrical profile, where the position of the core material was not completely centred but surrounded by a layer of gutta-percha in all the examined samples. A fluorescent sealer ring is noticeable around the canal wall in all sections of both obturation groups.

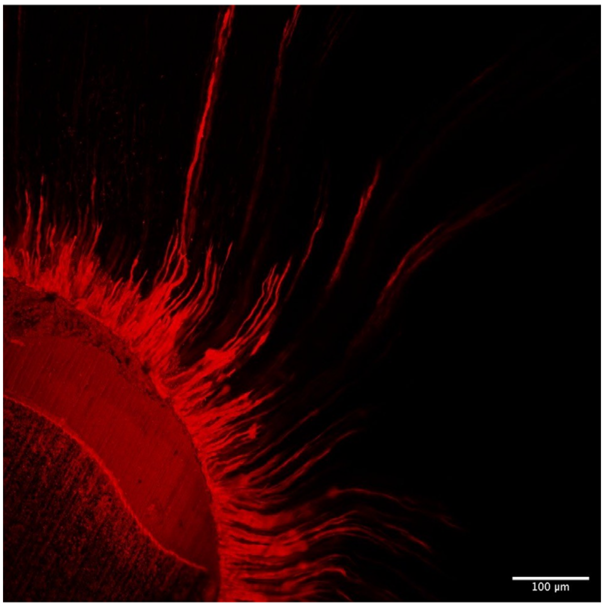

Figure 2 CLSM images of the HyFlex Obturator group at 5 mm from the apex. Resin tags of sealer labelled with Rhodamine B are visible.

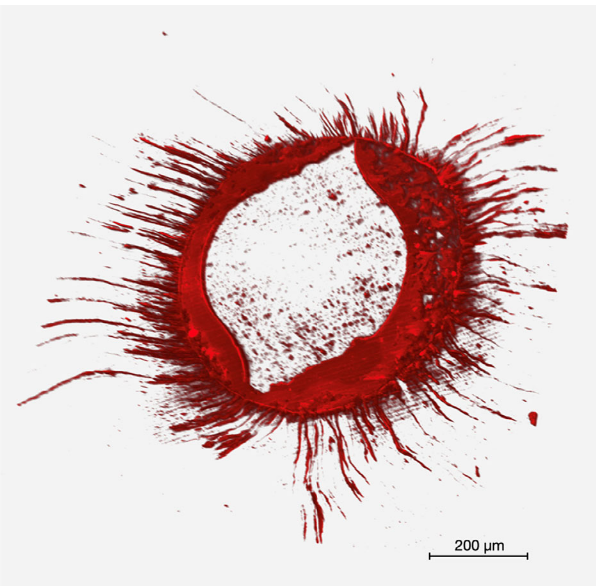

Figure 3 3D reconstruction of a representative 20 µm z-stack mosaic image of Obturator group obtained using the 3D tool in LasX software. Scale bar = 200 µm.

Table 3 Percentage of integrity of the sealer layer perimeter (%) at 2, 5 and 8 mm levels from the apex in Single-Cone Group and Obturator Group and percentage of sealer penetration (%) (Mean ± SD (µm)) at the same levels in both Groups

	Integrity (%)		Sealer penetration (%)	
Level from the apex	Single cone	Obturator	Single cone	Obturator
8 mm	99 ± 4	99 ± 3	90 ± 13*	90 ± 12*
5 mm	100	99 ± 2	77 ± 24	73 ± 24
2 mm	99 ± 3	94 ± 8	60 ± 23	59 ± 30
Total	99 ± 3	97 ± 5	76 ± 23	74 ± 26

*$P < 0.05$ versus 2 mm.

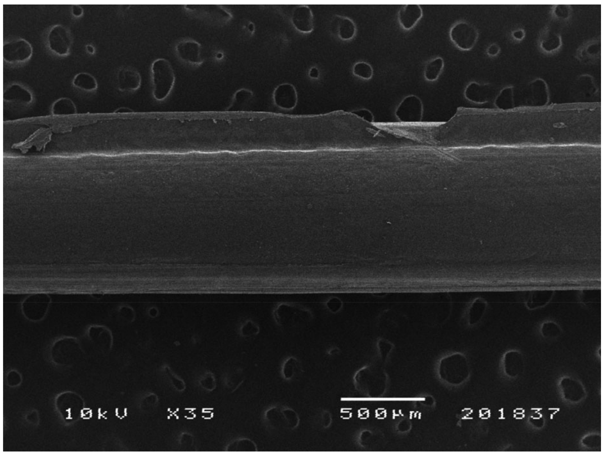

Figure 4 Representative SEM micrograph (35×) showing the middle portion of an Obturator. The superficial aspect is smooth with a longitudinal groove running from the tip to the coronal portion of the gutta-percha.

for clinical success of root canal treatment: the deeper a sealer can penetrate into dentinal tubules, the greater its mechanical retention to the dentinal walls would be (15).

GuttaFlow Bioseal was initially proposed by manufacturer to be used in combination with a gutta-percha master point (https://www.coltene.com/pim/DOC/IFU/docifu30003722-09-18-ifu-guttaflow-biosealsallaindv1.pdf). Being defined as a bioactive material for the presence of calcium silicate particles (8,16), GuttaFlow Bioseal was primarily conceived for cold obturation techniques. In fact, CS sealers have been shown to

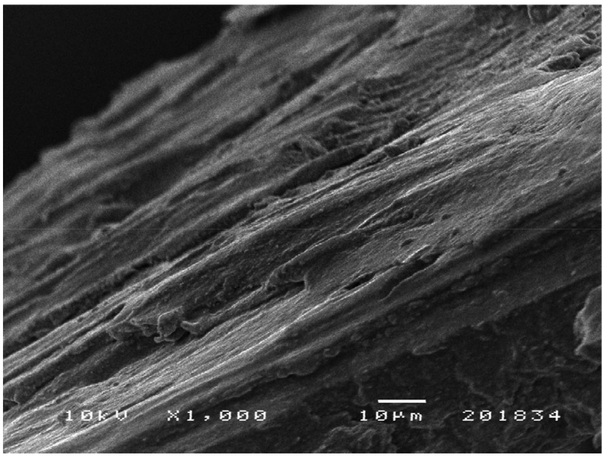

Figure 5 SEM micrograph (1000×) showing the outer surface of an Obturator. The superficial aspect is characterised by gutta-percha grooves oriented in parallel.

exhibit reduced setting time and flow when heated (17), and therefore their association with cold obturation techniques (i.e. laterally condensed gutta-percha or single cone) (17,18) was recommended. On the contrary, recent articles (19,20) reported that heat application resulted in improved physical properties of CS sealers, including setting time, flow, weight loss and chemical properties.

The current in vitro study investigates the effectiveness of GuttaFlow Bioseal sealer in association with a cold single cone or experimental carrier-based technique in terms of sealer penetration evaluated under confocal microscopy (CLSM).

Single cone obturation technique consists in a single matched gutta-percha cone used in association with sealer. The lack of condensation pressure during filling procedures generates a superior mass of sealer (21). Core obturators are a warm thermal root filling system,

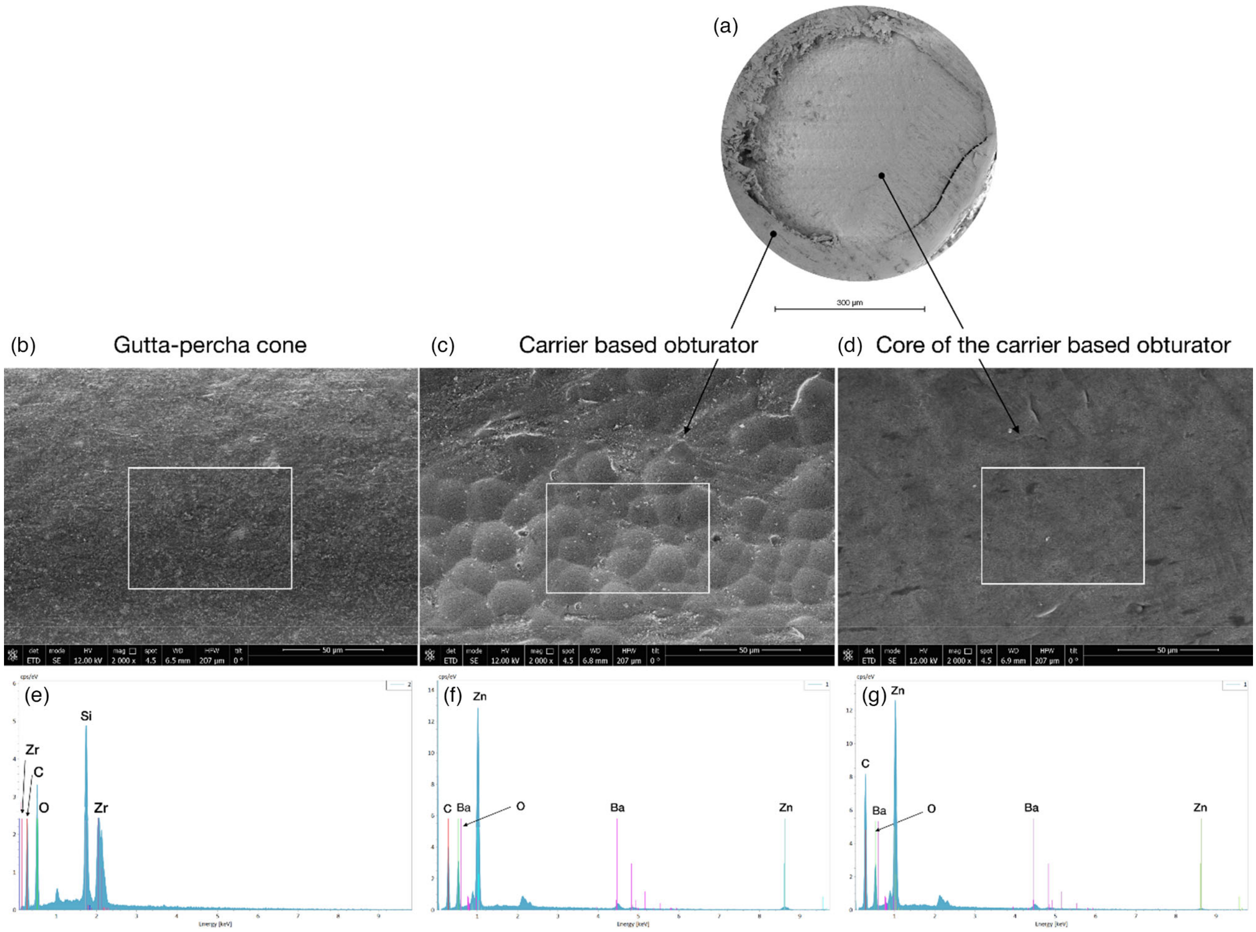

Figure 6 Representative EDX spectra of obturator and gutta-percha cones are reported and selected windows where the spectra were acquired are represented (white rectangles).

consisting of a core and a coating material based on resin and gutta-percha, which apply the heated gutta-percha three dimensionally into the root canal. Manufacturer' indications (https://idspr.coltene.com/fileadmin/Media/PR/IDS/docs/003283-03-17-EN-HyFlex-Obturators-Flyer-A4-VIEW.pdf) also suggest the complementarity with GuttaFlow Bioseal, but information is lacking on the effect of heat on this bioactive sealer. In fact, to the best of our knowledge, to date, no study has compared the effectiveness of the new warm obturator system in association with GuttaFlow Bioseal in terms of dentinal tubule penetration.

Samples were herein instrumented with HyFlex CM since the Core Obturators are matched for a perfect fit with the Hyflex systems. To avoid bias, the perfect fitting was also ensured in single-cone group, where precise single cone matched the canal preparation.

The penetration depth of material into dentinal tubules was evaluated under CLSM because it allows the detection of sealer penetration along the canal circumference of each sample using fluorescence (7). CLSM shows several advantages, such as not needing to destroy or dehydrate specimens (22), visualisation of optical sections below dentin surface under near normal conditions (23,24) at different depths (7). A small number of studies investigated the spread of sealer inside dentinal tubules using Thermafil obturators (13,14,25) or GuttaFlow Bioseal (26) but no one analysed the experimental Obturators, a new carrier-based system produced in 2020. The aim of the present research was to fulfil this gap of knowledge. In general, carrier-based systems are proven to represent a reliable obturation method with a good adaptation to canal anatomy with minimal voids (10) and adequate density of gutta-percha in the apical third of canals (27). These properties are essential for a positive outcome of root canal treatments and retreatments in the medium (11,28) and long term (12).

In the present research, the dentinal tubule penetration was evaluated by means of several parameters, such as depth, percentage of sealer penetration and integrity of the sealer layer perimeter. Sealer penetration into dentinal tubules has the benefit to prevent bacterial inactivation, to improve filling material retention thanks to mechanical interlocking and to block remaining bacteria within dentinal tubules (14,25). Therefore, sealer penetration into dentinal tubules may be considered clinically relevant. It depends on diameter of dentinal tubules and on the particle size of the material (7,29). The tested sealer, composed by zirconium, zinc, oxygen, silicon and calcium (18), has been previously reported with particle size in the range of 20–40 μm (26). Under EDX analysis, experimental carrier-based obturators were found to be mainly composed by gutta-percha in the outer and inner zones.

In the present study, penetration and sealing ability of GuttaFlow Bioseal in association with a warm obturation system revealed similar results when used with a cold technique in all regions of root canal. The sealer penetration was found to significantly decrease from the coronal and medium thirds to the apical third of the root, irrespective of the obturation technique. This aspect is ascribable to a minor tubule density and diameter in the apical root canal dentin (29). Moreover, the different diameter of dentinal tubules in mesiodistal and buccolingual cross-sections of roots was highlighted by a deeper sealer spread, suggesting the 'butterfly effect' (30) featured throughout the length of roots.

According to the present protocol, the sealer was in contact with warm obturators enhancing dentinal tubule penetration of the tested bioactive sealer. Indeed, it was recently demonstrated that the flow of the GuttaFlow (named in the text 'Guttaflow' but with characteristics and reference of the GuttaFlow Bioseal) was reduced by the effect of heat application (i.e. 100°C) (18) and therefore, it can be assumed that this deep penetration was related to the positive pressure exerted by the core-based material, more that to the effect of heat application on GuttaFlow Bioseal. It can be hypothesised that the increased pressure generated by obturators could equalise the sealer flow reduction, by explaining present findings where the sealer penetration was not affected by the filling technique. Nevertheless, further evaluation should assess the exact temperature of the warm obturators after heating, that can be supposed less than 100°C, and verify the properties of the sealer at the determined temperature.

Within the limits of this research, it can be concluded that warm carrier-based obturation technique does not seem to affect the bioactive sealer penetration when compared to single-cone cold technique. The null hypothesis was, therefore, accepted.

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Conflict of Interest

Chiara Pirani, Luigi Generali, Francesco Iacono, Francesco Cavani and Carlo Prati declare that they have no conflict of interest.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Author contributions

All authors have contributed significantly and all authors are in agreement with the manuscript.

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