

Review

Influence of sterilization procedures on the physical and mechanical properties of rotating endodontic instruments: a systematic review and network meta-analysis

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1. Abstract

The fracture of endodontic instruments inside the canal represents a problem that is not always easy to solve. The reutilization of endodontic instruments after sterilization procedures raises the question of how these processes affect their physical and mechanical properties. Alterations can involve the surface of the instruments, as well as their cutting effectiveness, shape and resistance to torsional and cyclic fatigue. The methodology adopted for this systematic review followed the PRISMA guidelines for systematic reviews. The following search terms were used in PubMed and Scopus: “endodontic sterilization”, “endodontic autoclave”, “cyclic fatigue”, “torsional”, “cutting efficiency”, “sterilization”, “surface characteristics” and “corrosion”. After the screening phase, the application of exclusion criteria and the removal of duplicates, 51 studies were identified

and divided into four outcomes: cyclic fatigue; deformation and torsional fatigue; corrosion or surface alterations; and cutting efficiency. Our study of the scientific literature highlights disagreements between studies on these effects. After autoclaving, instruments exhibit a reduction in the cutting efficiency, but NiTi alloy instruments have an improved resistance to cyclic and torsional fatigue.

2. Introduction

Over time, manufacturers have progressively perfected methods for the production of endodontic instruments for scouting, pre-flaring [1], preparing the glide path and shaping the endodontic canal [2]. Every endodontic file with a cutting capacity can also be employed for shaping. Here, the term “shaping step” refers to the shaping of the coronal, medium and apical parts following the scouting, pre-flaring and glide path steps [3].

The main steps of endodontic treatment are scouting, pre-flaring, glide path production, canal shaping and canal finishing, and each step requires an instrument that has different features that correspond to different physical and mechanical characteristics [4].

The sterilization process includes several phases: pre-sterilization, drying, packaging, heat sterilization and storage of the sterile material [5].

Pre-sterilization is the disinfection, decontamination and cleaning of dental instruments. This phase involves immersing the instruments in decontaminating and disinfecting liquids or cleansing them with washer-disinfectors. Decontamination reduces the microbial load on endodontic instruments, while cleansing removes organic and inorganic residues (to avoid the removal of debris by hand brushing, the use of ultrasonic trays is recommended) [6].

The subsequent phases involve the rinsing, drying and packaging of instruments, followed by heat sterilization (autoclave at 134 degrees at 2 atmospheres) to eliminate spores. The last phase is the storage of the instruments.

Other sterilization procedures after the pre-sterilization phase include glass bead sterilization or treatment with 2% glutaraldehyde (glutaraldehyde was used for high-level disinfection instead of sterilization but is not recommended because of its toxicity) [7].

An important limit to the reuse of endodontic instruments after sterilization lies in the inability to perfectly clean the blades from the debris created following the canal shaping phase [8]; the removal of debris by manual cleaning or with the help of ultrasonic trays does not allow to remove all debris of an organic and inorganic nature. And among these the prions which represent one of the contaminants that can resist decontamination procedures and are only partially inactivated and denatured at the temperatures reached by autoclaves used in dentistry; remember that prions are responsible for spongiform encephalopathy disease in humans [9–12].

Walker *et al.* [13] in 2007 they described the theoretical possibility of transmission of the pathology through prions through dental and endodontic procedures, indicating however low the risk of transmission [13–15]. Contamination by prions would potentially be triggered by the contact of the blades of the endodontic instruments with the pulp nerve tissue [16]; In addition, in patients with spongiform encephalopathy prions can be found at the trigeminal level, as demonstrated by Guiroy *et al.* [17, 18].

The WHO guidelines report the following methods as methods for the inactivation of the prion and its denaturation, as reported by Walker *et al.* [13]:

- ✓ Immersion in sodium hypochlorite (20,000 ppm of available chlorine) for one hour;
- ✓ Boiling in 1M sodium hydroxide for one hour;
- ✓ Autoclaving under vacuum at 121 °C for 30–90 minutes in the presence of 2M sodium hydroxide [19].

These methods alter the surface of the instruments and are not applicable to dental endodontic instruments as reported by Sonntag *et al.* [20].

The fracture of endodontic instruments inside the canal is a problem that is not always easy to solve [21–23]. The potential reuse of many endodontic instruments after sterilization procedures raises the question of how much these processes influence their physical and mechanical properties. There are three possible answers to this question: the instrument properties deteriorate, improve or are unaffected [24].

Some studies suggest that sterilization procedures have no influence on these instruments, but the results in the literature do not always agree. For example, D. Zhao *et al.* [25] reported an increase in the cyclic fatigue resistance of HyFlex CM, Twisted File and K3XF instruments (autoclave sterilization was performed at 134 °C with a pressure of 30 psi for 5 min), and Viana *et al.* [26] reported that the average number of cycles (916–950 cycles to failure) before failure was higher for instruments that were heat sterilized. In contrast, a recent study on NiTi instruments conducted by Masoud Khabiri *et al.* [27] indicated that sterilization had no influence on cyclic fatigue, while Silvaggio and Hicks [28] demonstrated that 10 autoclave cycles did not increase the risk of fracture of the files. Resistance to cyclic fatigue is not the only physical and mechanical property affected by sterilization procedures; other effects include resistance to torsional fatigue, on which recent studies disagree: Casper *et al.* [29] reported the improvement of M-Wire alloys on torsional fatigue strength, whereas King *et al.* [30] reported the deterioration of Gt Series X instruments. Furthermore, sterilization procedures can negatively affect the cutting efficiency and have corrosive effects on the surface of instruments, as demonstrated in a 1999 study by Rapisarda *et al.* [31], who reported a reduction cutting efficiency for ProFile instruments of 20%–50% after 7–14 cycles autoclave.

Thus, the literature reports conflicting findings about changes in endodontic instruments after sterilization procedures. These disagreements may be due to the multitude of instruments and methods used in endodontics and the different methods of sterilization and disinfection [32].

The heterogeneity of the results of the various studies could also depend on the high heterogeneity of the measurement apparatuses, in particular for the measurement of cyclic fatigue, Plotino [33] reports in a 2009 literature review as an ideal hypothetical model the curved canals of natural teeth, but the problem as set out by the author of the review and in the standardization of measurement.

All instruments for the measurement of cyclic fatigue that reproduce the artificial canal through a curved cylindrical tube are subject to wear and the endodontic instruments used within them have a degree of freedom following an unpredictable trajectory and without a precise measurement of radius and angle of curvature. In the realization of the measuring apparatus for cyclic fatigue, both

the angle and the radius of curvature of the artificial canal are fundamental, which differ in the various studies on the resistance to cyclic fatigue [33].

NiTi endodontic instruments consist of a crystalline lattice with a body-centered cubic shape which takes the name of austenite which, following thermal variations or the application of forces, becomes a monoclinic structure called martensite [34].

For NiTi alloys the martensitic transformations are of the displastic type, obtained by cooling the austenitic phase with the release of heat [35]. The transformation from austenite to martensite consists of 2 moments: in the first we have all the atomic movements necessary to produce the new structure and in the second the adaptation of shape and volume with a change in the crystal lattice that can take place with the slip, which is permanent, or the twinning which is reversible (and is preferable if you want to get a return of shape) [36].

Following a lowering of the temperature which varies according to the type of alloy, the crystal lattice acquires an easily deformable martensitic type configuration, with a low yield point; while upon heating, the alloy forms an austenitic lattice [37]; Under the application of a force, the austenitic alloy behaves in a similar way, passing to a martensitic crystalline form through an intermediate transformation phase that can accommodate greater stress without increasing the tension. Therefore, an endodontic instrument while working inside the endodontic canals is subjected to deformation forces in which there is the transition from the austenitic to martensitic phase through a transformation phase. In the transformation phase, the instrument will show superplasticity characteristics before reaching the martensitic phase which could be followed by yielding and rupture [38, 39].

Previous studies on the subject by the same research group have focused on the torsional property and surface alterations of endodontic instruments subjected to sterilization, focusing neither on cyclic fatigue nor deformation [7, 40, 41]. To date, no other systematic reviews have addressed these topics (cyclic fatigue, deformation, torsional fatigue, corrosion or surface alterations, cutting efficiency) in relation to sterilization.

The purpose of this review is to provide the widest and most up-to-date overview of the topic through a systematic methodology involving the screening and selection of articles [42, 43]. Our hypothesis is that not all alterations induced by the autoclave have a negative effect on rotating endodontic instruments.

3. Materials and methods

The following systematic review was performed in accordance with the PRISMA protocol (Preferred Reporting Items for Systematic reviews and Meta-Analyses): the outcomes, the data extraction process and the methods for

their quantitative and qualitative synthesis were previously agreed upon by 3 reviewers.

The protocol and methodology adopted for this review have been used for previous systematic reviews by the same research group.

Articles that were eligible for inclusion in this review were *in vitro* or clinical studies that evaluated the influence of disinfection and sterilization procedures on rotary endodontic instruments and endodontic files. Reports that were not written in English, were published before 1980 or were published in journals of national interest only were excluded. We selected only articles published in the last 4 decades because the instruments and sterilization methods have profoundly changed. Four research outcomes were defined:

- Primary outcome: influence of sterilization on the resistance to cyclic fatigue;
- Secondary outcome: influence of sterilization on the resistance to torsional fatigue;
- Tertiary outcome: alterations induced by sterilization procedures on the surface of endodontic instruments;
- Quaternary outcome: Reduction in cutting efficiency induced by sterilization procedures.

The studies were identified through bibliographic searches in electronic databases and the manual review of the biobibliography of previous systematic reviews conducted on the subject.

The literature search was conducted in the “PubMed” and “Scopus” search engines. The original search was conducted between 12.12.2020 and 01.03.2021, and a final search was conducted on 10.04.2021 for a partial update of the records.

We searched for different terms using the following combinations: “Endodontic sterilization” “endodontic autoclave” “cyclic fatigue” AND “sterilization”; “torsional” AND “sterilization”; “cutting efficiency” AND “sterilization” “surface characteristics” AND “sterilization”; “Corrosion” AND “sterilization”. Bibliographic citations of previous systematic reviews on the subject were also analyzed. Table 1 describes the research methodology for keyword searches in databases and article selection.

The “records” obtained from the search were subsequently screened by two independent reviewers (M.D. and D.S.). Situations of uncertainty or disagreement between the 2 reviewers were resolved by a third supervisor. Agreement between the 2 screening reviewers was measured by the K statistic, and $K = 0.68$.

After applying the preliminary eligibility criteria (articles in English and published after 1979) through the application of filters in search engines, duplicates were removed with the aid of the EndNote X8 software (Thomson Reuters, Clarivate Analytics, Philadelphia, PA, USA). Then, the two reviewers independently removed articles and studies that were not related to the topics of the re-

Table 1. Inclusion and exclusion criteria.

Category	Exclusion criteria	Inclusion criteria
Publication language	Not English	English
Study type	Reviews, systematic reviews , case reports, case series,	<i>in vitro</i> studies
Data characteristics	I. Articles that do not report data on physical and mechanical characteristics (resistance to cyclic and torsional fatigue, alterations of the surface of the instruments, cutting efficiency) following sterilization procedures; II. To exclude all studies that measured the variation in resistance to torsional and cyclic fatigue as well as surface alterations and cutting efficiency after shaping procedures of artificial or natural canals.	A. To include all studies reporting data on cyclic and torsional fatigue after sterilization; B. To include all studies that report data on the variation of the topographical surface after sterilization; C. To include all studies reporting data on cutting efficiency after sterilization.
Year of publication	Published before 1979	Published after 1979
further inclusion and exclusion criteria applied to the network meta-analysis		
Data characteristics	I. To exclude those data and studies in which the sterilization procedure was performed not in an autoclave; II. To exclude those studies that report data without a control (instruments not subjected to autoclaving cycles or other sterilization procedures); III. To exclude all data on cyclic fatigue obtained on artificial canals with a curvature angle and a radius of curvature different from 60 degrees and 5 mm.	A. The cyclic fatigue measurement must have been performed with a curvature angle of 60 degrees and a radius of curvature of 5 mm; B. The sterilization procedure must have been carried out by means of an autoclave (10 cycles).

view (endodontics and sterilization) by analyzing the title and abstract. The inclusion and exclusion criteria applied to the studies are shown in the Table 1.

Subsequently, studies were included in the qualitative analysis based on the 4 outcomes decided in advance.

The extraction of data from the included studies involved the development of tables and was performed by the two reviewers.

The characteristics of the data were the following: first author, date of publication, bibliographic reference, type of endodontic instrument, method of sterilization and alterations in the instruments induced by sterilization.

4. Results

A total of 1195 records were identified in the PubMed and Scopus databases. After screening the articles through the use of filters (restriction by year and language), 996 articles were obtained, and 578 were retained after the removal of duplicates. After the application of the eligibility criteria (all articles relating to the topic of sterilization in endodontics), we arrived at 161 articles, and 103 articles remained after the elimination of duplicates. After the application of exclusion and inclusion criteria, we obtained 51 articles, which were divided according to the topics of interest: outcome 1 (16 studies that investigate the influence of disinfection and sterilization procedures on resistance to cyclic fatigue); outcome 2 (7 articles investigating the influence of disinfection and sterilization procedures on resistance to torsional fatigue); outcome 3 (23 articles investigating the effects of sterilization and disinfection on the surface characteristics of endodontic instruments); outcome 4 (7 articles investigating changes in the cutting efficiency of instruments after sterilization and disinfection

procedures). Some articles were included in multiple outcomes.

The studies included in the quantitative analysis were as follows. First outcome: Khabiri *et al.* 2017 [27], Arias *et al.* 2014 [44], Zhao *et al.* 2016 [25], Plotino, *et al.* 2012 [45], Mize *et al.* 1998 [46], Viana *et al.* 2006 [26], Sharroufna and Mashyakh, 2020 [47], Das *et al.* 2019 [48], Alshwaimi 2019 [49], Arias *et al.* 2020 [50], Kim *et al.* 2020 [51], Li *et al.* 2015 [52], Yang *et al.* 2018 [53], Pedullà *et al.* 2011 [54], Bulem *et al.* 2013 [55], Özyürek *et al.* 2017 [56], Second outcome: Iverson *et al.* 1985 [57], Chenail *et al.* 1986 [58], Hilt *et al.* 2000 [59], Silvaggio *et al.* 1997 [28], Canalda-Sahli *et al.* 1998 [60], King *et al.* 2011 [30], Casper *et al.* 2011 [29]. Third outcome: Razavian *et al.* 2016 [61], Alexandrou *et al.* 2005 [62], Qaed *et al.* 2018 [63], Li *et al.* 2015 [52], Alexandrou *et al.* 2006 [64], Yang *et al.* 2018 [53], Yılmaz *et al.* 2017 [65], Nair *et al.* 2015 [66], Valois *et al.* 2008 [67], Spagnuolo *et al.* 2012 [68], Inan *et al.* 2007 [69], Can Saglam *et al.* 2015 [70], Das *et al.* 2019 [48], Cai *et al.* 2017 [71], Prasad *et al.* 2014 [72], Saglam *et al.* 2012 [73], Topuz *et al.* 2008 [74], Ametrano *et al.* 2011 [75], Fayyad and Mahrar [76], Uslu *et al.* 2018 [77], Shahi *et al.* 2012 [78], Stokes *et al.* 1999 [79], Casella *et al.* 2011 [80]. Fourth outcome: Schafer *et al.* 2002 [81], Haikel *et al.* 1996 [82], Rapisarda *et al.* 1999 [31], Seago *et al.* 2015 [83], Haikel *et al.* 1998 [84], Morrison *et al.* 1989 [85], Neal *et al.* 1983 [86]. There are 4 articles included in the network meta-analysis for the first outcome: Sharroufna and Mashyakh, 2020 [47], Kim *et al.* 2020 [51], Özyürek *et al.* 2017 [56], Plotino, *et al.* 2012 [45].

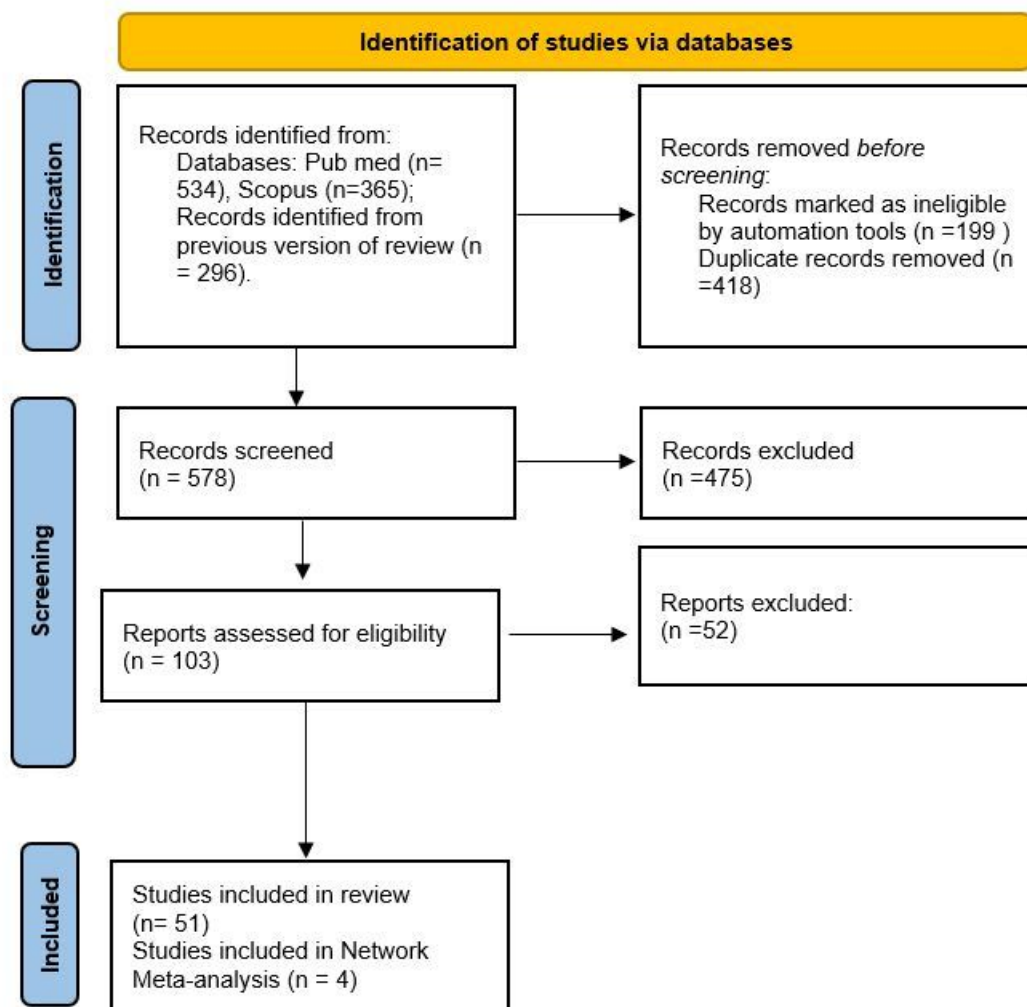


Fig. 1. Flowchart of the different phases of the systematic review.

The entire selection and screening procedure, as described in Table 2 (Ref. [7, 40, 41, 87–90]), is presented in the flowchart in Fig. 1.

5. Discussion

5.1 Cyclic fatigue

Cyclic fatigue is one of the main causes of fractures of rotating instruments made of NiTi. With the advent of new NiTi alloys and the introduction of new production methods, the occurrence of cyclic fatigue has been increasingly reduced [36, 91–93]. NiTi alloys today have equal atomic concentrations, which renders the austenitic phase very stable. A change in the proportion between titanium and nickel makes the alloy more unstable, and transformation into the super-elastic martensitic phase is favored under stress. After the super-elastic martensitic phase, the instrument undergoes compression and bending cycles, as is usually the case for instruments rotating in curved canals, after which it deforms and breaks [92, 94, 95]. The studies in the

literature can be divided into two subgroups: those that investigated the effects of hypochlorite on cyclic fatigue (Table 3, Ref. [54, 55]) and those that investigated the effects of hot sterilization procedures on cyclic fatigue resistance (Table 4, Ref. [25–27, 44–53]).

The studies conducted by Pedullà *et al.* [54] and Bulem *et al.* [55] agree that 5 percent sodium hypochlorite has no influence on cyclic fatigue, neither worsening nor improving it, regardless of whether the chemical is used as a canal irrigant or as a disinfectant agent for the instruments.

Other studies that support a lack of influence on cyclic fatigue are those conducted by Mize *et al.* [46], Khabiri *et al.* [27] and Kim *et al.* [51]. In these studies, there was no statistically significant increase or decrease. The investigated instruments were a Profile rotary file, Pro-Taper Universal, K3XF, HyFlex EDM and TF adaptive.

Conversely, other studies describe an increase in resistance to cyclic fatigue for the K3, K3XF, Mtwo, Pro-Taper, GT, GTX, Vortex, EdgeFile X7 and Twisted file instruments [26, 45, 47, 52].

Table 2. Complete overview of the search methodology Total articles after screening = 1095, after removed overlaps = 578 article included = 51.

Database - Provider	Key words and search details	Number of records	Number of records) after restriction by year of publication and language (last 40 years)	After remove overlaps articles	Number of remaining articles after screening for the latest review topic	Article remaining after application of the inclusion and exclusion criteria
Pub-med	Search: “endodontic” AND “sterilization” Sort by: Most Recent	224	161			
Pub-med	“endodontic” [All Fields] AND “sterilization” [All Fields] Search: “endodontic” AND “autoclave” Sort by: Most Recent	45	42			
Pub-med	“endodontic” [All Fields] AND “autoclave” [All Fields] Search: “cyclic fatigue” AND “sterilization” Sort by: Most Recent	29	27			
Pub-Med	“cyclic fatigue” [All Fields] AND “sterilization” [All Fields] Search: “torsional” AND “sterilization” Sort by: Most Recent	34	31			
Pub-med	“torsional” [All Fields] AND “sterilization” [All Fields] Search: “cutting efficiency” AND “sterilization” Sort by: Most Recent	17	17			
Pub-med	“cutting efficiency” [All Fields] AND “sterilization” [All Fields] Search: “surface characteristics” AND “sterilization” Sort by: Most Recent	34	33			
Pub-med	“surface characteristics” [All Fields] AND “sterilization” [All Fields] Search: “Corrosion” AND “sterilization” Sort by: Most Recent	151	107			
Scopus	“Corrosion” [All Fields] AND “sterilization” [All Fields] TITLE-ABS-KEY (endodontic AND sterilization)	301	229			
Scopus	TITLE-ABS-KEY (“endodontic” AND “autoclave”)	64	60			
Dioguardi <i>et al.</i> 2021 [88]	Systematic review	65	62			
Silva <i>et al.</i> 2020 [87]	Systematic review	40	40			
Dioguardi <i>et al.</i> 2020 [7]	Systematic review	97	95			
Jena <i>et al.</i> , 2020 [89]	Review	25	24			
Dioguardi <i>et al.</i> 2019 [40]	Systematic review	28	28			
Rajpurohit and Chowdary, 2019 [90]	Review	16	16			
Dioguardi <i>et al.</i> 2019 [41]	Systematic review	25	24			
Total records		1195	996	578	103	51

In contrast to these data, Alshwaimi [49] report a reduction in resistance to cyclic fatigue for Proflexendo rotary files. A decrease was also found for HyFlex EDM in a study by Arias *et al.* [50].

There is an apparent contrast among the results of different studies. Thus, for a definitive analysis, a meta-analysis should be carried out by extracting data from reports, such as the average number of cycles to failure and the length of fragments. However, there is excessive diversity in the studies, such as different apparatuses to measure cyclic fatigue, different angles of curvature of the artificial canals with which the cyclic fatigue was measured, the heterogeneity of instruments and different numbers of sterilization cycles. These differences can lead to results with a high heterogeneity index, as also indicated by a recent systematic review conducted by Silva *et al.* [87].

5.1.1 Network meta-analysis

In light of the high heterogeneity of the instruments investigated and of the investigation methods that are not always standardized (variations in the radius and angle of curvature), the 2 reviewers decided not to perform a meta-analysis of the data (variation in the number of cycles upon failure after sterilization) comparing instruments never autoclaved and instruments with 10 autoclave cycles, but to perform a Network meta-analysis comparing the different endodontic instruments through an indirect and direct comparison of the various rotating instruments. The data included in the network meta-analysis (mean difference, of the variation of the Number of Cycles to Failure) respects the principle of similarity (only the studies that reported data on NFC on non-sterilized instruments and after 10 sterilizations, obtained with artificial canals with a radius of curvature of 5 mm and a curvature angle of 60 degrees) since the methodology adopted is similar in each study and therefore comparable (Table 5, Ref. [45, 47, 51, 56]). The network diagram shown in Fig. 2 shows us how the main data of the direct comparisons are mainly on the K3XF, Vortex and PTU nodes with a similar robustness of the lines towards the various nodes.

The general consistency test reports at $\chi^2(1) = 30.17$, $p = 0.0001$.

For the analysis of the effect size, we proceeded through the representation by forest plot Fig. 3.

We then evaluated the instruments that after 10 autoclave cycles exhibit the lowest reduction in the number of turns at failure as shown in Fig. 4 and Table 6, the probability that the EDM endodontic instrument is the best (with the least reduction of NFCs) is approximately 71.8% and the probability that it is at least the second best is 6.4%. In the SUCRA (surface under the cumulative ranking), in fact, the surface for the EDM instrument reaches almost 90%, while we have that the PTG endodontic instrument has the probability that it is the best is 20.4% and 51.2% that it is the second, with a SUCRA also of 90%.

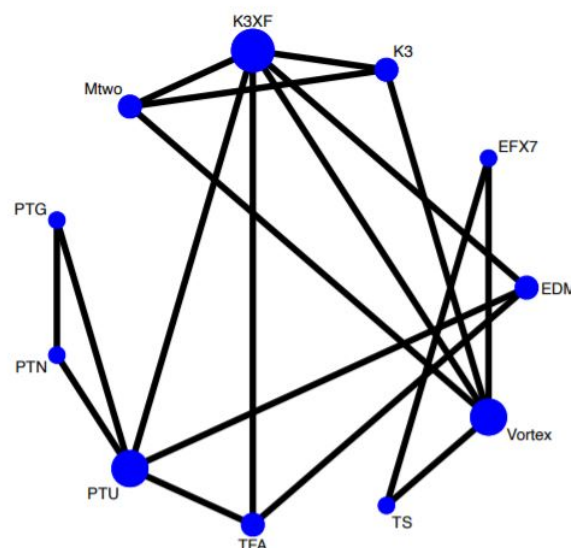


Fig. 2. Network geometry. The size of the 11 nodes, one for each treatment, indicates the number of studies included in the corresponding nodes, while the thickness of the lines connecting 2 nodes indicates the amount of relevant data (PTG, Protaper Gold; PTN, Protaper Next; PTU, Protaper Universal; TFA, TF adaptive file; TS, TRUShape; Vortex; EDM, HyFlex EDM; EFX7, EdgeFile X7; K3, K3XF, Mtwo).

5.2 Deformations and torsional fatigue

Among the fundamental properties that an endodontic instrument must have in order to be safely used in the endodontic canal is torsion resistance.

Torsion is defined as the stress to which an elongated body is subjected when one of its sections is rotated with respect to another or the effect of such stress [96].

In the most up-to-date methods, instruments such as K-files, NiTi files, Unifile and flex-or-files (generally 2% tapered instruments) are used to explore the endodontic canal and execute the glide path [97]; These instruments must exhibit characteristics of flexibility and resistance to torsion and at the end of each clinical use, it is important to inspect and eliminate instruments that have deformations or show stretching of the blades [98].

Iverson *et al.* [57] investigated the K-Flex and Burns Unifile instruments and reported that the torsion resistance of K-Flex files increased by 12.7% and 9.7% after heat and cold sterilization, respectively, compared with that of the controls and files subjected to other sterilization methods. These data are in contrast to the results reported by Chenail *et al.* [58], who studied the same instruments but observed no statistically significant influence, and agree with the findings of Hilt *et al.* [59], who reported no negative or positive influence on NiTi K-files.

These studies show that, for manual endodontic instruments with a constant taper of 2% made of both steel and nickel titanium (K-file, NiTi file), sterilization has neither a positive nor negative influence.

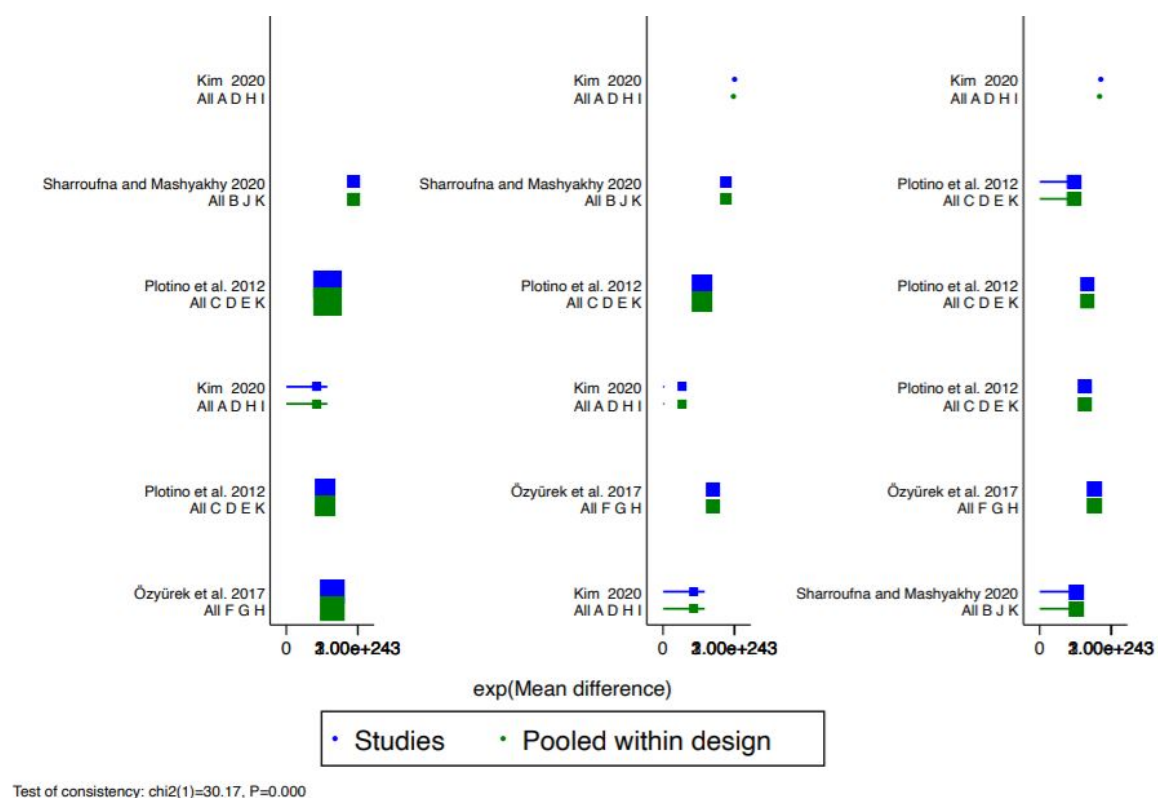


Fig. 3. Network Forest plot. All direct and mixed comparisons. A: EDM, B: EFX7, C: K3, D: K3XF, E: Mtwo, F: PTG, G: PTN, H: PTU, I: TFA, J: TS, K: Vortex.

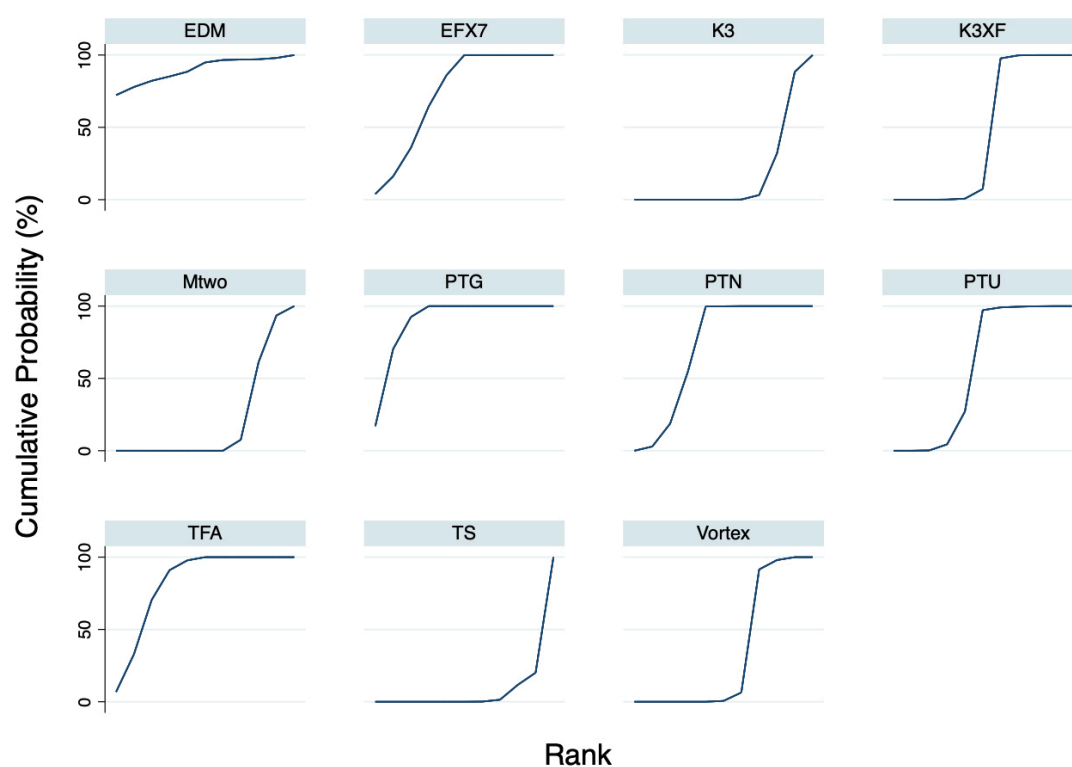


Fig. 4. Results of the network rank test. Graphical ranking; SUCRA: surface under the cumulative ranking. The higher the SUCRA value, the greater the probability that one instrument is better than the others with a lower reduction of NCFs after 10 cycles of sterilization.

Table 3. Studies that investigated the effects of hypochlorite on cyclic fatigue.

Autor, data	Instruments	Sterilization methods	Influence on resistance to cyclic fatigue
Pedullà <i>et al.</i> 2011 [54]	Twisted Files, Revo S, Mtwo files	5% NaOCl solution	No influence
Bulem <i>et al.</i> 2013 [55]	ProFile, FlexMaster, Mtwo and TwistedFiles	immersed in NaOCl; immersed in NaOCl and sterilized in one autoclave cycle; 5 cycles immersed in NaOCl and sterilized in autoclave and not immersed in NaOCl and not sterilized	No influence

Table 4. Studies that investigate the effects of sterilization procedures on cyclic fatigue.

Autor, data	Instruments	Sterilization methods	Influence on resistance to cyclic fatigue in relation to sterilization cycles
Khabiri <i>et al.</i> 2017 [27]	90 Nickel-Titanium 642 rotary files #30 with 0.06 tape	134 °C, 30 psi for 36 minutes (autoclave)	No influence
Arias <i>et al.</i> 2014 [44]	GT, GTX files	immersed in NaOCl and Autoclave	Increase resistance
Zhao <i>et al.</i> 2016 [25]	HyFlex CM, TF and K3XF	autoclave sterilization was performed at 134 °C, with a pressure of 30 psi, for 5 min	Increase resistance
Plotino, <i>et al.</i> 2012 [45]	K3, Mtwo, Vortex, and K3 XF	autoclave	Increase resistance (only k3 XF)
Mize <i>et al.</i> 1998 [46]	Ni-Ti instruments	135 °C and 30 to 35 psi for 5 minutes	No influence
Viana <i>et al.</i> 2006 [26]	ProFile	Dry heat sterilization cycles 170 °C, 40 minutes	Increase resistance
Sharroufna and Mashyakhy, 2020 [47]	EdgeFile X7, Vortex Blue, TRUShape	autoclave sterilization (10 cycles) at a temperature of 134 °C for 35 minutes	Increase resistance (only EdgeFile X7)
Das <i>et al.</i> 2019 [48]	Hyflex CM, Hyflex EDM	autoclave sterilization at 121 °C at 15 psi for 15 min	Not applicable
Alshwaimi 2019 [49]	Proflexendo rotary files	134 °C for 25 min,	Reduced the cyclic fatigue resistance of Proflexendo files
Arias <i>et al.</i> 2020 [50]	Hyflex EDM, TRUShape	136 °C, 4 minutes; Gamma irradiation	Gamma-irradiation decreased cyclic fatigue resistance of TRUShape; sterilization decreased fatigue resistance Hyflex EDM
Kim <i>et al.</i> 2020 [51]	ProTaper Universal, K3XF, HyFlex EDM, and TF adaptive	autoclave (10 cycles) 132 °C for 15 min	No influence
Li <i>et al.</i> 2015 [52]	K3, Mtwo, ProTaper	134 °C, 30 psi for 36 minutes (autoclave)	Increase resistance
Yang <i>et al.</i> 2018 [53]	K3XF, K3	autoclave	No influence

For rotary instruments, the torsional resistance of Profile instruments after sterilization was investigated for the first time by Silvaggio *et al.* [28] in 1997, who observed an improvement in resistance, and further studies on Twisted files and M-wire alloys also showed improvements [29, 36].

Alazemi *et al.* [99] reported that HyFlex instruments recovered their shape following clinical use and subsequent autoclaving.

Deterioration was only reported in one study, which was conducted by King *et al.* [30] on GT Series X files (Table 7, Ref. [28–30, 51, 57–60, 99–101]).

A recent systematic review of the literature conducted by this research group identified a meta-analysis of data on the variations in the deflection angle (the angle that is generated at the moment of fracture) and in the torsional

moment of NiTi and steel instruments after sterilization procedures. The data reveal reductions in the torsional fatigue resistance (reduction of the torsional moment) and the deflection angle, but with low statistical significance. The authors of the review cite the high heterogeneity among the included studies as a limitation [40].

5.3 Corrosion, topographic and surface alterations

An undesired effect of heat sterilization (autoclave and dry heat sterilization) on the surface of instruments is the formation of surface irregularities or micro-pitting [102]. Corrosion can also occur following the use of sodium hypochlorite or other dental disinfectants [103].

In the reviewed literature, three types of methods were used to analyze the level of corrosion: atomic force microscopy (AFM) [66], scanning electron mi-

Table 5. Data extracted from the 4 articles included in the network meta-analysis are reported.

	Intruments	Cycles autoclave	Number	N.C.F.	S.D.	Mean difference	S.D.M.D.
Sharroufna and Mashyakhy, 2020 [47]	EFX7	0	12	755.8	± 175.4	-443.0	219.2
		10	12	1198.8	± 255.6		
	Vortex	0	12	568.7	± 174.6	-37.4	160.53
		10	12	606.1	± 145.1		
	TS	0	12	442.2	± 80.9	45.6	80.8
		10	12	487.8	± 80.7		
Kim 2020 [51]	PTU	0	10	1712.9	± 321.6	270.1	330.26
		10	10	1442.8	± 338.7		
	EDM	0	10	11645.3	± 1768.0	-290.6	1702.75
		10	10	11935.9	± 1634.9		
	TFA	0	10	1344.7	± 297.00	78.7	330.71
		10	10	1266.0	± 361.3		
	K3XF	0	10	1726.8	± 383.1	473.9	329.99
		10	10	1252.9	± 266.5		
Plotino <i>et al.</i> 2012 [45]	K3	0	12	439	± 64	15	54.52
		10	12	424	± 43		
	Mtwo	0	12	419	± 45	10	48.09
		10	12	409	± 51		
	Vortex	0	12	454	± 95	-26	99.60
		10	12	480	104		
	K3XF	0	12	651	± 149	-111	175.79
		10	12	762	± 199		
Özyürek <i>et al.</i> 2017 [56]	PTG	0	20	1045.21	± 198.24	-261.13	224.62
		10	20	1306.34	± 248.22		
	PTN	0	20	525.44	± 94.67	-98.82	104.09
		10	20	624.26	± 112.72		
	PTU	0	20	248.12	± 32.52	-3.97	33.06
		10	20	252.09	± 33.59		

Table 6. The columns show the probability expressed as a percentage that each instrument is the best.

Rank	EDM	EFX7	K3	K3XF	Mtwo	PTG	PTN	PTU	TFA	TS	Vortex
Best	71.8	2.4	0.0	0.0	0.0	20.4	0.0	0.0	5.4	0.0	0.0
2nd	6.4	10.9	0.0	0.0	0.0	51.9	3.6	0.0	27.2	0.0	0.0
3rd	4.1	20.3	0.0	0.0	0.0	21.5	16.7	0.7	36.7	0.0	0.0
4th	2.7	29.5	0.0	0.0	0.0	6.2	36.8	4.7	20.1	0.0	0.0
5th	1.9	21.7	0.0	0.4	0.0	0.0	42.8	24.1	9.1	0.0	0.0
6th	7.2	15.2	0.0	7.6	0.0	0.0	0.1	68.3	1.5	0.0	0.1
7th	1.8	0.0	0.0	90.0	0.1	0.0	0.0	1.9	0.0	0.2	6.0
8th	0.7	0.0	4.2	2.0	5.7	0.0	0.0	0.2	0.0	1.6	85.6
9th	0.3	0.0	31.2	0.0	53.5	0.0	0.0	0.1	0.0	9.1	5.8
10th	0.4	0.0	53.2	0.0	34.0	0.0	0.0	0.0	0.0	9.9	2.5
Worst	2.7	0.0	11.4	0.0	6.7	0.0	0.0	0.0	0.0	79.2	0.0
Mean rank	2.2	4.0	9.7	6.9	9.4	2.1	4.2	5.7	3.0	10.7	8.0
SUCRA	0.9	0.7	0.1	0.4	0.2	0.9	0.7	0.5	0.8	0.0	0.3

croscopy (SEM) [64] and electrochemical methods (saturated calomel electrode (SCE), open circuit potential (OCP) and Electrochemical Impedance Spectroscopy (EIS)) [78].

We divided the articles into three sub-groups and extracted the corresponding data, which are reported in three different tables.

5.3.1 Scanning electron microscopy

SEM analysis showed the presence of topographical alterations on the surface. These alterations are already visible after the first autoclave cycle and can repre-

sent points where microcracks are induced in the structure, indicating cyclic or torsional fatigue failure.

Corrosion resistance is higher for alloys with equal atomic concentrations of nickel and titanium, and corrosion phenomena are more evident for alloys (46 titanium alloys, 56 nickel) with chromium inclusions, which facilitate corrosion through the formation of intermediate alloys [104].

Qaed *et al.* [63] observed these superficial alterations on AlphaKite and Revo-S NiTi rotary files, with an increase in surface changes after three cycles at a tempera-

Table 7. Characteristic of the main studies on the influence of sterilization procedures on resistance to torsional fatigue.

Autor, data	Sterilization methods	Instruments	Torsion resistance
Iverson <i>et al.</i> 1985 [57]	Hot sterilization	K flex, Burs unifile	Increase for burns unifile after 10 sterilization cycles
Chenail <i>et al.</i> 1986 [58]	Cold and dry heat sterilization	K flex, Burs unifile	No influence
Hilt <i>et al.</i> 2000 [59]	Hot sterilization	K file in acciaio and in NiTi	No influence
Silvaggio <i>et al.</i> 1997 [28]	Hot sterilization	Profile	Increase resistance
Canalda-Sahli <i>et al.</i> 1998 [60]	Autoclaving	Nitiflex Naviflex Microtitane Flexofile Flex-R	Increase for NiTi
King <i>et al.</i> 2011 [30]	Autoclaving	Gt serie x twisted file	Reduction Gt serie x Increase twisted file
Casper <i>et al.</i> 2011 [29]	Autoclaving	Profile Vortex Twisted Files CM Wire file	Increase CM Mwire
Alazemi <i>et al.</i> 2014 [99]	Autoclaving	HyFlex CM	Form recovery after sterilization
Kim <i>et al.</i> 2020 [51]	Autoclaving	ProTaper Universal, K3XF, HyFlex EDM, and TF adaptive	No influence
Testarelli <i>et al.</i> 2003 [100]	Autoclaving 124 °C 2.2 bar, for 20 min	Hero	No influence
Haïkel <i>et al.</i> 2003 [101]	hot sterilization, 180 °C 2.2 bar 2 h	Unifile, Flexofile and H-File	No influence

Table 8. Characteristic of studies performed with SEM analysis. The superficial alterations found on the instruments are highlighted following the various disinfection and sterilization processes.

Autor, data	Instruments	Sterilization methods	Results
Razavian <i>et al.</i> 2016 [61]	S-Files	1, 5 and 10 autoclave Cycles	O = Debris and roughness 1 = No significant 5 = Significant increase in roughness 10 = Significant increase in roughness
Alexandrou <i>et al.</i> 2005 [62]	Mani NRT NiTi	1, 6 and 11 autoclave cycles	1 = No significant 6 = No significant 11 = Significant increase in roughness
Qaed <i>et al.</i> 2018 [63]	Revo-S NiTi rotary	Control each instrument 3 shaped channels with a single autoclave cycle each instrument 9 shaped channels with a 3 autoclave cycle	show a smoother surface with few machining grooves dulling and blunting of the cutting edges is predominant showed plastic deformation, disruption in the surface with metal flakes
	AlphaKite	control each instrument 3 shaped channels with a single autoclave cycle each instrument 9 shaped channels with a 3 autoclave cycle	gross machining grooves on their surface with no pits, strips or disruption disruption of cutting edges microcracks, metal stripping
Li <i>et al.</i> 2015 [52]	K2, mtwo, protaper	10 cycles of sterilization autoclave	surface and inner imperfections in all instruments were intensified greatly after 10 cycles of sterilization
Alexandrou <i>et al.</i> 2006 [64]	ProFile, Flexmaster	11 autoclave cycles	Increased roughness was observed in all samples that were exposed to repeated sterilizations
Yang <i>et al.</i> 2018 [53]	K3XF, K3	10, 20 and 30 Autoclave cycles	The surface roughness of K3XF was increased after autoclave

ture of under 121 °C at 15 psi for 30 minutes. These data were confirmed by the SEM studies of Alexandrou *et al.* in 2005 and 2006 [62, 64] and Razavian *et al.* in 2016 [61] (Table 8, Ref. [52, 53, 61–64]).

Studies on longitudinal sections were conducted by Alexandrou *et al.* [62], who noted a statistically significant increase in the surface roughness of NiTi instruments

after 11 autoclave cycles. Moreover, SEM on the longitudinal sections of the inclusions and holes indicated a significant increase from 1 to 11 autoclave cycles [62].

5.3.2 Atomic force microscopy

The atomic force microscope consists of a cantilever with a sharp tip (tip) mounted on the end, typically

Table 9. Characteristic of studies performed with AFM analysis.

Autor, data	Instruments	Sterilization methods	Results
Yılmaz <i>et al.</i> 2017 [65]	HyFlex EDM	1, 5 and 10 autoclave cycles	Significant increase in roughness
Nair <i>et al.</i> 2015 [66]	Mani NRT NiTi, M two file	1, 5 and 10 autoclaves cycles	Significant increase in roughness
Valois <i>et al.</i> 2008 [67]	Greater Taper, ProFile	1, 5 and 10 autoclaves cycles	Significant increase in roughness
Spagnuolo <i>et al.</i> 2012 [68]	ProTaper, TiN -coated AlphaKite	1, 5 and 10 autoclaves cycles	Significant increase in roughness
Inan <i>et al.</i> 2007 [69]	ProTaper	1 autoclave cycles	Significant increase in roughness
Can Saglam <i>et al.</i> 2015 [70]	ProTaper retreatment, R-endo, and Mtwo	autoclaved five times and, 2% NaOCl or Chloroform	Surface alteration
Das <i>et al.</i> 2019 [48]	Hyflex CM, Hyflex EDM	autoclave sterilization at	Higher surface roughness
Cai <i>et al.</i> 2017 [71]	HyFlex, M3	5.25% NaOCl, 17% Edta	The surface roughness of 5.25% NaOCl and 17% EDTA immersed M3 files significantly increased compared to the control groups
Prasad <i>et al.</i> 2014 [72]	ProTaper and iRaCe	5.25% NaOCl, 17% EDTA	Short-term contact with 17% EDTA and 5% NaOCl can cause significant surface deterioration
Saglam <i>et al.</i> 2012 [73]	ProTaper	2.5% NaOCl, 5% NaOCl, 2% CHX, and MTAD	All tested solutions caused surface alterations
Topuz <i>et al.</i> 2008 [74]	RaCe	NaOCl solution for 5 min	NaOCl causes deterioration on the surface of RaCe
Ametrano <i>et al.</i> 2011 [75]	ProTaper	5.25% NaOCl, 17% EDTA	short-term contact between NaOCl and EDTA endodontic irrigants and ProTaper instruments caused alterations in the surface of instruments
Fayyad and Mahran [76]	GTX, TF, RaCe and Hero Shaper	5.25% NaOCl, 17% EDTA	17% EDTA did not significantly affect the surface roughness of the instruments, whilst 5.25% NaOCl increased the surface roughness
Uslu <i>et al.</i> 2018 [77]	HyFlex EDM, HyFlex CM	5.25% NaOCl, 17% EDTA	EDTA increase roughness, NaOCl not increase

Table 10. Characteristic of studies performed with Electrochemical methods. Endodontic instruments were treated with hypochlorite at different concentrations.

Autor, data	Means of investigation	Instruments	Results
Shahi <i>et al.</i> 2012 [78]	Electrochemical Impedance Spectroscopy (EIS)	RaCe, Mtwo	Corrosion resistance was significantly higher in Mtwo files compared to RaCe files in sizes #25, #30, and #35 and these properties might affect clinical efficacy of these instruments
Stokes <i>et al.</i> 1999 [79]	open circuit potential (OCP) (NaClO 5.25% for 1 Hour)	K-Flex, Flex-O, Flex-R, NiTiFile	No significant difference between stainless steel and NiTi
Casella <i>et al.</i> 2011 [80]	saturated calomel electrode (SCE) 5% NaClO aerated solution at 37 uC	GT Rotary, K3, K file	The sterilisation treatment had no significant influence on the corrosion resistance (Only k3 decrease corrosion)

composed of silicon or silicon nitride, which has a radius of curvature of the order of nanometers. Flexing the detector tip causes it to interact with the sample through van der Waals forces [105].

Three main parameters are taken into consideration when analyzing the surface with AFM: arithmetic mean roughness (AMR), maximum height (MH) and root mean square (RMS).

Data from studies conducted with atomic force microscopy are in agreement with those conducted with SEM. They report a statistically significant increase in surface imperfections after five autoclave cycles. The analyzed studies and their results are shown in Table 6. The corrosive effects of sodium hypochlorite and EDTA were studied using AFM by Uslu *et al.* [77], Prasad *et al.* [72], Cai *et al.*

[71], Saglam *et al.* [73], Topuz *et al.* [74], Ametrano *et al.* [75], Fayyad and Mahran [76] and Can Saglam *et al.* [70] (Table 9, Ref. [48, 65–77]).

A previous literature review with meta-analysis conducted by our research group [41] was carried out on the surface alterations induced by autoclaving, sodium hypochlorite and EDTA. The AMR and RMS were used as parameters in the AFM analysis of the surface. The results suggest that five autoclave cycles induce statistically significant corrosive phenomena, called micro-pitting, and corrosion occurs after only 5 minutes of hypochlorite exposure and after 10 minutes of EDTA exposure [41].

The scientific literature is in full agreement that both intra-canal disinfectants and autoclave sterilization cause surface alterations.

Table 11. Characteristics of the studies that relate the cutting efficiency of endodontic instruments in relation to disinfection and sterilization procedures.

Author data	Instruments	Method sterilization	Reduction of cutting capacity, expressed as a percentage of the control
Schafer <i>et al.</i> 2002 [81]	Niti Kfile PVD	Autoclave 5–10 cycles 135°	No reduction
		NaOCl 5.25% 30 minutes	No reduction
	Niti Kfile	Autoclave 5–10 cycles	50.6%–16.1%
		NaOCl 5.25%	No reduction
Haikel <i>et al.</i> 1996 [82]	Unifile	Dry hot sterilization 5–10 cycles 180° per 2 hours	No reduction (115.9%–104.9% efficacy)
		NaOCl 2% 12–48 hours	29.7%–36.7%
		Ultrasonic cleaning 1–4 hours, NaOCl (2.5%) and NH 4 (5%)	61.8%–61.8%
		NH4 1–4 hours	62.6%–62.2%
		Chemiclave 5–20 cycles per 20 minutes	63.9%–67.1%
		Glass bed sterilization 10–40 cycles per 40 seconds at 250°	5.4%–14.1%
	Flexofile	Dry hot sterilization 5–10 cycles per 2 hours 180°	1.1%–1.7%
		NaOCl 2% 12–48 hours	57.9%–67.6%
		Ultrasonic cleaning 1–4 hours NaOCl (2.5%) and NH 4 (5%)	66.8%–65.7%
		NH4 1–4 hours	68.5%–68.5%
		Chemiclave 5–20 cycles per 20 minutes	77%–73%
		Glass bed sterilization 10–40 cycles per 40 seconds at 250 °C	67.4%–66.9%
	H-File	Dry hot sterilization 5–10 cycles per 2 hours	49.8%–54.1%
		NaOCl 2% 12–48 hours	66.3%–62.9%
		Ultrasonic cleaning 1–4 hours NaOCl (2.5%) and NH 4 (5%)	65.3%–97.8%
		NH4 1–4 hours	50%–53.5%
		Chemiclave 5–20 cycles per 20 minutes	68.1%–50.4%
		Glass bed sterilization 10–40 cycles per 40 seconds at 250 °C	76%–59%
Rapisarda <i>et al.</i> 1999 [31]	ProFile instruments	7–14 cycles autoclave	20%–50%
Seago <i>et al.</i> 2015 [83]	Hyflex CM NiTi rotary files	From 1 to 9 sterilization cycles by autoclave after each single use	no statistical decrease after 1, 4, 5, and 6 cycles. A statistically significant decrease in cutting efficiency was for 2, 3, 7, 8, and 9 cycles
Haikel <i>et al.</i> 1998 [84]	Niti file Maillefer	2.5% NaOCl. 12–48 hours	(109.8% efficacy)–24.36%
	Niti file Brasseler	2.5% NaOCl. 12–48 hours	16.42%–32.87%
	Niti file JS Dental	2.5% NaOCl. 12–48 hours	29.97%–25.35%
	Niti file McSpadden	2.5% NaOCl. 12–48 hours	3.25%- no reduction
Morrison <i>et al.</i> 1989 [85]	K file	Sterilization after 1, 5, 10 use	No statistically significant change compared to the control group
	Flexofiles	Sterilization after 1, 5, 10 use	No statistically significant change compared to the control group
Neal <i>et al.</i> 1983 [86]	K-type, stainless steel files		autoclave sterilization resulted in a small but significant decrease in cutting ability of the files

5.3.3 Electrochemical methods

Shahi *et al.* [78], Stokes *et al.* [79] and Casella *et al.* [80] used electrochemical systems to investigate the effects of hypochlorite on the surfaces of instruments. The specific methods with the results are reported in Table 10 (Ref. [78–80]).

According to these studies, immersion in sodium hypochlorite at concentrations equal to 5.25% at a temperature of 37 degrees does not cause a significant decrease in corrosion resistance, although both SEM and AFM reveal effects on the investigated surface, as previously reported. If the exposure times exceed 12–24 hours, the corrosive effects will be apparent not only microscopically but also macroscopically.

5.4 Cutting efficiency

The first studies conducted on changes in cutting efficiency following sterilization were conducted by Neal *et al.* in 1983 [86], who described a significant decrease in the cutting capacity of K-files after 10 cycles of autoclaving. Subsequently, these data were partially disproved by Morrison *et al.* [85], who stated that there was no reduction in the cutting depth after five autoclaving cycles and that decreases were only due to clinical use.

Similar to Morrison, Seago *et al.* [83] did not observe any statistically significant reductions in the cutting capacity of HyFlex (rotary NiTi instruments) after one or two autoclave cycles.

Rapisarda *et al.* [31] reported that the cutting capacity of Profile NiTi rotary tools was reduced, with decreases ranging from 20% for 7 cycles to 50% for 14 cycles (heat sterilization). Corrosive phenomena were induced by the oxidation of surface layers with the formation of NiTi oxides [31].

The data reported by Schafer *et al.* [81] and Haikel *et al.* [82, 84] support a reduction in cutting efficiency. Schafer observed a reduction in the cutting efficiency of K-wires (NiTi) equal to 16.1% after 5 sterilization cycles and 50.8% after 10 autoclaving cycles at a temperature of 135 °C [81]. Haikel noted a reduction in cutting efficiency following immersion in 2.5% sodium hypochlorite for a time ranging from 12 to 48 hours and also reported that the use of ultrasonic trays resulted in a reduction of 20%–60%.

The effects of heat sterilization can appear after 5 sterilization cycles, but in most studies, they became evident after 10 cycles [31, 81, 82]. Sodium hypochlorite with concentrations ranging from 2.5% to 5.25% for 30 minutes showed no effect on shear capacity, as reported by Schafer *et al.* [81], but if the duration reached 12–48 hours, corrosive phenomena were triggered, resulting in a net reduction in efficiency [84, 106] (Table 11, Ref. [31, 81–86]).

6. Conclusions

The study of the literature shows that there are conflicting findings regarding the different effects of sterilization procedures. In sum, sterilization and disinfection procedures produce changes in physical and mechanical characteristics, but conclusions about whether the effects are favorable or unfavorable for clinical use are not always consistent between studies.

The alterations can be summarized as follows:

- There is a reduction in cutting capacity after autoclaving, and it becomes statistically significant after five autoclaving cycles;
- A corrosive effect is observed after the use of disinfectants based on sodium hypochlorite (12–48 hours) and after the application of heat sterilization;
- There is a potential shape recovery effect with regard to deformation and increased resistance to torsional and cyclic fatigue after sterilization, but not all studies agree.

7. Author contributions

Conceptualization, MD, AD, KZ, RA, GI; methodology, MD and DS; software, GI; validation, MD and AD; formal analysis, MD and AD; investigation, DS and AD and VCAC; resources, LLM; data curation, EL, BR and LL; writing—original draft preparation, MD and GT; writing—review and editing, MD, GT, CA and LLM.

8. Ethics approval and consent to participate

Not applicable.

9. Acknowledgment

Not applicable.

10. Funding

This research received no external funding.

11. Conflict of interest

The authors declare no conflict of interest.

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Abbreviations: PRISMA, Preferred Reporting Items for Systematic reviews and Meta-Analyses; SEM, scanning electron microscopy; AFM, atomic force microscopy; AMR, arithmetic mean roughness; MH, maximum height; RMS, root mean square.

Keywords: Cyclic fatigue; Endodontics; NiTi alloy; Endodontic instruments; Torsional resistance; Flexural fatigue; Corrosion; Cutting efficiency; Mechanical property; Fatigue properties

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