Apical microleakage evaluation of a new proposal for endodontic sealer associated with hydroxyapatite: an ex vivo study

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Abstract — The filling of the root canal system has a fundamental role in endodontic treatment, since it will take the place of the root pulp, thus the material must act by isolating the root canal system, preventing the penetration of microorganisms and their toxic by-products, which can compromise the prognosis of the procedure. The present study was carried out to evaluate the apical microleakage of a hydroxyapatite modified sealer. After removal of crowns and endodontic instrumentation, 40 selected maxillary single root teeth were randomly divided into two experimental groups (n = 15 each) according to the sealer: GENDO – Endomethasone Sealer / GENDOHX - Endomethasone Sealer +5% hydroxyapatite, and two control groups (n = 5 each). The root canals of specimens from the experimental group were filled with the cold lateral condensation technique. To assess apical microleakage, the apical linear dye penetration was measured microscopically from apex to most coronal part and data were statistically analyzed. Descriptive analyses were performed, followed by the Mann-Whitney test. The mean values of leakage observed in the groups were GENDO – 0,95± 1,80 and GENDOHX – 1,01± 1,82. No significant differences were found between experimental groups (p>0,05). Conclusion: The addition of hydroxyapatite to the endomethasone sealer did not interfere with its apical sealing capacity.

1. INTRODUCTION

For the endodontic treatment to be successful, a sequence of procedures must be performed inside the root canals, starting with the removal of all organic and inorganic content present inside the root canal [1]. Although the chemical-mechanical preparation is a very important step in the endodontic treatment, modeling and decontamination of the canal will be of no use if the sealing promoted at the end by the endodontic filling is faulty [2].

A three-dimensional root canal filling has a fundamental role in endodontic treatment, since it will take
the place of the root pulp, thus the filling must act by isolating the root canal system, preventing the penetration of microorganisms and their toxic by-products, which can compromise the prognosis of the procedure [3]. The majority of endodontic failures are caused by the microleakage resulting from incomplete obturation [4].

The obturation technique using a main gutta-percha cone wrapped in sealer is the most widely used in endodontics worldwide [5].

Root canal sealers are important in achieving a three-dimensional filling by reducing apical and coronal microleakage [6]. Thus, such material must meet the following requirements: biocompatibility, allow healing and an adequate apical sealing [7]. Therefore, new possibilities may arise regarding the active principle of new endodontic cements, and hydroxyapatite represents one of these alternatives.

Hydroxyapatite within the dental area is used to prevent bone loss in alveolar regions after extraction of one or several dental elements, as well as recovery of areas with bone resorption. Hydroxyapatite coated titanium pins are used in the implant for root replacement and are being researched in other areas such as endodontics [7]. Other dental specialties can benefit from the use of this hydroxyapatite-based biomaterial, such as endodontics. Therefore, the possibility of formulating a new material or integrating hydroxyapatite into an existing sealer is justified.

In order to analyze the apical sealing promoted by endodontic cements, several methodologies have been proposed, but the most widely used analysis is the linear measurement of dye penetration in the apical region through the foramen [8]. Thus, new possibilities for endodontic sealers must still be tested, with the objective of comparing and observing better possibilities of materials to be applied clinically.

Thus, the aim of this study was to evaluate the apical sealing capacity of Endomethasone N (Saint-Maur-des-Fossés Cedex - Franca) associated with hydroxyapatite in 5%. The null hypothesis tested in this study was that there is no difference in the apical sealing capacity between the endodontic sealers groups tested.

II. MATERIALS AND METHODS

Specimen selection and preparation:

Once approval from the Human Research Ethics Committee of the Pontifical Catholic University of Campinas had been obtained (no. 3.653.397), 40 freshly maxillary incisors had been extracted for various reasons were included in the present study based on the inclusion and exclusion criteria. Tooth with root caries, fracture line, open apex, external/internal resorption, calcified canals or curved roots were replaced. Teeth were selected and disinfected by soaking in 1% chloramine-T trihydrate solution for ten days.

The crowns of the all teeth were coronated at cementoenamel junction with a diamond disc (Horico Dental HpF; Ringleb, Berlin, Germany) coupled to a slow-speed handpiece powered by a micromotor, under constant refrigeration, standardizing roots segments of 15 mm in length. Working length was determined by passing a size K#15 (Dentsply Maillefer, Ballaigues, Switzerland) into the canal until the tip of the file was just visible through the apical foramen. The final working length was obtained after shortening 1 mm from the real root canal length.

The biomechanical preparation was carried using WaveOne Gold Large 45.05 (Dentsply Maillefer, Ballaigues, Switzerland) reciprocating system following the manufacturer's recommendations. A crown-down approach was employed in preparing the root canals using the X-Smart Plus (Dentsply Maillefer, Ballaigues, Switzerland) electric motor.

The canals were irrigated with 5 ml of 2.5% sodium hypochlorite (NaOCl) solution for each preparation per root third to rinse the canal and remove organic residues. In all groups, after each cycle of instrumentation and irrigation, foramen patency was controlled with a #10 K-file advanced 1 mm beyond the foramen. After instrumentation was completed, 5mL of 17% EDTA were introduced and ultrasonically activated in 3 cycles of 20 seconds [10]. Next, a final flush with 5 ml of NaOCl followed by 5.0 mL of saline was performed. The root canals were dried with paper point size 45.05 (Dentsply Maillefer, Ballaigues, Switzerland).

Group allocation:

The samples were randomly allocated into two experimental groups (n=15) and two control groups (n=5) using a computer algorithm (www.random.org). The endodontic sealer used in endodontic filling represented each experimental group:

- **GENDO = Endomethasone N Sealer**
- **GENDOHX = Endomethasone N Sealer associated with 5% hydroxyapatite**
- **Control Group (+) = roots were obturated with gutta-percha but without sealer;**
- **Control Group (-) = Samples in the negative control group did not receive root canal fillings.**
Root Canal Filling:

Obturation of the root canal was performed using lateral compaction technique associated with the main cone of gutta percha wave one gold large 45.05 (Dentsply Maillefer, Ballaigues, Switzerland) and the respective sealers.

The Endomethasone sealer was manipulated according to the manufacturer’s instructions. In group GENDOHX a 5% amount of hydroxyapatite was added to the endomethasone sealer powder using a precision analytical balance.

The tip of each pre-selected master cone was slightly coated with its respective sealer and inserted into the prepared canal using in-and-out pumping motion until reaching the full working length.

Lateral compaction was done and accessory cones with light coats of sealer around them were placed. The spreader was placed beside the gutta-percha to create sufficient space for the accessory cones.

Excess gutta percha from the canal orifice was removed by using a heated endodontic plugger and then vertically condensed with other could endodontic pluggers to the level of the canal orifice. The access cavity was sealed with glass-ionomer cement.

The quality of root canal filling was assessed radiographically. The specimens were stored in saline (100% humidity) at 37°C for 1 week to allow complete setting of sealers.

Preparation of Specimen for Microleakage measurement:

After 1 week, teeth (experimental and positive control groups) were air-dried and the external root surface was coated with 3 layers of nail varnish, except for the apical 4 mm. The roots including apical foramen in the negative control group were entirely covered with nail varnish.

Apical leakage was estimated using a dye penetration test then all samples were immersed in 1% methylene blue dye and stored at 37°C for 72 h.

After a diamond disk was used to longitudinally section the root in a bucco-lingual direction.

The split segments were examined using an operative microscope (8X magnification) to evaluate the linear dye penetration from the apex to the most coronal part of the root in millimeters using Image J software program (Fig.1).

A single operator completed all preparations and testing procedures.

Statistical analysis:

The results were statistically analyzed by Shapiro-Wilk and Mann-Whitney test using IBM SPSS version 20 (IBM Corporation 1 New Orchard Road Armonk, New York 10504-1722, United States), at a significance level of 5%.

III. RESULTS

The mean values and standard deviations of apical dye penetration for experimental groups are presented in Table 1.

Table 1: Mean apical linear dye penetration values (in mm)

| Group     | Minimum | Maximum | MA (SD)       | MD (IQD) |
|-----------|---------|---------|---------------|----------|
| GENDO     | 0.00    | 4.21    | 1.44 (1.30)   | 0.95 (1.80) |
| GENDOHX   | 0.11    | 4.02    | 1.48 (1.35)   | 1.01 (1.82) |

(P-MW) 0.4098

Abbreviations: MD, Median; IQD, interquartile deviation; MA, Mean; SD, standard deviation.
The evaluation of the dye linear infiltration indices showed no statistical difference between the experimental groups (p>0.05).

The negative control group showed no leakage, while the positive control groups showed complete leakage through the canal space, which confirms and validates the experimental method (Fig. 2).

No specimens were damaged in the split process.

Fig. 2: Microscope images of tested samples showing linear dye penetration: A: GENDO (GP/Endomethasone), B: GENDOHX (GP/Endomethasone + 5% Hydroxyapatit), C: Negative control group, D: Positive control group.

IV. DISCUSSION

Incomplete endodontic filling of the root canal system with inadequate apical and coronal sealing has been pointed out as one of the main causes of endodontic failures [11,12]. Therefore, the root canal filling should seal the canal space both apically and coronally to prevent microorganisms and tissue fluids from entering the canal space.

Thus, this study was carried out to evaluate the apical sealing capacity of a variation of endodontic sealer in which 5% hydroxyapatite was added with the possibility of later analyzing a possible improvement in its biocompatibility.

In the present study, the linear measurement of dye penetration technique with 1% methylene blue dye was used to compare the apical infiltration in root canals after endodontic filling. Linear measurement of dye penetration is the one such method that is most common, relatively easy and fast to gauge the microleakage of the sealers [13].

A range of methodologies to assess microleakage of the root canal space is reported in the literature. Among them the use of scanning electron microscopy, radioisotope penetration, bacterial penetration, electrochemical analysis, fluid filtration and dye penetration employed in the study. In the analysis performed by Wu et al. 1993 [14], radioisotope penetration or dye infiltration has been used in more than 80% of the sealing studies performed in endodontics.

Different dyes are used in sealing studies such as India ink, Eosin, Procion, brilliant blue, 50% silver nitrate, Pelican ink and, most common, methylene blue. In the case of Methylene Blue, the most used concentrations are 0.25, 1 and 2%. In present study, was used 1% as it is the most commonly indicated as in studies 15, 16. Ahlberg KM et al. 1995 [17] noted that methylene blue at 1% is superior to other options in terms of penetration and has a low molecular weight comparable to some bacterial by-products [18, 19, 20, 21, 22].

The cold lateral gutta-percha condensation technique was used and has been considered a gold standard filling technique by many studies, reflecting the good clinical results observed [23,24]. Some studies in the literature do not mention significant differences in the cold technique compared to other filling techniques used [25,26,27].

After the filling of the specimens, the roots were stored at 37 °C and 100% humid for seven days, as in other researches, to promote the complete setting of the sealer and provide an environment similar to the oral cavity [28,29,30,31].

The results of the study mention that in both groups, regardless of the sealer formulation tested, they did not totally prevent the apical infiltration of the dye. The positive control group resulted in higher levels of infiltration, indicating that the sealing ability of single-cone gutta-percha is deteriorated when used without a root canal sealer. On the other hand, the negative control group did not show apical leakage, which confirms the sealing of the varnish used in this methodology.

The incorporation of hydroxyapatite to zinc oxide sealer can be an alternative in the condition of improved biocompatibility with endodontic sealer.

According to the results of the present study regarding dye infiltration, the zinc oxide eugenol sealer and its
version incorporated at 5% hydroxyapatite did not differ from each other. This result demonstrates that such biocompatible substrate extracted from the dental element itself did not minimize its apical sealing capacity of the root canal system.

A chemical process of decalcification and reduction of substrates from the tooth itself or even from eggshells, bovine bone or even fish scales can be sources for obtaining hydroxyapatite [32,33,34,35].

The endodontic sealer Endomethasone N has a vast amount of publications in the literature and, even though it is not a resin or even bioceramic sealer, it presents satisfactory results in several aspects when evaluated [36,37,38,39,40].

As observed in the literature, endodontic sealers based on zinc oxide and eugenol have a disadvantage in terms of biocompatibility, and from this point came the possibility of incorporating a substrate to their compound that could provide a better condition for interaction and less cytotoxicity with the periapical tissues [40,41,42].

The zinc oxide eugenol sealer was chosen to be added to hydroxyapatite due to its previous presentation in powder condition. Paste-paste sealers such as resinous ones would make it difficult to pre-handle hydroxyapatite to its previous weight.

It is known that other tests need to be carried out in relation to the possible new sealer formulation, including the variation of the percentage added to the material.

Hydroxyapatite has been used in several fields in the health area and, specifically in dentistry, as a biomaterial applied in the condition of grafts or bone defects [43,44,45,46,47, 48].

New formulations of endodontic sealers must be tested, mainly with active principles of biomaterials that allow the sealing of the root canal system and concomitantly a biocompatibility, stimulating apical repair when necessary.

V. CONCLUSION

According to the methodology employed, it was possible to conclude that the addition of hydroxyapatite to the endomethasone sealer did not interfere with its apical sealing capacity.

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REFERENCES

[1] AL-Hadlaq SMS, AlDosari MAM, Al-Maneei KK. Comparison of the Shaping Ability of WaveOne and Reciproc NiTi Files. J Endod. 2013 Feb;39 (3):1-39.
[2] Arikatla, S.K.; Chalasani U.; Mandava J.; Yelisela R.K. Interfacial adaptation and penetration depth of bioceramic endodontic sealers, Journal of Conservative Dentistry, India, v.21, n.4, p.373-377, 2018.
[3] Hiller, K.; Buchalla, W.; Grillemeier, I.; Neubauer, C.; Schmalz, G. In vitro effects of hydroxy apatite containing tooth pastes on dentin permeability after multiple applications and ageing. Scientific Reports, Estados Unidos, v.8, n.4888, p.1-13, 2018.
[4] Mohamed El Sayed MA, Al Hussein H. Apical dye leakage of two single-cone root canal core materials (hydrophilic core material and gutta-percha) sealed by different types of endodontic sealers: An in vitro study. J Conserv Dent 2018;21:147-52.
[5] Candeiro GT, Correia FC, Duarte MA, Ribeiro-Siqueira DC; Gavani G. Evaluation of radiopacity, ph, release of calcium ions, and flow of a bioceramic root canal sealers, J Endod, 2012;38:842-45.
[6] De Almeida WA, Leonardo MR, Tanomaru Filho M, Silva LA. Evaluation of apical sealing of three endodontic sealers. Int Endod J 2000;33:25-7.
[7] Pawar SS, Pujar MA, Makandar SD. Evaluation of the apical sealing ability of bioceramic sealer, AH plus & epiphany: An in vitro study. J conserve Dent. 2014;17:579-82.
[8] Costa, C.C.R.; Rocha, V.G.N.; Habitante, S.M.; Raldi, D.P.; Marques, J.L.L. Análise da infiltração apical de um novo cimento endodôntico a base de mta. Cienc Odontol Bras. abr./jun.; 12 (2): 35-40, 2009.
[9] Lone, M.M.; Khan, F.R.; Lone, M.A. Evaluation of microleakage in single-rooted teeth obturated with thermoplasticized gutta-percha using various endodontic sealers: An in-vitro Study, Journal Coll Physicians Park, Inglaterra, v.28, n.5, p.339-343, 2018.
[10] Van der Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J 2007 Jun;40(6):415-26. doi: 10.1111/j.1365-2951.2007.01243.x. Epub 2007 Apr 17.
[11] Ballullaya SV, Vinay V, Thumu J, Devalla S, Bollu IP, Balla S. Stereomicroscopic Dye Leakage Measurement of Six Different Root Canal Sealers. J Clin Diagn Res. 2017 Jun;11(6):ZC65-ZC68.
[12] Thoden van Velzen SK, Duivenvoorden HJ, Schuurs AH. Probabilities of success and failure in endodontic treatment: A Bayesian approach. Oral Surg Oral Med Oral Pathol 1981;52:85-90.
[13] Roggendorf MJ, Ebert J, Petchelt A, Frankenberger R. Influence of moisture on the apical seal of root canal fillings with five different types of sealer. J Endod. 2007;33:31-33.
[14] Wu MK, De Gee AJ, Wesselink PR, Moorer WR. Fluid transport and bacterial penetration along root canal fillings. Int Endod J 1993;26(4):203–8.
[15] Baker PS, Oguntebi BR. Effect of apical resections and reverse fillings on Thermafil root canal obturations, J

www.ijaers.com
derived hydroxyapatite. J Basic Med Sci. 2020 Nov 2;20(4):430-437. doi: 10.17305/jbms.2020.4888.

[10] Oduoke JK, Danyu Y, Baruwa AD, Azeez AAJ. Synthesis and characterization of hydroxyapatite from bovine bone for production of dental implants. Appl Biomater Funct Mater. 2019 Apr-Jun;17(2):22800800193836829.

[11] Liu Y, Li J, Wang D, Yang F, Zhang L, Ji S, Wang S. Enhanced extraction of hydroxyapatite from bighed carp (Aristichthys nobilis) scales using deep eutectic solvent. J Food Sci. 2020 Jan;85(1):150-156. doi: 10.1111/1750-3841.14971. Epub 2019 Dec 26.

[12] Ricucci D, Röças IN, Alves FR, Loghin S, Siqueira JF Jr. Apically Extruded Sealers: Fate and Influence on Treatment Outcome. J Endod. 2016 Feb;42(2):243-9. doi: 10.1016/j.joen.2015.11.020. Epub 2015 Dec 24.

[13] Suzuki P, Souza Vd, Holland R, Gomes-Filho JE, Murata SS, Dezan Junior E, Passos TR. Tissue reaction to Endomethasone sealer in root canal fillings short of or beyond the apical foramen. J Appl Oral Sci. 2011 Oct;19(5):511-6. doi: 10.1590/1678-775720100500013.

[14] Jeannecu C, Giraud T, Milan JL. About Investigating unset endodontic sealers’ eugenol and hydrocorisone roles in modulating the initial steps of inflammation. I Clin Oral Investig. 2020 Feb;24(2):639-647. doi: 10.1007/s10268-019-02957-2. Epub 2019 May 21.

[15] Lucena-Martín C, Ferrer-Luque CM, González-Rodríguez MP, Robles-Gijón V, Navajas-Rodríguez de Monedo JM. A comparative study of apical leakage of Endomethasone, Top Seal, and Roeko Seal sealer cements. J Endod. 2002 Jun;28(6):423-6.

[16] Teixeira ABV, de Castro DT, Schiavon MA, Dos Reis AC. Cytotoxicity and release ions of endodontic sealers incorporated with a silver and vanadium base nanomaterial. Odontol. 2020 Oct;108(4):661-668. doi: 10.1007/s10266-020-00507-x. Epub 2020 Mar 21.

[17] Komabayashi T, Colmenar D, Cvach N, Bhat A, Primus C, Imai Y. Comprehensive review of current endodontic sealers. Dent Mater J. 2020 Sep;29(5):703-720. doi: 10.4017/dmj.2019-288. Epub 2020 Mar 24.

[18] Benetti F, de Azevedo Queiroz IÖ, Oliveira PHC, Conti LC, Azuma MM, Oliveira SHP, Cintra LTA. Cytotoxicity and biocompatibility of a new bioceramic endodontic sealer containing calcium hydroxide. Braz Oral Res. 2019;33:e042. doi: 10.1590/1807-3107bor-2019.vol33.0042. Epub 2019 May 16.

[19] Guillen-Romero LD, Oropesa-Guzmán MT, López-Maldonado EA, Iglesias AL, Paz-González JA, Ng T, Serena-Gómez E, Villarreal-Gómez LJJ. Synthetic hydroxyapatite and its use in bioactive coatings. Appl Biomater Funct Mater. 2019 Jan-Mar;17(1):2280800018817463.

[20] Kumar A, Biswas K, Basu B. Hydroxyapatite-titanium bulk composites for bone tissue engineering applications. J Biomed Mater Res A. 2015 Feb;103(2):791-806. doi: 10.1002/jbmr.35198. Epub 2014 Apr 25.

[21] Singh R, Ismail PMS, Kambil V, Kumar R, Singh KD, Kochhar AS, Babaji P. Evaluation of Hydroxyapatite Granules, CERAMENT, and Platelet-rich Fibrin in the
[46] Washio A, Morotomi T, Yoshii S, Kitamura C. Bioactive Glass-Based Endodontic Sealer as a Promising Root Canal Filling Material without Semisolid Core Materials. Materials (Basel). 2019 Nov 29;12(23):3967. doi: 10.3390/ma12233967.

[47] Andrade ALM, Rennó ACM. Bone substitutes and photobiomodulation in bone regeneration: A systematic review in animal experimental studies. J Biomed Mater Res. 2021;1–11. https://doi.org/10.1002/jbm.a.37170

[48] Zaen El-Din AM, Hamama HH, Abo El-Elaa MA, Grawish ME, Mahmoud SH, Neelakantan P. The effect of four materials on direct pulp capping: An animal study. Aust Endod J. 2020 Aug;46(2):249-256. doi: 10.1111/aej.12400. Epub 2020 Mar 4.