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Técnicas in vitro para evaluar la remoción de barrillo dentinario por agentes irrigantes endodónticos: Revisión de la literatura.

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Abstract. Introduction: The purpose of this review is to address the most commonly used techniques for evaluating smear layer removal ability or chelating capacity of root canal irrigants, including Energy Dispersive X-Ray Spectroscopy (EDS or EDX), Atomic Absorption Flame Spectrometry (AASF), wavelength dispersive X-ray fluorescence spectrometry (WDXRF), inductive coupled plasma emission spectroscopy (ICP-AES), Scanning Electron Microscopy (SEM), and Fourier Transform Infrared Spectroscopy (FTIR). **Methods:** An electronic literature search was conducted in the Pub Med / MEDLINE database of indexed journals from 1992 to 2020. The search terms included chelating, chelation, calcium chelation, smear layer, smear layer removal, and demineralizing effect. **Results:** All the techniques were classified in terms of their results, both quantitatively and qualitatively. Even though smear layer removal and chelating capacity are not the same parameters, most of the studies included both techniques to correlate their results. SEM is the most commonly used technique for evaluating smear layer removal using various root canal irrigants. Ethylenediaminetetraacetic acid (EDTA) (17%) was the most widely studied root canal irrigant. **Conclusion:** Different techniques can be used to evaluate smear layer removal and chelating capacity of root canal irrigants. All of these methods have their corresponding advantages and disadvantages. This study aimed to provide researchers with a background

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for the selection of technique(s) to study the irrigant´s capacity for calcium chelation, which is applicable to smear layer removal.

Key words: smear removal, root canal irrigants, evaluation techniques, chelating capacity, calcium chelation.

1. Introduction

Chelation is defined as a process in which chemical agents interact to form soluble complexes with certain metal ions, binding the ions such that they do not react with other molecules or ions. During endodontic therapy, calcium chelating agents, such as 17% ethylenediaminetetraacetic acid (EDTA), are used to dissolve the inorganic components of the smear layer that sodium hypochlorite (NaOCl) cannot dissolve (Violich & Chandler, 2010).

The smear layer is produced by mechanical preparation of the root canal system. This amorphous structure is composed of both organic and inorganic components and can block the entrance of the dentinal tubules. Many investigations propose removal of the smear layer because it prevents the penetration of irrigating agents, medication, and sealing materials into the tubules, and even prevents their contact with the canal walls (Vasudev Ballal et al., 2011).

Additional studies have shown that the smear layer includes fragments of odontoblastic processes, microorganisms, and necrotic materials. The generation of a smear layer is inevitable during root canal instrumentation (Violich & Chandler, 2010).

In this article, the authors have attempted to discuss *In-vitro* techniques for the evaluation of chelating capacity of root canal irrigants in terms of specificity and sensitivity while identifying their advantages and disadvantages. This has been done to help clinicians, specialists, and researchers select the technique most suitable for their particular needs.

An electronic literature search was conducted in the Pub Med / MEDLINE database of indexed journals from 1992 to 2020. Terms used for the search included chelating, chelation, calcium chelation, smear layer, smear layer removal, and demineralizing effect. Articles included in the review met the criteria of being primary research articles, reflecting a variety of research designs, and having been undertaken to test the effects of various chelating agents/irrigants on smear layer removal or calcium chelation of root canal systems. Review articles, literature reviews, non-research articles, and studies on primary dentition were excluded from this review. Following this process, 32 studies were included in the review.

For easier reading and understanding, we classified the techniques under the following headings.

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- Quantitative techniques.
- Qualitative techniques.

1.1.- Quantitative techniques

The aim of these techniques is to classify features, count them, and construct statistical models to explain what is observed.

Energy Dispersive X-Ray Spectroscopy (EDS). This technique provides fast and non-destructive chemical analysis of a specimen with micrometric-scale resolution. The information generated by this analysis consists of a spectrum where it shows peaks corresponding to the elements in the sample. This technique can be qualitative, semi-quantitative, or quantitative, providing information on the spatial distribution of elements through mapping.

The EDS technique is non-destructive, and if required, specimens of interest can be examined in situ with little or no sample preparation (Vasudev Ballal et al., 2011). Different authors (Doğan, 2001; Mathew et al., 2017; Ozdemir et al., 2012; Vasudev Ballal et al., 2011) have used this technique to evaluate the decalcifying effect of different irrigating agents, such as EDTA, maleic acid, or citric acid, on dentin, mainly seeking changes in the levels of Ca, P, and Mg. When EDTA was compared with maleic acid, the specimens were treated for 0, 1, 5, 10 and 15 minutes, and as a result, maleic acid reduced the maximum amount of calcium and phosphorus at all-time intervals, but was significant only up to 5 minutes (p<0.001) (Vasudev Ballal et al., 2011). When EDTA was combined with NaOCl irrigation, the mineral content of the root dentin was altered, whereas the use of EDTA alone did not change it significantly. These findings were similar to those of two other studies (Doğan, 2001; Ozdemir et al., 2012). In another study (Mathew et al., 2017), chemical changes on the tooth surface were evaluated using 17% EDTA, 0.3% chitosan, and 0.5% chitosan. EDS analysis showed that the Ca/P ratio of the root dentin in the EDTA group was significantly lower than that in the chitosan group. One of the disadvantages of this technique is that the measurements cannot be made exactly at the same point; therefore, an average of the measurements must be made. In addition, the porosity of the dentin can produce secondary diffraction, so the dentinal surface has to be polished. Another disadvantage is that new measurements must be performed to obtain measurements of several elements (Spanó et al., 2009a).

Atomic Absorption Flame Spectrometry (AASF). This technique allows the determination of concentrations of a particular metallic element within a liquid sample up to mg/L. The information generated by this analysis consists of a spectrum that shows peaks corresponding to the elements that constitute the sample. This technique has been used to measure the concentration of Ca ions in irrigating agents (15% EDTA, 10% citric

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acid, 10% sodium citrate, apple cider vinegar, 1% acetic acid, 5% acetic acid, 5% maleic acid, 1% NaOCl, and 0.2% chitosan) after contact with dental samples (P. V. Silva et al., 2013; Spanó et al., 2009a). Both studies had the same findings: The highest Ca ion concentrations were observed with 15% EDTA and 0.2% chitosan, followed by 10% citric acid. The disadvantages of this technique are that all samples must be in a liquid state and the test has a low sensitivity.

Wavelength dispersive X-ray fluorescence spectrometry (WDXRF). This technique allows for the quantification and identification of the elements present in the sample at a scale of ppm (mg/L). For the analysis of dental samples, it is necessary to obtain dentin chips using Gates Glidden drills and sieve them to ensure homogeneity, which does not allow them to recover and return to their original state. This technique was used to evaluate changes in the mineral content (Ca, P, Mg, and K) and Ca/P ratio of root dentin after laser irradiation and five different irrigating agents (saline solution, 5.25% NaOCl, 3% H_2O_2, 15% EDTA, and 2% chlorhexidine gluconate). Their findings suggest that all irrigating agents decrease the calcium level with significant differences when compared with the control group (saline solution) (Gurbuz *et al.*, 2008).

Inductively coupled plasma-emission spectroscopy (ICP-AES). This technique allows for the quantification and identification of metallic elements present in a solution at a scale of ppb (µg/L). This technique has been used to evaluate the effect of different irrigating solutions (0.2% chlorhexidine, 3% H_2O_2, 17% EDTA, 5.25% NaOCl, and 2.5% NaOCl) on the mineral content of root dentin (Ari & Erdemir, 2005). Other authors have used this technique to evaluate the effects of 10% citric acid, 18% etidronate, 2.25% paracetic acid, and 17% EDTA on the levels of Ca, P, K, Mg, Na, S, Mn, and Zn in dentin samples (Cobankara et al., 2011).

Both studies agree that when compared with a control group, 17% EDTA significantly decreased Ca levels, and when compared with other experimental groups, there was no significant difference in calcium levels after treatment with the irrigation solutions except for 5.25% NaOCl. One of the main advantages is that polishing of the sample is not necessary, and that dentin chips obtained with Gates Glidden drills are sufficient for testing using ICP-AES. In addition, multiple elements can be measured simultaneously and repeated for a second element. To analyze dental samples, it is necessary to digest them in a solution of HNO_3, which means that they cannot be recovered. Another disadvantage of this technique can be observed during sampling, since the authors proposed to obtain dentin chips with the use of Gates Glidden burs, which does not allow standardization of their amount or weight, and thus leads to false positives. Table 1 shows a summary of all quantitative techniques.

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Table 1. Summary of quantitative techniques used to study smear layer removal.

Quantitative techniques		
Method	**Advantages**	**Disadvantages**
Energy Dispersive X-Ray Spectroscopy	-Fast and non-destructive. -Samples need less or no preparation.	-Measurements cannot be made exactly at the same point. -Porosity of the dentin can produce secondary diffraction and thus lead to false positive results. -Only one element can be measured at the time.
Atomic absorption flame spectrometry	-Allows to quantify and determine the elements present in the sample at concentration up to mg/L.	-Only metallic elements can be measured -All samples must be in liquid state.
Wavelength dispersive X-ray fluorescent spectrometry	-Allows to quantify and identify the elements present in the sample at a scale of ppm (mg/L).	-It is necessary to obtain dentin chips, which does not allow them to be recovered and returned to their original state.
Inductive coupling plasma emission spectroscopy	-Allows to quantify and identify the metallic elements present in a solution, at a scale of ppb (µg/L).	-Samples cannot be recovered because they need to be digested in a solution of HNO_3. -It´s hard to standardize weight of samples using Gates-Glidden burs.

1.2.- Qualitative Techniques.

The aim of this techniques is to evaluate the presence or absence of certain features, rather than measure them.

Scanning electron microscopy (SEM).

The most commonly used technique to evaluate the chelating capacity or smear layer removal of root canal irrigants is scanning electron microscopy (See Table 2). This technique allows detailed analysis of the morphology of the surface of the sample. It facilitates the analysis of dental materials, study of the shape and characteristics of dentin, quantification of dentinal tubules, and the effects of different irrigating solutions on dentin, among others.

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The resolution of SEM is 25 nm (approximately) and depends on the penetration of the electron beam. The samples of dental materials to be observed using SEM require some preparation. The best images can be obtained from clean, dry surfaces, free of organic pollutants, with a small amount of moisture on the surface, resistant to high vacuum and with good electrical conductivity. Samples that do not have these characteristics require a coating of duct material, preferably 10 nm thick gold or palladium-gold.

The method of evaluating the chelating capacity or smear layer removal of irrigating agents with SEM has been reported by several authors (Arslan et al., 2016; Connell et al., 2000; Da Costa Lima et al., 2015; del Carpio-Perochena et al., 2015; Doğan, 2001; Geethapriya et al., 2015; Ghisi et al., 2015; Gurbuz et al., 2008; Hennequin et al., 1994; Hennequin & Douillard, 1995; Kaufman et al., 1997; Kim et al., 2013; Mancini et al., 2013; Nassar et al., 2015; Pimenta et al., 2012; Schmidt et al., 2015; P. V. Silva et al., 2012, 2013; Spanó et al., 2009a; Turk et al., 2015; Vasudev Ballal et al., 2011; Zhou et al., 2018), which consists of acquiring micrographs of the canal wall in the desired area at magnification of x500, x1000, x2000, or x5000.

These micrographs are generally evaluated for the amount of smear layer by blinded (external) examiners through a scoring system that most of them are as follows:1) absence of smear layer; 2) few areas covered with smear layer (<33.3%), and many dentinal tubules visible; 3) most areas covered with smear layer (33.3-66.6%), and only a few dentinal tubules visible; and 4) surface completely covered with smear layer (100%), and no visible dentinal tubules.

With this technique, it is not possible to compare the same dentin surfaces before and after contact with chelating agents; therefore, the experimental groups must be compared with a control group, such as distilled water or NaCl solution. Another disadvantage is that the samples cannot be recovered because of dehydration and sputter coating before SEM analysis.

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Table 2. Main smear layer removals (root canal irrigants) studied by SEM.

Chelating agent	Findings	References
Acetic Acid	5% Acetic acid was not as effective in removing smear layer compared to 15% EDTA or 10% citric acid, with a contact time of 5 min.	(Spanó et al., 2009a)(Spanó et al., 2009b)
Apple Vinegar	Apple vinegar was not as effective in removing smear layer compared to 15% EDTA or 10% citric acid, with a contact time of 5 min.	(Spanó et al., 2009b)
Boric Acid	5% Boric acid was more effective in removing smear layer compared to 5% EDTA or 2.5% citric acid, with a contact time of 1 min.	(Turk et al., 2015)
Chitosan	0.2% chitosan was more effective in smear layer removal than MTAD, especially in the apical third, with a contact time of 3 min. When comparing 15% EDTA, 0.2% chitosan and 10% citric acid, all of them were associated with little smear layer remaining on dentine walls, having similar results to each other, with a contact time of 3 min.	(P. V. Silva et al., 2013; Zhou et al., 2018)
Citric Acid	10% citric acid and 15% EDTA are more effective in removing smear layer on dentin walls than 10% sodium citrate, apple vinegar, 5% acetic acid and 5% malic acid, with a contact time of 1 min. 5% Boric acid was more effective in removing smear layer compared to 5% EDTA or 2.5% citric acid, with a contact time of 1 min. When comparing 15% EDTA, 0.2% chitosan and 10% citric acid, all of them were associated with little smear layer remaining on dentine walls, having similar results to each other, with a contact time of 3 min.	(P. V. Silva et al., 2013; Spanó et al., 2009b; Turk et al., 2015)
Clorhexidine	2% chlorhexidine gluconate was not effective in removing smear layer on dentin walls, when compared with 3% H_2O_2 and 15% EDTA, with a contact time of 15 min.	(Gurbuz et al., 2008)
Etidronic Acid	9% and 18% etidronic acid were more effective in removing smear layer on dentin walls, when compared with 17% EDTA, 0.5%, 1% and 2% peracetic acid, with a contact time of 1 min.	(Ulusoy & Görgül, 2013)
EDTA	15% EDTA was as effective as 10% citric acid in removing smear layer on dentin walls, and better than 5% acetic acid, apple vinegar and 5% boric acid, with a contact time of 5 min. When comparing 15% EDTA, 0.2% chitosan and 10% citric acid, all of them were associated with little smear layer remaining on dentine walls, having	(Connell et al., 2000; Gurbuz et al., 2008; Kim et al., 2013; Nassar et al., 2015; Schmidt et al.,

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	similar results to each other, with a contact time of 3 min. 17% EDTA or 9% and 18% etidronic acid were more effective in removing smear layer on dentin walls than 0.5%, 1% and 2% peracetic acid with a contact time of 1 min. Three solutions of EDTA – a 15% concentration of the alkaline salt, a 15% concentration of the acid salt, and a 25% concentration of the alkaline salt – effectively removed most of the smear layer on dentin walls but did not remove it completely. 15% EDTA was as effective as 3% H_2O_2 in removing smear layer on dentin walls and better than 2% chlorhexidine gluconate, with a contact time of 15 min. 5% EDTA was not as effective as 5% boric acid in removing smear layer on dentin walls, with a contact time of 1 min. 17% EDTA was as effective in removing smear layer on dentin walls as 1% phytic acid, with a contact time of 30 sec or 1 min. 17% EDTA combined with passive ultrasonic irrigation was as effective as conventional irrigation, with a contact time of 1 min. When comparing liquid-type or gel-type EDTA, there were no difference in the remaining smear layer on dentin walls.	2015; P. V. Silva et al., 2013; Spanó et al., 2009b; Turk et al., 2015; Ulusoy & Görgül, 2013)
H_2O_2	3% H_2O_2 was as effective as 15% EDTA in removing smear layer on dentin walls with a contact time of 15 min.	(Gurbuz et al., 2008)
Malic acid	5% Malic acid was not as effective to remove smear layer compared to 15% EDTA or 10% citric acid, with a contact time of 5 min.	(Spanó et al., 2009b)
MTAD	MTAD was not as effective in smear layer removal than 0.2% chitosan, with a contact time of 3 min.	(Zhou et al., 2018)
Peracetic Acid	0.5%, 1% and 2% peracetic acid were not as effective to remove smear layer on dentin walls, when compared with 17% EDTA or 9% and 18% etidronic acid with a contact time of 1 min.	(Ulusoy & Görgül, 2013)
Phytic Acid	1% Phytic acid was as effective to remove smear layer on dentin walls as 17% EDTA, with a contact time of 30 sec or 1 min.	(Nassar et al., 2015)
Sodium Citrate	10% Sodium citrate was the least effective to remove smear layer compared to any other chelator, with a contact time of 5 min.	(Spanó et al., 2009b)

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Fourier-transform infrared spectroscopy (FTIR). This technique is widely used to characterize biomaterials because it analyzes the presence or absence of functional groups in a sample to be identified. One advantage is that it is fast and easy to perform measurements. It has been proposed to characterize the chemical degradation of the main components of human dentin after exposure to irrigating solutions such as 2.5% NaOCl, 17% EDTA, or RCPrep by changes in the intensity of the characteristic bands of collagen and phosphate (Ramírez-Bommer et al., 2018; Verdelis et al., 1999). One of the disadvantages of this technique is that grounded and sieved dentin must be taken for a correct reading and that the intensity of the bands relies on the amount of the sample; therefore, the same weight samples must be analyzed. Table 3 shows a summary of all qualitative techniques.

Table 3. Summary of qualitative techniques used to study smear layer removal.

Qualitative techniques		
Method	**Advantages**	**Disadvantages**
Scanning electron microscopy	-Allows to analyze in detail the morphology of the surface of the sample with a resolution of 25 nm.	-Samples must be dehydrated and require a coating of ductil material. -It is not possible to compare the same dentin surfaces before and after the contact with chelating agents.
Fourier transform infrared spectroscopy	-It is fast and easy to perform the measurements. -Samples requires little preparation.	-Grounded and sieved dentin must be taken for a correct reading. -Intensity of the bands relies on the amount of the sample.

2.- Conclusions & final considerations

SEM was the technique mostly used by researchers to evaluate smear layer removal from root canal irrigants. To obtain more accurate findings, other researchers have used techniques that provide precise results, such as EDX, AASF, or ICP. These techniques can determine the concentration of ions, such as Ca or P, making it easier to construct statistical models and compare the performance of several root canal irrigants. Researchers must consider their aim of study so that they can decide which techniques suit their necessities.

Some considerations may be helpful when selecting samples and interpreting results.

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1.- Mineralization rates vary depending on the type and anatomical location of dentin tissue samples (Erdemir et al., 2004).

2.- Age-induced sclerotic dentin shows lower collagen content, which renders highly mineralized old peritubular dentin more quickly dissolvable in acids. This might explain the excessive tubular erosion in old dentin specimens, as evidenced by the increase in the tubular diameter and area (Ozdemir et al., 2012).

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