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Review

Surface Alterations Induced on Endodontic Instruments by Sterilization Processes, Analyzed with Atomic Force Microscopy: A Systematic Review

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Abstract: Endodontic canal disinfection procedures that use sodium hypochlorite, and subsequently, heat sterilization procedures can alter the surface of endodontic instruments, described as corrosion and micropitting. These phenomena can be visualized on the surface of the instruments by SEM and atomic force microscopy analyses. The endodontic instruments used in probing, pre-enlargement, and shaping phases are made of steel alloy or nickel-titanium alloy (NiTi) and are subject to torsional, flexor, and cyclic fatigue; indeed, reuse of these instruments must be done with the knowledge that these instruments are subject to fracture following stress caused during their use. Fracture of the instrument within the canal is an eventuality that can lead to failure of the treatment, and therefore it is important to try to reduce situations that can contribute to the fracture. This review was performed based on the PRISMA protocol. Studies were identified through bibliographic research using electronic databases. A total of 1036 records were identified on the PubMed and Scopus databases. After screening the articles, restricted by year of publication (1979 to 2019), there were 946 records. With the application of the eligibility criteria (all the articles pertaining to the issue of sterilization in endodontics), there were 228 articles. There were 104 articles after eliminating overlaps. There were 50 articles that discussed the influence of sterilization procedures on the surface characteristics of endodontic instruments, and 26 articles that measured parameters on surface alteration. Applying the inclusion and exclusion criteria resulted in a total of eleven articles for quantitative analysis. Four articles were in reference to the primary outcome, eight articles to secondary outcome, and five articles to tertiary outcome. The meta-analysis showed a statistically significant surface alteration effect after five autoclaves and after immersion in the canal irrigants after 10 min.

Keywords: autoclave; endodontic sterilization; atomic force microscopy; NiTi alloy; endodontics; corrosion

1. Introduction

Endodontic instruments are commonly used in dental practice to perform endodontic treatments of vital and necrotic teeth, endodontic retreatments, pulpotomies, pulpectomy and specification procedures. Depending on the phase of treatment, endodontic instruments are divided into instruments for probing the endodontic canal for the pre-enlargement, and glidepath for shaping the canal or instruments for the closure and three-dimensional sealing of endodontic canals [1,2]. Many of these tools are reusable after performing cleaning, disinfection, and sterilization procedures by autoclaving [3].

Endodontic canal disinfection procedures that use sodium hypochlorite [4], and subsequently, heat sterilization procedures, can alter the surface of endodontic instruments, described as corrosion and micropitting, phenomena [5] that can be visualized by SEM and atomic force microscopy analyses.

The endodontic instruments used in the probing, pre-enlargement, and shaping phases are made of steel alloy or nickel-titanium alloy (NiTi) and are subject to torsional, flexor, and cyclic fatigue, indeed the reuse of these instruments must be done with the knowledge that these tools are subject to fracture following stress caused during their use [6]. Fracture of the instrument within the canal is an eventuality that can lead to failure of the treatment, and therefore it is important to try to reduce situations that can contribute the fracture.

On the surface subject to fatigue, surface alterations can give rise to microcrack which can lead to fracture of the instrument, and also reduce the cutting capacity of the blades on the endodontic files [7]. Therefore, in order to maintain the same cutting efficiency, the endodontist has to exert greater pressure on the instrument with an increase in torsional fatigue stress [6].

An atomic force microscope is an instrument capable of analyzing the surface of instruments. It consists of a cantilever with a pointed tip (tip) mounted on the end, typically composed of silicon or silicon nitride and having a radius of curvature of the "order of nanometers". The sample to be scanned, through the Vand Der Waals forces, interacts with the tip of the detector by flexing it. There are several methods to detect any cantilever movement. The majority of atomic force microscopy (AFM) systems use laser beam detection, which is an optical system with position sensitive detectors called photodiodes. The laser light is reflected by the cantilever on the position-sensitive photodiode. Very small forces are produced between the probe and the surface to be scanned, and these are the forces that allow the AFM system to record the deflection of the cantilever. The cantilever deflection is called "cantilever rigidity". This rigidity can be measured by Hooke's law. Rigidity is recorded visually and can be viewed on the computer in real time. The surface scan of endodontic instruments is used both in non-contact mode and in contact mode and, in general, the scanned surfaces start at 3 mm from the tip of the instrument up to 6 mm. The parameters which are considered with AFM for the analysis of a surface are the arithmetic mean roughness(AMR) of the maximum height (MH) and root mean square (RMS). The atomic force microscopy, therefore, provides detailed information with measurable parameters of possible alterations and irregularities present on the surface of an instrument [8].

Surface alterations can represent a problem in the use of endodontic instruments. A study by Ylmaz, in 2018, identified surface alterations described as surface roughness with statistically significant results for instruments constructed with new M-wire and EDM alloys [9].

One problem of reusing endodontic instruments that are subject to fatigue is the deterioration they suffer that results from their use in the dental canal for the removal of dentin, as well as the corrosive action by the root canal irrigants such as sodium hypochlorite, and subsequently, the action of the temperature and steam induced by the autoclave sterilization process. The surface alterations are well described in a study by Inan, in 2007, on the universal ProTaper, after clinical use and sterilization [10].

Fayyad and Mahran, in a 2013 study, demonstrated by AFM analysis that the alterations on Twisted Files [11], Hero Shaper, RaCe, and GTX instruments were statistically significant after immersion in 5% sodium hypochlorite, however, the alterations were not statistically significant after EDTA immersion. In contrast, Ametrano et al., in 2010, reported significant results for instruments immersed in EDTA [12]. Other studies have report conflicting data, such as the study conducted by Casella, in 2011, in which there was no variation in corrosion resistance for some instruments (K FILE and GT-rotary) unlike the K3 knife immersed in 5% sodium hypochlorite [13]. In addition, studies conducted at the Sem da Razavianet, in 2015, reported an increase in roughness directly related to the number of sterilization cycles performed on endodontic instruments [14].

In contrast, there is debate within the scientific community regarding whether there are statistically significant surface alterations induced by the autoclave or the canal irrigants. This review aims to try to clarify this aspect by investigating the literature to extrapolate the data on surface alterations in endodontic instruments in order to statistically analyze them in a meta-analysis.

Previous systematic reviews on this topic have not included the effect of surface alterations of endodontic instruments subjected to heat sterilization. There is only one systematic review that analyzes the variations in torsional properties subjected to autoclave sterilization.

This review could help endodontists who perform endodontic therapy and reuse endodontic instruments daily. Awareness of the greater or lesser risk of potential fracture triggered by surface variations due to heat or use of canal irrigants on the instruments could be helpful.

2. Materials and Methods

This systematic review was conducted based on the Prisma protocol.

The study was constructed using the following PICO elements for questions: Population (endodontic instruments); intervention (surface alterations induced by sterilization processes and root canal irrigation); control (new endodontic instruments not subject to sterilization; and outcome (surface alterations induced by the sterilization process by autoclave, and by root canal irrigants such as sodium hypochlorite and EDTA).

The following PICO question was formulated: To what extent, statistically significant, the sterilization processes and the used canal irrigants alter the surface of the rotating endodontic instruments with respect to the control?

After an initial selection phase of article identification in the databases, the potentially eligible articles were qualitatively evaluated in order to investigate the surface alterations of endodontic instruments resulting from the sterilization of instruments and disinfection of endodontic canals.

2.1. Eligibility Criteria

This literature review took into consideration in vitro and clinical studies that concerned the subject of sterilization and the influence of the latter on the physical and chemical properties of endodontic instruments. In particular, articles that dealt with the corrosive phenomena and surface alterations considered by microscopy methods (atomic force microscopy), conducted in recent years, and published with abstracts in English, were considered potentially eligible.

Articles from the last 40 years were chosen, because disinfection and sterilization procedures have changed in light of new discovered infectious contaminants, such as HIV and HCV viruses and the prong of spongiform encephalopathy. Furthermore, the methods used to manufacture the instruments have changed with the introduction of new alloys and new instruments. Therefore, in summary, potentially eligible articles included studies that investigated the influence of sterilization and disinfection procedures on endodontic canals, as well as on the physical and chemical characteristics of endodontic instruments, however, articles published more than 40 years ago and those that did not present an abstract in English were excluded.

Finally, the articles that were potentially eligible were subjected to a full text analysis to verify their use for a qualitative and quantitative analysis.

The inclusion and exclusion criteria applied in the full text analysis are the following:

- Include all those studies that describe the alterations induced by the sterilization methods of the endodontic instruments, analyzed using atomic force microscopy;
- Include all the articles that describe the alterations induced by root canal irrigants (sodium hypochlorite and EDTA), analyzed using atomic force microscopy;

- The exclusion criteria are to exclude all those studies that do not report data (average and standard deviation) on surface irregularities (AMR, MH, and RMS).

2.2. Research Methodology

The studies were identified through a bibliographic research on electronic databases.

The literature search was conducted using the search engines "PubMed" and "Scopus". The search on the providers was conducted between 12 September 2019 and 18 September 2019 and the last search for a partial update of the literature was conducted on 1 October 2019.

The following search terms were used on PubMed and Scopus: "Endodontic sterilization" PubMed 333 and Scopus 269; "endodontic autoclave" PubMed 38 and Scopus 52; "atomic force microscopy" AND "endodontic" PubMed 21 and Scopus 33; "roughness" AND "endodontic" Pub Med 42 and Scopus 67; "roughness" AND "ethylenediaminetetraacetic acid" PubMed 15 and Scopus 40; "roughness" AND "sodium hypochlorite" PubMed 1 and Scopus 1; "sodium hypochlorite" AND "atomic force microscopy" PubMed 40 and Scopus 80; "atomic force microscopy" AND "NiTi rotary instruments" PubMed 1 and Scopus 2 (Table 1).

2.3. Screening Methodology

The records obtained were, subsequently, examined by two independent reviewers (M.D. and S.D), and a third reviewer (E.L.) acted as a decision maker in situations of doubt. The screening included the analysis of the title and the abstract to eliminate the recordings not related to the topics of the review. After the screening phase, the overlaps were removed and the complete texts of the articles were analyzed, from which the ones eligible for the qualitative analysis and the inclusion in the meta-analysis for the three results were identified. The results sought by the two reviewers were:

(1) Primary outcome, variations of the root mean square root (RMS) of endodontic instruments subjected to five autoclave cycles as compared with non-autoclaved control;
(2) Secondary outcome, variations of the root mean square (RMS) of endodontic instruments exposed to sodium hypochlorite 5% as compared with the control group;
(3) Tertiary outcome, variations of the root mean square (RMS) of the endodontic instruments described at EDTA 10% as compared with the control group.

The fourth reviewer, with supervisory duties, was L.Lo.M. The K agreement between the two screening reviewers was 0.8464 (Table 2). The K agreement was based on the formulas of the *Cochrane Handbook for Systematic Reviews* [15].

The Newcastle–Ottawa scale for case-control studies was used to assess the risk of bias in the included studies. The quantitative analysis was performed with the Rev Manager software 5.3 (Cochrane Collaboration, Copenhagen, Denmark [16].

Table 1. Complete overview of the search methodology.

Database-Provider	Key Words	Search Details	Number of Records	Number of Records after Restriction by Year of Publication (Last 40 Years)	Number of Articles Remaining after the Elimination of Records not Related to the Issue of Sterilization Influence on Endodontic Instruments	Articles After Remove Overlaps Articles	Number of Articles Dealing with the Problem of the Influence of Sterilization Procedures on the Surface Characteristics of Endodontic Instruments	Number of Articles that Have Analyzed the Surface Alterations with Methods Different from the Atomic Force Microscopy	Number of Articles that Analyzed Surface Alterations with Atomic Force Microscopy	Numbers of Articles Included in the Quantitative Analysis for the 3 Outcomes
PubMed	"endodontic sterilization"	"endodontic" [All Fields] AND ("sterilization"[All Fields] OR "sterilization", reproductive"[MeSH Terms] OR ("sterilization"[All Fields] AND "reproductive"[All Fields]) OR "reproductive sterilization"[All Fields] OR "sterilization"[All Fields] OR "sterilization"[MeSH Terms])	333	291	46					
PubMed	"endodontic autoclave"	"endodontic" [All Fields] AND "autoclave" [All Fields]	38	38	25					
PubMed	"atomic force microscopy" AND "endodontic"	"atomic force microscopy" [All Fields] AND "endodontic" [All Fields]	21	21	9					
PubMed	"roughness" AND "endodontic"	"roughness" [All Fields] AND "endodontic" [All Fields]	42	41	11					
PubMed	"roughness" AND "ethylenediaminetetraacetic acid"	"roughness" [All Fields] AND ("ethylenediaminetetraacetic" [All Fields] AND "acid" [All Fields])	15	15	2					
PubMed	"roughness" AND "sodium hypochlorite"	"roughness" [All Fields] AND ("sodium" [All Fields] AND "hypochlorite" [All Fields])	1	1	1					
PubMed	"sodium" "hypochlorite" AND "atomic force microscopy"	"sodium hypochlorite" [All Fields] AND "atomic force microscopy" [All Fields]	40	40	13					
PubMed	"atomic force microscopy" AND "NiTi rotary instruments"	"atomic force microscopy" [All Fields] AND "NiTi rotary instruments" [All Fields]	1	1	1					
Scopus	"endodontic sterilization"	TITLE-ABS-KEY (endodontic AND sterilization)	269	225	56					
Scopus	"endodontic autoclave"	TITLE-ABS-KEY (endodontic AND autoclave)	52	52	25					
Scopus	"atomic force microscopy" AND "endodontic"	TITLE-ABS-KEY ("atomic force microscopy" AND "endodontic")	33	33	13					
Scopus	"roughness" AND "endodontic"	TITLE-ABS-KEY ("roughness" AND "endodontic")	67	65	12					
Scopus	"roughness" AND sodium "hypochlorite"	TITLE-ABS-KEY ("roughness" AND "sodium" AND "hypochlorite")	1	1	1					
Scopus	"roughness" AND "ethylenediaminetetraacetic acid"	TITLE-ABS-KEY ("roughness" AND "ethylenediaminetetraacetic acid")	40	40	2					
Scopus	"sodium hypochlorite" AND "atomic force microscopy"	TITLE-ABS-KEY ("sodium hypochlorite" AND "atomic force microscopy")	80	80	9					
Scopus	"atomic force microscopy" AND "NiTi rotary instruments"	TITLE-ABS-KEY ("atomic force microscopy" AND "NiTi rotary instruments")	2	2	2					
Total records			1036	946	228	104	50	24	26	11

Table 2. K agreement calculation, Po = 0.94 (proportion of agreement), Pe = 0.6092 (agreement expected), K agreement = 0.8464 (<0 no agreement, 0.0 to 0.20 slight agreement, 0.21 to 0.40 fair agreement, 0.41 to 0.60 moderate agreement, 0.61 to 0.80 substantial agreement, and 0.81 to 1.00 almost perfect agreement). The K agreement was calculated from the 50 articles and included eleven articles with the application of the inclusion and exclusion criteria.

/	/	Reviewer 2	Reviewer 2	Reviewer 2	
		Include	Exclude	Unsure	Total
Reviewer 1	include	11	0	0	11
Reviewer 1	exclude	2	36	0	38
Reviewer 1	unsure	1	0	0	1
	total	14	36	0	50

3. Results

A total of 1036 records were identified on the PubMed and Scopus databases (Table 1). After screening the articles, with the restriction by year of publication (1979 to 2019), there were 946 records. With the application of the eligibility criteria (all the articles pertaining to the issue of sterilization in endodontics), there were 228 articles. There were 104 articles after eliminating overlaps. There were 50 articles that discussed the influence of sterilization procedures on the surface characteristics of endodontic instruments, and 26 that measured parameters on surface alteration.

Applying the inclusion and exclusion criteria resulted in a total of eleven articles for quantitative analysis.

Four articles were in reference to the primary outcome, eight to the secondary outcome, and five to the tertiary outcome. The entire selection and screening procedures are described in the flow chart (Figure 1).

3.1. Study Characteristics and Data Extraction

The studies included for quantitative analysis were:

- First outcome: Yılmaz et al., 2017 [9]; Spagnuolo et al., 2012 [17]; Inan et al., 2007 [10]; and Can Saglam et al., 2015 [18];
- Second outcome: Uslu et al., 2018 [19]; Can Saglam et al., 2015 [18]; Fayyad et al., 2013 [11]; Ametrano et al., 2010 [12]; Topuz et al., 2008 [20]; Cai et al., 2017 [21]; Saglam et al., 2012 [22]; and Prasad et al., 2014 [23];
- Third outcome: Uslu et al., 2018 [19]; Fayyad et al., 2013 [11]; Ametrano et al., 2010 [12]; Cai et al., 2017 [21]; and Prasad et al., 2014 [23].

The extracted data included the magazine (author, data, and journal); the endodontic instrumentation object of measurement (name, taper, and diameter at tip); the method of sterilization by heat (temperature, pressure, and time); the number autoclave cycles or irrigants; the number of instruments (control and experimental); the number of surfaces scanned by the instrument; the number of total scans; the size of the scanning surface; and the data concerning the root mean square (RMS) ± standard deviation.

The data extracted for the tree outcomes are shown in Tables 3 and 4.

3.2. Risk of Bias

The risk of bias was assessed through the Newcastle–Ottawa case-control scale. The results are reported in detail in Table 5. For each category, a value of one to three was assigned (one = low and three = high).

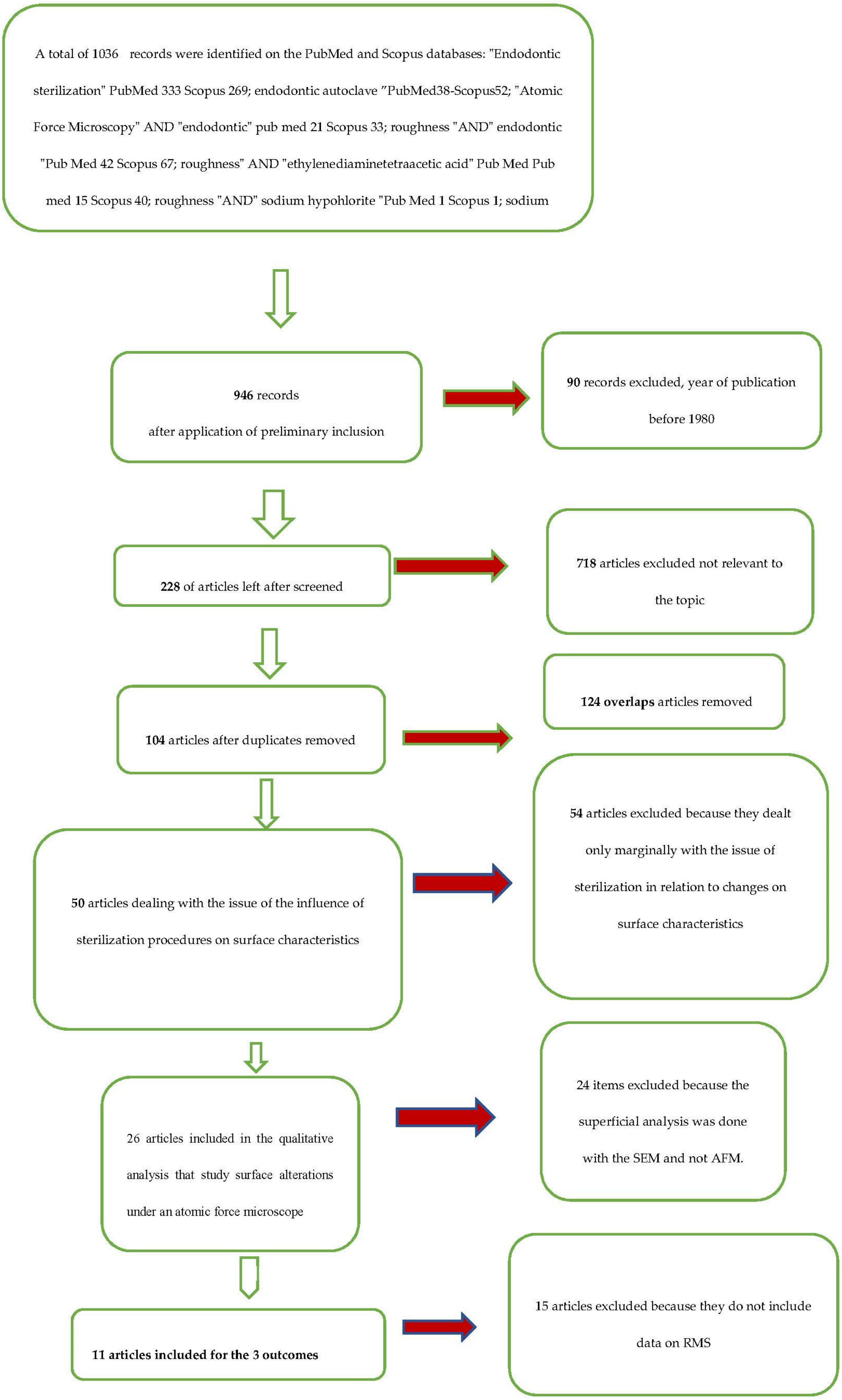

Figure 1. Flow chart of the different phases of the systematic review.

Table 3. Primary outcome (extraction of data relating to the root mean square detected on the surface of the endodontic instruments subjected to atomic force microscopy examination, with respect to control and after 5 cycles of autoclaving).

Autor, Data, Journal	Sterilization Method (Autoclave Temperature, Pressure Exposure Time)	Endodontic Instruments (Diameter and Taper at the Tip)	Autoclave Cycles	Number of Instruments	Surfaces Scanned by Instrument	Number of Total Scans	Scanning Surface	Root Mean Square (RMS) ± Standard Deviation
Yılmaz et al., 2017, Clin. Oral. Investig. [9]	Autoclave 134 °C, 30 psi for 20 min	HyFlex EDM (25/08)	0	2	15	30	5 × 5 µm	48.62 ± 7.76 nm
			5	2	15	30	5 × 5 µm	57.84 ± 6.94 nm
Spagnuolo et al., 2012, Int. Endod. J. [17]	Autoclave 121 °C, 15 psi, for 15 min	ProTaper F2 (25/08)	0	15	15	225	15 × 15 µm	203.75 ± 35.81 nm
			5	15	15	225	15 × 15 µm	715.22 nm ± 37.71
Can Saglam et al., 2015, Microsc. Res. Tech. [18]	Autoclave 121 °C for 20 min	ProTaper retreatment D1 (30/09)	0	1	11	11	2 × 2 µm	1.33 ± 0.558 nm
			5	1	11	11	2 × 2 µm	1.75 ± 0.940 nm
Inan et al., 2007, J. Endod. [10]	Autoclave 134 °C for 18 min	ProTaper F2 (25/08)	0	1	11	11	1 × 1 µm	1.46 ± 0.45 nm
			1	1	11	11	1 × 1 µm	7.29 ± 0.88 nm

Table 4. Extracted data relating to the secondary outcome and tertiary outcome, the data extracted are the root mean square (RMS), the total number of surfaces scanned, the endodontic instruments being scanned by the atomic force microscopy, and the irrigants used with the relative concentrations.

Autor, Data, Journal	Endodontic Instruments (Diameter and Taper at the Tip)	Irrigant Used (Concentration and Exposure Time)	Number of Instruments	Surfaces Scanned by Instrument	Number of Total Scans	Scanning Surface	Root Mean Square (RMS) ± Standard Deviation
Uslu et al., 2018, Microsc. Res. Tech. [19]	HyFlex EDM (25/.08)	control	4	20	80	5 × 5 µm	42.44 ± 4.51 nm
		NaOCl 5.25% for 5 min	4	20	80	5 × 5 µm	57.05 ± 8.55 nm
		EDTA 17% for 5 min	4	20	80	5 × 5 µm	60.65 ± 7.27 nm
Can Saglam et al., 2015, Microsc. Res. Tech. [18]	ProTaper retreatment D1 (30/09)	Control	1	11	11	2 × 2 µm	1.33 ± 0.558 nm
		NaOCl 2% for 5 min	1	11	11	2 × 2 µm	2.24 ± 0.555 nm
Fayyad et al., 2013, Int. Endod. J. [11]	RaCe	control	4	15	60	20 × 20 µm	83.3 ± 3.1 nm
		NaOCl 5.25% for 5 min	2	15	30	20 × 20 µm	92.3 ± 23.5 nm
		EDTA 17% for 5 min	2	15	30	20 × 20 µm	90 ± 7.5 nm
Ametrano et al., 2011, Int. Endod. J. [12]	ProTaper F2 (25/08)	control	1	20	20	1 × 1 µm	2.88 ± 0.72 nm
		NaOCl 5.25% for 5 min	1	20	20	1 × 1 µm	4.10 ± 1.13 nm
		EDTA 17% for 5 min	1	20	20	1 × 1 µm	4.79 ± 0.74 nm
Topuz et al., 2008, Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod. [20]	RaCe rotary NiTi files (30.06)	control	1	11	11	1 × 1 µm	2.06 ± 0.49 nm
		NaOCl 5.25% for 5 min	1	11	11	1 × 1 µm	6.99 ± 2.18 nm
Saglam et al. Microsc. Res. Tech. 2012 [22]	ProTapar f3 (30.08)	control	1	12	12	5 × 5 µm	1.31 ± 0.558 nm
		NaOCl 5% for 10 min	1	12	12	5 × 5 µm	3.20 ± 1.280 nm
Prasad et al., 2014, J. Conserv. Dent. [23]	iRaCe-R3	control	1	9	9	1 × 1 µm	1.35 ± 0.29 nm
		NaOCL 5%	1	9	9	1 × 1 µm	4.74 ± 1.09 nm
		EDTA 17%	1	9	9	1 × 1 µm	3.90 ± 0.58 nm
Cai et al., 2017, Int. Endod. J. [21]	HyFlex (25.06)	control	1	15	15	1 × 1 µm	10.12 ± 1.88 nm
		NaOCl 5.25% for 10 min	1	15	15	1 × 1 µm	9.35 ± 2.05 nm
		control	1	15	15	1 × 1 µm	10.47 ± 2.34 nm
		EDTA 17% for 10 min	1	15	15	1 × 1 µm	13.88 ± 3.78 nm

Table 5. Assessment of risk of bias within the studies (Newcastle–Ottawa scale) with scores 7 to 12 = low quality, 13 to 20 = intermediate quality, and 21 to 24 = high quality.

	Selection				Comparability	Exposure			Score
Reference	**Definition of Cases**	**Representativeness of Cases**	**Selection of Controls**	**Definition of Controls**	**Comparability of Cases and Controls on the Basis of the Design or Analysis**	**Ascertainment of Exposure**	**Same Method of Ascertainment for Cases and Controls**	**Non-Response Rate**	
Cai et al., 2017 [21]	3	3	3	3	3	3	3	0	21
Prasad et al., 2014 [23]	2	2	2	2	2	2	3	0	15
Saglam et al., 2012 [22]	3	2	3	3	3	3	3	0	20
Topuz et al., 2008 [20]	3	2	3	3	3	3	3	0	20
Ametrano et al., 2011 [12]	2	2	2	2	3	3	3	0	17
Fayyad et al., 2013 [11]	2	2	2	2	2	3	3	0	16
Can Saglam et al., 2015 [18]	3	2	3	3	3	3	3	0	20
Uslu et al., 2018 [19]	3	3	3	3	3	3	3	0	21
Inan et al., 2007 [10]	3	2	3	3	3	3	3	0	20
Yılmaz et al., 2017 [9]	3	3	3	3	3	3	3	0	21
Spagnuolo et al., 2012 [17]	3	3	3	3	3	3	3	0	21

The risk of bias within the individual studies was low enough that the methods of investigation adopted for the controls were identical to the cases included in the meta-analysis. The Prasad study [23] was the only study of the exposure time of endodontic instruments to canal irrigants that was not well defined, exposing the study to a bias.

The risk of bias between the various studies was considered high, and therefore partly limited the importance of the results. The heterogeneity of the studies depended mainly on the diversity of the instruments, which were similar, in some cases, only in terms of tip diameter, taper, and type of metal alloy.

The heterogeneity of the studies was represented by funnel plots of the four outcomes, as shown in Figure 2.

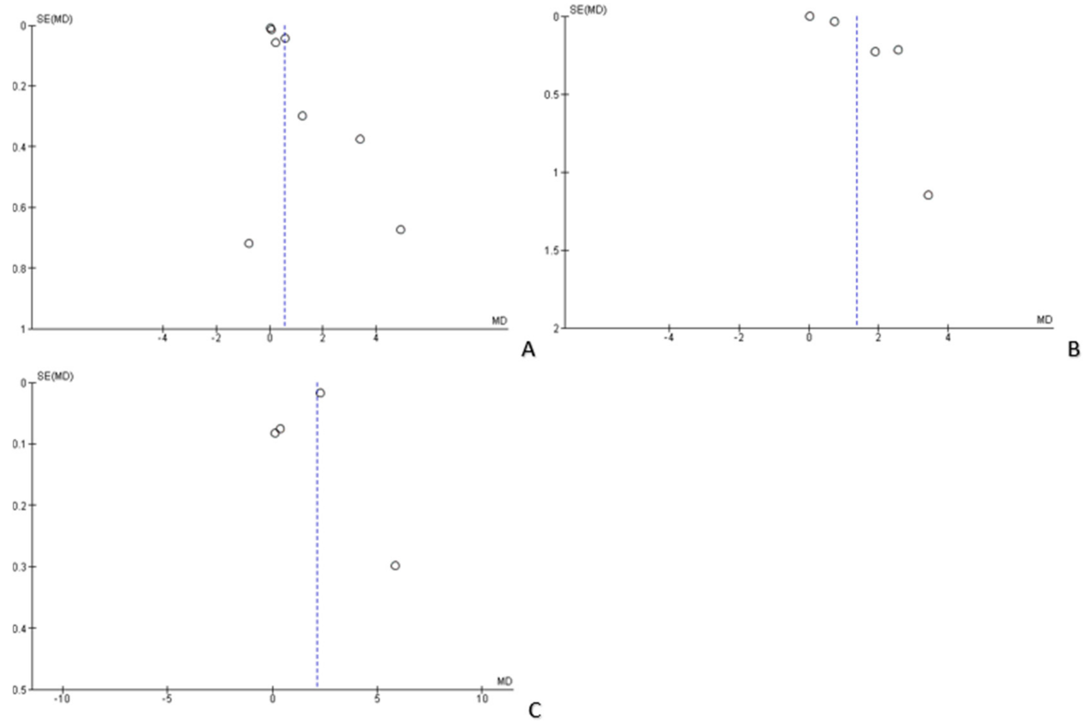

Figure 2. Funnel plots of the evaluation of heterogeneity for the (**A**) first, (**B**) second, (**C**) third outcomes.

3.3. Data Analysis

The statistical analysis of the data was performed using the Rev Manager 5.3 software (Copenhagen, 153 Denmark, The Nordic Cochrane Centre, The Nordic Cochrane Collaboration, 2014) and the results were represented by forest plots for each of the outcomes.

For the primary outcome, variations of the root mean square root (RMS) of endodontic instruments subjected to five autoclave cycles as compared with the non-autoclaved control, the comparison showed high heterogeneity of the studies, with an I2 equal to 100%. For this reason, a random effects model was used. Overall, for the primary outcome, meta-analysis was favorable for the control group. The studies that present data with a statistically significant difference are Inan et al., 2007 [10] and Spagnuolo et al., 20012 [17]. The studies by Ylmaz et al., 2018 [9] and Can Saglam et al., 2015 [22] are exactly at the center of the line of no effect. The studies by Ylzam and Can Saglam are exactly at the center of the line of no effect, however, the remaining two studies are favorable for the group subjected to control, their confidence intervals do not intercept the line of no effect (Figure 3).

Study or Subgroup	autoclave Mean	autoclave SD	autoclave Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Can Saglam 2015	0.4375	0.235	11	0.3325	0.1395	11	25.2%	0.10 [-0.06, 0.27]
Inan I 2007	7.29	0.88	11	1.46	0.45	11	24.3%	5.83 [5.25, 6.41]
Spagnuolo 2012	3.17875	0.1676	225	0.9055	0.19515	225	25.3%	2.27 [2.24, 2.31]
Yılmaz 2018	2.3136	0.2776	30	1.9448	0.3104	30	25.2%	0.37 [0.22, 0.52]
Total (95% CI)			**277**			**277**	**100.0%**	**2.11 [0.65, 3.57]**

Heterogeneity: Tau² = 2.19; Chi² = 1363.03, df = 3 (P < 0.00001); I² = 100%
Test for overall effect: Z = 2.83 (P = 0.005)

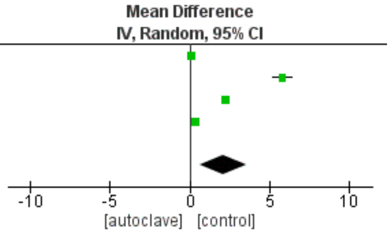

Figure 3. Forest plot of the random effects model of the meta-analysis of the primary outcome.

For the secondary outcome, variations of the root mean square (RMS) of endodontic instruments exposed to sodium hypochlorite 5% as compared with the control group, the comparison showed high heterogeneity among the studies, with an I2 equal to 98%. For this reason, for the second outcome, a random effects model was applied to avoid minimizing the roles of smaller-dimension studies. For the second outcome, the forest plot is in favor of the subject group control.

The studies that reported statistically significant data in favor of the control group are Ametrano, 2011; Prasad, 2014; Topuz, 2008; and Uslu, 2018. The Cai's study was the only study that was in favor of the group subjected to sodium hypochlorite, even though its confidence interval crosses the line of no effect. The other studies report statistically insignificant data (Figure 4).

Study or Subgroup	NaOCl Mean	NaOCl SD	NaOCl Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Ametrano 2011	4.1	1.13	20	2.88	0.72	20	7.4%	1.22 [0.63, 1.81]
Cai 2017	9.35	2.05	15	10.12	1.88	15	1.8%	-0.77 [-2.18, 0.64]
Can Saglam 2015	0.56	0.13875	11	0.3325	0.1395	11	20.0%	0.23 [0.11, 0.34]
Fayyad 2013	0.23075	0.05875	30	0.20825	0.00775	60	21.5%	0.02 [0.00, 0.04]
Prasad 2014	4.74	1.09	9	1.35	0.29	9	5.3%	3.39 [2.65, 4.13]
Saglam 2012	0.128	0.0512	12	0.0524	0.02232	12	21.4%	0.08 [0.04, 0.11]
Topuz 2008	6.99	2.18	11	2.06	0.49	11	2.0%	4.93 [3.61, 6.25]
Uslu 2018	2.282	0.342	80	1.6976	0.1804	80	20.7%	0.58 [0.50, 0.67]
Total (95% CI)			**188**			**218**	**100.0%**	**0.54 [0.35, 0.74]**

Heterogeneity: Tau² = 0.05; Chi² = 314.28, df = 7 (P < 0.00001); I² = 98%
Test for overall effect: Z = 5.42 (P < 0.00001)

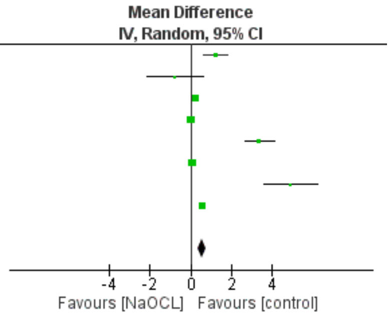

Figure 4. Forest plot of the random effects model of the meta-analysis of the secondary outcome.

For the tertiary outcome, variations of the root mean square (RMS) of the endodontic instruments exposed to EDTA 10% as compared with the control group, the comparison showed high heterogeneity between the studies, with an I2 of 99%, and therefore a random effects model was applied. For the tertiary outcome, the forest plot is in favor of the control group except for the study by Fayyad which is positioned in the line of no effect (Figure 5).

Study or Subgroup	EDTA Mean	EDTA SD	EDTA Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Ametrano 2011	4.79	0.74	20	2.88	0.72	20	22.0%	1.91 [1.46, 2.36]
Cai 2017	13.88	3.78	15	10.47	2.34	15	5.7%	3.41 [1.16, 5.66]
Fayyad 2013	0.225	0.01875	30	0.20825	0.00775	60	25.1%	0.02 [0.01, 0.02]
Prasad 2014	3.9	0.58	9	1.35	0.29	9	22.3%	2.55 [2.13, 2.97]
Uslu 2018	2.426	0.2908	80	1.6976	0.1804	80	25.0%	0.73 [0.65, 0.80]
Total (95% CI)			**154**			**184**	**100.0%**	**1.37 [0.76, 1.98]**

Heterogeneity: Tau² = 0.38; Chi² = 555.09, df = 4 (P < 0.00001); I² = 99%
Test for overall effect: Z = 4.41 (P < 0.0001)

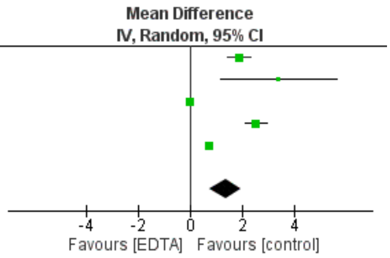

Figure 5. Forest plot of the random effects model of the meta-analysis of the tertiary outcome.

4. Discussion

The results of the meta-analysis for the three outcomes are in agreement in establishing that the superficial alterations induced by autoclave, from the sodium hypochlorite and from the EDTA, are statistically significant surface alterations that represent points where instrument fractures can be triggered. In addition, the alterations induced on the surface analyzed by SEM and AFM show that the alterations can also be expressed on the cutting surface, altering, in a pejorative sense, the cutting efficacy.

For the first outcome, the studies, in the literature, that supported a statistically significant alteration are:

1. In 2007, Inan reported statistically significant data for all the instruments of the ProTaper series (S1, S2, F1, F2), and reported that the superficial deterioration induced by the autoclave is greater for ProTaper finished than for ProTaper shaping;
2. In 2012, Spagnuolo confirmed, in agreement with Inan's data, that multiple cycles (autoclave sterilization) modified the surface topography and chemical composition of conventional NiTi (F2 ProTaper) and TiN-coated (alpha kit) instruments, in a statistically significant way (after five autoclave cycles).

Sodium hypochlorite certainly alters the surface of NiTi instruments and innumerable studies are in agreement such as Uslu et al., 2018 [19]; Ametrano et al., 2010 [12]; Topuz et al., 2008 [20]; and Prasad et al., 2014 [23]. Furthermore, a study conducted by Yokoyama et al., 2004 [24] stated that the action of sodium hypochlorite causes a worsening of the surface in endodontic instruments that facilitates their rupture following flexor and torsional stress.

The statistical analysis, in a similar way but with fewer studies, also confirms that the EDTA determines an increase in surface irregularities in a statistically significant way, and studies that confirm it after 10 min of exposure are well highlighted in the forest plot (Figure 5). Studies that are in contrast to the present meta-analysis report conflicting data regarding the action of EDTA on the surface. It seems that for exposures less than 5 min they do not alter the surface, however, according to Bonaccorsa et al. [25], a passivation phenomenon could lead to the creation of a complex between the metallic ions and the EDTA at a PH lower than four which renders the instrument resistant.

5. Conclusions

In conclusion, based on the present systematic analysis we affirm that autoclave induces a statistically significant corrosive phenomena, called micropitting, after five cycles of autoclave and determined by the heat, and comparatively, hypochlorite determines corrosion after only 5 min of exposure and EDTA after 10 min of exposure.

Superficial alterations, which are widely discussed in the literature, can determine the triggering of fractures in instruments subjected to cyclic fatigue and torsional fatigue. Therefore, it is important for endodontist to have knowledge of such corrosive phenomena, induced by irrigants such as sodium hypochlorite and EDTA, on instruments that can be reused and autoclaved.

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