In-vitro evaluation of apical microleakage of two obturation methods of immature permanent teeth: orthograde apical plug of Mineral Trioxide Aggregate and root canal filling combining custom gutta-percha cone with Calcium Silicate-based sealer

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GIE-129; No. of Pages 7
ARTICLE IN PRESS

Giornale Italiano di Endodonzia (2017) xxx, xxx—xxx

Società Italiana di Endodonzia

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/gie

ORIGINAL ARTICLE/ARTICOLO ORIGINALE

In-vitro evaluation of apical microleakage of two obturation methods of immature permanent teeth: Orthograde apical plug of mineral trioxide aggregate and root canal filling combining custom gutta-percha cone with calcium silicate-based sealer

Valutazione in vitro della infiltrazione apicale di due metodi di trattamento degli apici immaturi: chiusura ortograda con apical plug e MTA e chiusura con coni customizzati di guttaperca e sealer calciuo-silicatico Bioroot

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Received 14 May 2017; accepted 25 July 2017
Available online xxxxxx

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Peer review under responsibility of Società Italiana di Endodonzia.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.gien.2017.09.001

KEYWORDS
Apexification;
Custom gutta-percha cone;
Open apex;
Tricalcium silicate cement.

Abstract
Aim: The aim of this study was to assess whether an obturation, combining a custom gutta-percha cone with the BIOROOT™-RCS sealer, displays similar sealing quality to the orthograde apical plugs of MTA CAPS® in immature teeth with irregular wide apices.
Methodology: Thirty-four immature permanent premolars with apical diameter varying between (1—3 mm) were chosen for this study and were divided into two groups. They were imbedded in wet sponge, which simulated the periapex. In the first group; 5 mm orthograde plugs of MTA were placed using an appropriate plugger. In the second group; a custom gutta-percha cone was fabricated and used for root canal filling with the BIOROOT™-RCS sealer. The specimens were stored at 37 °C and 100% humidity during five weeks to allow the complete set of the filling materials. The apical leakage was evaluated using a dye penetration test with 50%-weight silver-nitrate. The teeth were then embedded in a transparent resin and sectioned transversally at 1 and 3 mm from the apex. The slices were examined under optical microscope and were given scores from (0) to (4). When scoring a slice was difficult, spectroscopy for energy dispersion using a scanning electron-microscope was used to confirm the score. The results were compared using the Fisher test with $p < 0.05$.
Results: Silver-nitrate was found in both groups in all slices at 1 mm. At 3 mm, the difference of micro-leakage was not significant.
Conclusions: The custom gutta-percha cone combined with BIOROOTtm-RCS sealer displays similar leakage resistance to the orthograde MTA plugs.

PAROLE CHIAVE
MTA. BIOROOT;
Sigillo apicale;
Tecnica ortograda;
Apici immature;
Tricalcio-silicati.

Riassunto
Obiettivi: Lo scopo del presente studio è stato valutare le capacità di sigillo marginale del BIOROOT™-RCS sealer utilizzato in combinazione con un cono di guttaperca customizzato e di confrontarlo con il sigillo ottenuto con la tecnica di chiusura ortograda con il sistem T CAPS® utilizzando elementi con apice irregolare ed immaturo.
Materiali e metodi: **Trenta quattro premolari** permanenti **immaturi** con diametro apicale variabile da 1 a 3 mm sono stati selezionati a divisi in due gruppi. Entrambi sono stati immersi in una spugna bagnata che ha simulato la area apicale. Nel primo gruppo è stata eseguita un otturazione ortograda di 5 mm con MTA CAPS utilizzando un adeguato plugger. Nel secondo gruppo è stato fabbricato un cono custom di guttaperca e applicato nel canale con il sealer BIOROOT. I campioni sono stati mantenuti a 37° in 100% di umidità per completare la reazione di indurimento. La penetrazione apicale del colorante è stata valutata con il nitrato di argento al 50%. I denti così trattati c sono stati inglobati in una resina trasparente e sezionati trasversalmente a 1 e 3 mm dall'apice. Le sezioni erano poi valutate al microscopio ottico e ad ognuna attribuito uno score da 0 a 4. Quando risultava difficile dare uno score, la sezione era valutata al microscopio elettronico a scansione collegato con un EDS (spettroscopio a dispersione di energia) per confermare il risultato. La valutazione statistica è stata effettuata con il Fisher test al valore $p < 0.05$.
Risultati: Il nitrato di argento (ovvero il colorante) è stato rilevato in entrambi i gruppi nelle sezioni a 1 mm. Nelle sezioni a 3 la differenza non era significativa.
Conclusioni: La tecnica custom-made con guttaperca e BIOROOT sealer ha dimostrato valori di penetrazione apicale del colorante al nitrato di argento simile alla tecnica ortograda tradizionale con MTA:

Introduction

Complete sealing of the root canal system with a biological inert material is essential[1] to prevent the re-infection of the root canal which is a major factor influencing the treatment outcome.[2] Root canal filling of immature teeth with thin dentinal walls and open apices needs a particular management; it is technically difficult to control the compaction of the root filling material.[3] Conventional root canal filling of the immature teeth with gutta-percha is not adequate as it does not reinforce the remaining root.[4,5]

In the literature, several therapeutic methods are proposed to solve this problem: partial pulpotomy, revascularization, apexogenesis and apexification.[6] Apexification is a method to induce a calcified barrier in a root with an open apex or the continued apical development of an incomplete root in teeth with necrotic pulp.[7] After apexification, the tooth is usually sealed with a root canal sealer and gutta-percha. Many studies demonstrated the efficacy of calcium hydroxide pastes to form a hard apical barrier[8] but this method requires a long period of treatment (mean time needed 12.9 month).[9] Such an extended time of treatment increases the tooth fracture risk.[10] Apexification with mineral trioxide aggregate MTA is a trustworthy technique to manage open apices[3,11,12] and is recommended by numerous studies to accomplish the apical closing.[3,13,14] The regenerative treatment of teeth with pulp necrosis and incomplete root formation is becoming part of the therapeutic endodontic arsenal of immature teeth. Despite the promising results of many published case reports, the protocol of revitalization procedure has not been established yet.[15] The regenerative procedures appear to develop an instructed endodontic rehabilitation instead of physiological-like tissue regeneration.[16]

Numerous in vitro studies have tried to assess the ability of different methods and materials to manage the root canal filling with immature teeth. However, most of these studies were performed on simulated immature apices, i.e. the apex was enlarged by using a Gates Glidden drill,[17] diamond bur[18] or Ni-Ti files.[19] Thus, the resulting shape of the simulated immature apex remains regular. The shape irregularities of the open apex in in vivo situations increase the difficulty of managing the root canal filling.

New sealing materials based on tricalcium silicate are getting an increased interest in the endodontic research. It was shown that these cements are biocompatible,[20,21] ensure a good root canal sealing[22,23] and increase the root fracture resistance.[24] A major disadvantage is the retreatability, these cements become hard after the setting and it is not possible to eliminate the cement completely by using the conventional retreatment methods.[25,26] Using tricalcium silicate cement with immature tooth could be interesting considering the reinforcement of the root structure, the good quality of sealing and the low risk of extrusion in the periapex as manufacturers recommend the use of single cone or lateral compaction techniques. When used for orthograde obturation, the calcium silicate-based cements showed similar marginal adaptation to the orthograde MTA plugs with simulated open apices.[19]

Recently, Septodont (Saint-Maur Des Fossés, France) has introduced a new tricalcium silicate sealer named (BIOROOT™ RCS). According to the manufacturer, this cement is made from pure calcium silicate and is monomer-free ensuring zero shrinkage, contains pure mineral formulation that will not stain teeth. It offers an excellent adhesion to dentin and to gutta-percha points, a great ability to seal auxiliary canals due to its high flowability and hydrophilic behavior that allows a continuous sealing in the presence of moisture.

The objective of this study was to assess whether a root canal filling, combining a fabricated custom gutta-percha cone with the BIOROOT™ RCS cement, displays comparable sealing quality to orthograde plugs of MTA CAPS® (Acteon, Pierre Roland, Mérignac-France) in immature teeth with irregular wide apices. The null hypothesis tested in this study is that there is no difference in apical leakage between these two methods using a dye penetration test with silver nitrate.

Materials and methods

Thirty-four immature permanent maxillary and mandibular premolars which where extracted for orthodontic reasons were selected for this study. The criteria for tooth selection were: wide apex, no visible root caries and fractures. The anatomical difficulty was evaluated by measuring the apical diameter by taking photos of the apices placed next to a millimeter paper with a camera Canon EOS 600D Digital SLR equipped with macro Tamron® SP 90MM F/2.8 Di VC USD 1:1. The teeth were divided into two groups (MTA group and B.C. group) with respect to their measured apical diameters to create two balanced groups.[27] All teeth were coated by two coats of two different nails polish. The crowns of all teeth were then removed to obtain a standardized length of 15 mm.

The working length (WL) is determined to be short 0.7 mm of the point where the K-file size 15 was first visible with a binocular at ×16 magnifications. In all specimens, the root canal preparation was chemical shaping rather than mechanical shaping. The teeth were irrigated with at least 5 mL of 2.5% sodium hypochlorite and 3 mL of 17% EDTA solution and were dried with paper points.

The samples were imbedded in wet sponge which simulated the periapex and prevented the extrusion of filling material out of the apex.[28]

In group 1 (the MTA group): 5 mm orthograde MTA plugs were placed in all teeth using an appropriate plugger. The teeth were then temporarily filled with a moist paper point and Cavit (ESPE, Cergy Pontoise, France). Two days later, the Cavit was removed and the remaining part of the canal was filled with GC-Fuji-IX GP Fast® (GC Corporation, Japan).

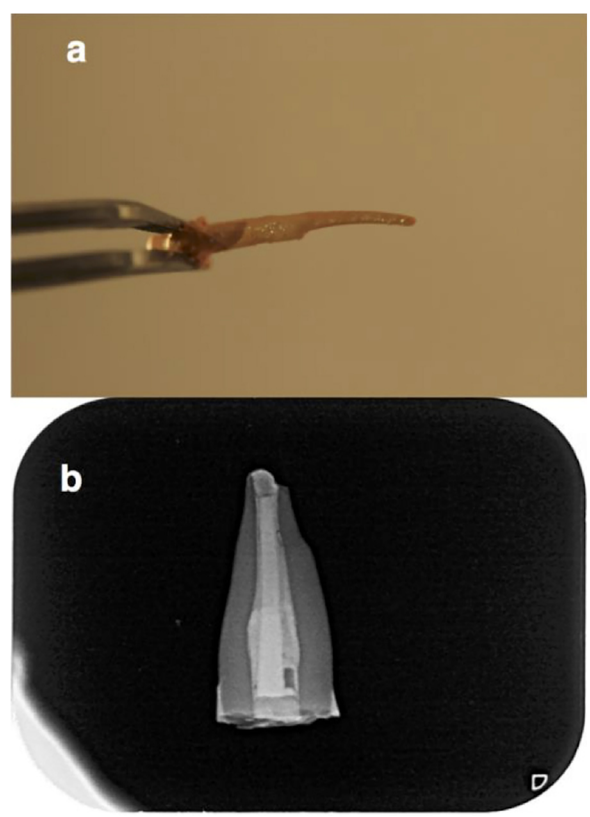

Figure 1 (a) The fabricated master gutta-percha cone. (b) The custom cone is fitted to the working length (Control radiography).

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In group 2 (the B.C. group): A non-standardized gutta-percha cone was fitted to within 3—4 mm of the working length. In very wide canals, two non-standardized gutta-percha cones were placed on a cold glass slap and were then rolled and merged by another heated glass slap to form one gutta-percha cone. The new cone was fitted to within 3—4 mm of the working length. The apical 3—4 mm of the fabricated cone were immersed in the Eucalypti-Aetheroleum® solution (Fagron, Rotterdam, Netherlands) for 15 s and the cone was then inserted, with light pressure, into the root canal which was filled with sodium hypochlorite 2.5%. The maneuver was repeated until the fabricated cone was fitted to the working length. The gutta-percha cone was rinsed and left in sodium hypochlorite 2.5% for few minutes to remove any Eucalypti residuals and to harden its surface. The canal was flushed with at least 5 mL of sodium hypochlorite 2.5% for and dried with paper points. The BIOROOT™ RCS cement was prepared in accordance with the manufacturers recommendations and placed in the root canal using the fitted gutta-percha cone (Fig. 1). The coronal part of canal was sealed with GC-Fuji-IX GP Fast® (GC Corporation, Japan). Radiographs were taken for all teeth after the root canal filling. The samples were then stored at 37 °C and 100% humidity for five weeks to allow the complete set of the sealer and the MTA.

Apical micro-leakage: A dye penetration test using 50% weight silver nitrate solution during one hour[29] and in absence of light was executed to all samples except for two teeth in each group which were randomly chosen to serve as a control groups. The teeth were then rinsed with distilled water for 5 min to remove any traces of sliver nitrate. All samples were then immersed in a photo-developing solution (Kodak Professional D-76, Germany) for 24 h in presence of light. The teeth were then washed in distilled water and scaled with ultrasonic hand piece to eliminate any residuals of silver nitrate. All specimens were embedded in a transparent polyester resin (Neovents, Sainte-Gemme, France) and were then transversally sectioned at 1 and 3 mm from the apex using a slow-speed saw with water cooling. The photographs of all slices were taken using a microscope (Leica—WILD M3B) at ×16 magnification and a digital camera Canon EOS 600D Digital SLR equipped with macro Tamron® SP 90MM F/2.8 Di VC USD 1:1. The photographs were evaluated by two experimented examiners; the dye penetration test was scored (0, 1, 2, 3, 4) when (0%, <25%, 25—50%, 50—75%, 75—100%) respectively of the canal circumference were concerned by the leakage of silver-nitrate; score 0 means absence of leakage, score 4 means complete infiltration (Fig. 2).

Scanning electron microscope analyses: Scanning Electron Microscopy (JEOL JSM-5310LV) was chosen to confirm the presence/absence of silver-nitrate in the interface material/dentin or in dental tubules using spectroscopy for energy dispersion EDS (Figs. 3 and 4).

Statistical analysis: Scores at 1 and 3 mm were analyzed using the Fisher test with ($p < 0.05$). Kappa values were calculated to assess the inter-examiners agreement regarding slices scores.

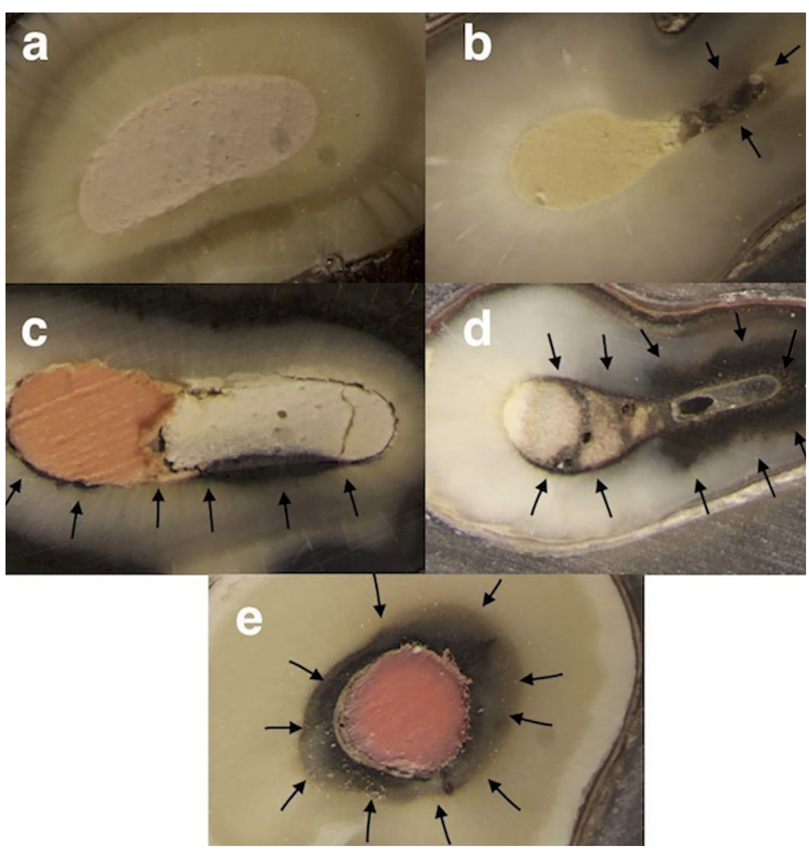

Figure 2 Scores of the dye test. (a) Score 0: Absence of leakage. (b) score 1: <25% of canal circumference were concerned by the leakage of silver nitrate. (c) Score 2: 25—50% were concerned. (d): Score 3: 50—75% were concerned. (e) Score 4: complete infiltration.

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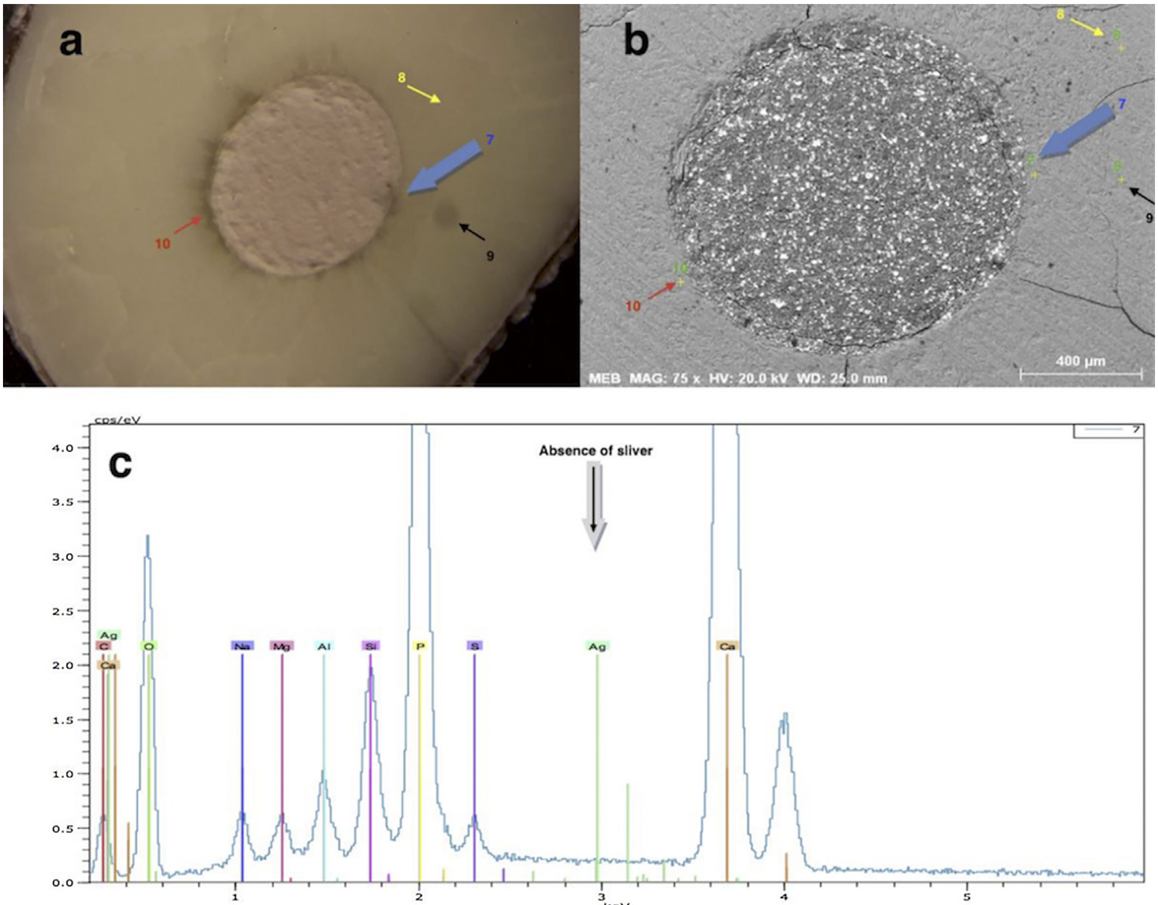

Figure 3 Scanning Electron Microscope analysis of this slice. (a) Choosing multiple points of interest. (b) Choosing the same points on the SEM image. (c) Energy Dispersion Spectroscopy EDS of the point n°7 indicates the absence of the dye in this point. The same procedure was repeated for all points of interest and the final score given to this slice was 0 due to the absence.

Results

The minimum and the maximum apical diameters were nearly 1 mm and nearly 3 mm respectively. In the MTA group, the Kappa values were 1 at 1 mm and 0.73 at 3 mm. In the B.C. group, the Kappa values were not calculable at 1 mm and 0.59 at 3 mm; a strong agreement was found between examiners.

For the first examiner, the *p*-values between two groups were 1 at 1 mm and 0.05 at 3 mm.

For the second examiner, the *p*-values between two groups were 1 at 1 mm and 0.26 at 3 mm.

No significant difference in leakage was proved between the two methods.

For both examiners at 1 mm, none of the slices of the B.C. group obtained a score of 0 whereas only one slice of the MTA group obtained a score of 0.

Discussion

When the root filling material can prevent the leakage of small molecules, it would probably prevent the passage of microorganisms.[30] A dye penetration test using sliver-nitrate was used in this study to evaluate the apical leakage since it offers clear results and the possibility of the SEM analysis.[31] The fluid transport method is a sensitive technique, and if not standardized, the precision of the results is operator dependent.[32] The bacterial leakage model was criticized since possible microbial leakage pathways can exist and thus results are incorrect.[33] Analyzing marginal adaptation using SEM is also used to evaluate the resistance to leakage.[19,29] However, samples sectioning can possibly shift the filling material and can create hiatus.

The apical sealing of both methods was assessed in more physiologically accurate conditions and not in enlarged apices where the shape remains relatively regular. The interruption of radicular edification results in a large variety of apical shapes.[3] The results of the current study highlight a considerable difficulty to manage successfully the orthograde root canal filling of immature teeth with irregular wide apices. Both methods showed unsatisfying apical sealing and the null hypothesis was accepted. When the apical diameter exceeds 1 mm, conventional orthograde obturation methods seem to be unable to prevent the apical leakage.

In the current study, silver-nitrate was found nearly in all slices at 1 mm whereas at 3 mm, a slight superiority was found in the MTA group over the B.C. group. That can be explained by the compaction of MTA with a suitable plugger, whereas in the B.C. group, no compaction was executed. The fabricated custom gutta-percha cone combined with a

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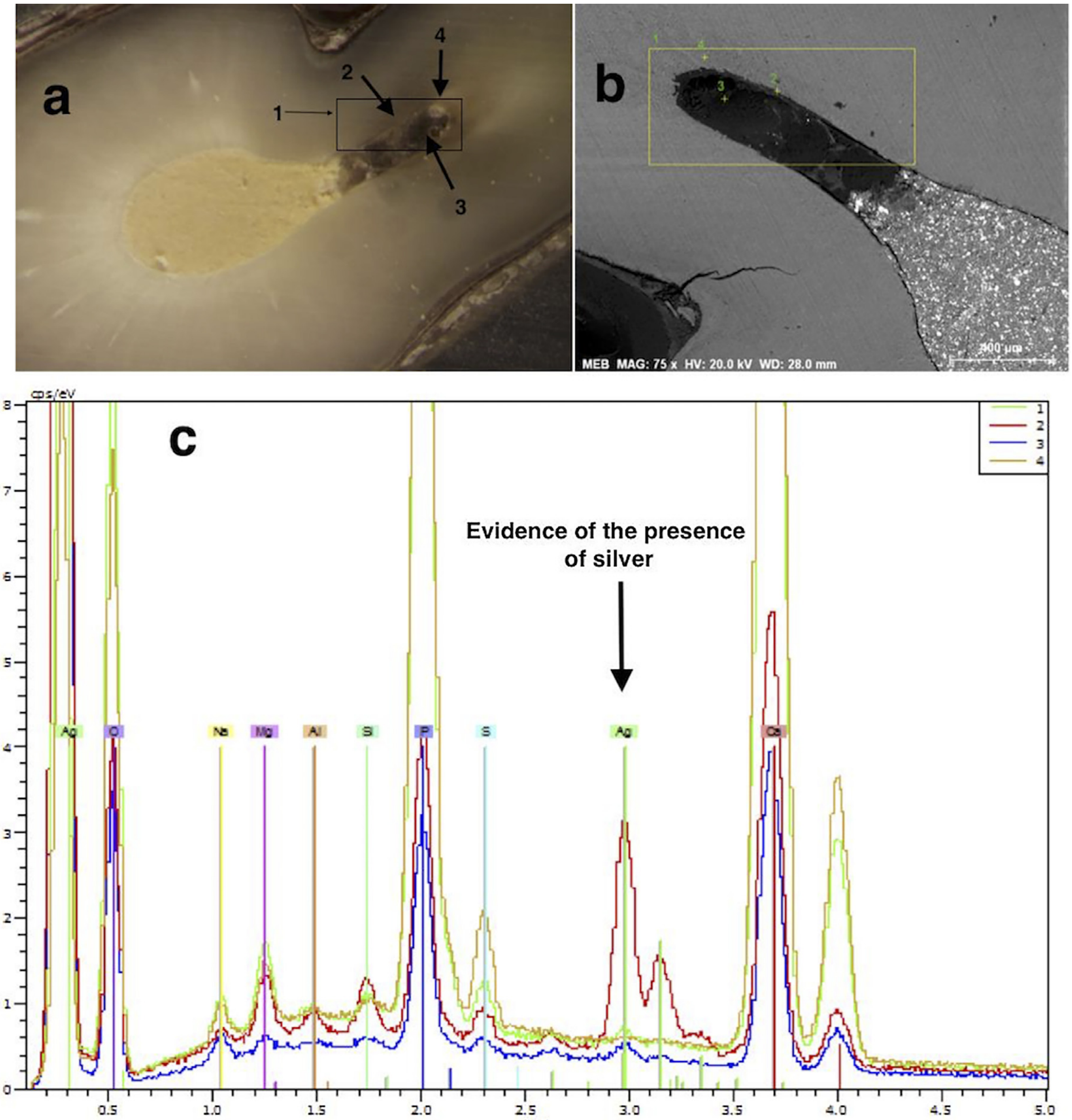

Figure 4 Scanning Electron Microscope analysis of this slice. (a) Choosing a zone of interest 1 and multiple points 2, 3, 4. (b) Choosing the same points on the SEM image. (c) Energy Dispersion Spectroscopy EDS of these points indicates the presence of Silver at least with point 2.

tricalcium silicate sealer (BIOROOT^{TM} RCS) displays similar leakage results to the trustworthy method of the MTA plugs, and can be considered for the everyday practice due to its easiness where no specific materials are needed.

Only regenerative procedures allow the radicular edification to be resumed. Future investigations of regenerative dentistry would possibly establish an authentic treatment of immature teeth with wide irregular apices.

Conclusion

Within the limits of this study, it can be concluded that a fabricated custom gutta-percha cone combined with tricalcium silicate sealer BIOROOT^{TM} RCS displays similar leakage resistance to the orthograde MTA plugs and, due to its easiness, can be taken into consideration to manage immature teeth with wide irregular apices.

Declaration

All authors have contributed significantly and agree with the content of the manuscript.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors deny any conflict of interest.

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